SALT PAN ECOLOGY AND ITS IMPACT ON COMMUNITY STRUCTURE OF HALOPHILIC ARCHAEA

THESIS SUBMITTED TO
GOA UNIVERSITY
FOR THE
AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY
BY

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NOVEMBER 2006

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DECLARATION

I, Ms. Caroline F. E. Fernandes, hereby declare that this thesis, submitted for the Ph. D. Degree, on the topic "SALT PAN ECOLOGY AND ITS IMPACT ON COMMUNITY STRUCTURE OF HALOPHILIC ARCHAEA", represents the research work carried out under the guidance of Dr. Irene J. Furtado, Reader, Department of Microbiology, Goa University, and that it has not been submitted to any other University or Institute for the award of any Degree, Diploma or other similar titles.

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I hereby certify the above and state that the thesis is a record of research work done by the candidate under my guidance, at the Department of Microbiology, Goa University.

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MY PARENTS

ACKNOWLEDGEMENTS

Most of all I would like to thank THE ALMIGHTY without whose strength, the thesis would not come to light.

A huge thank you goes to my Guide, Dr. Irene Furtado, who has been so generous to me, with both her knowledge and time in the laboratory, and for giving me the opportunity to do research on the topic suggested by her.

I heartily thank the University Grants Commission for award of assistance on Faculty Improvement Programme which enabled me to complete 80% of the work during the leave period of August 2004-2006.

I would like to thank the Management and the Principal, Dr. A. S. Dinge of my Parent Institution PES's College of Arts and Science, Farmagudi, Ponda.

I would also like to thank the Goa Government for approval, for availing the Teachers Fellowship by the University Grants Commission.

I would like to thank Dr. S. B. Bhosle, Head, Department of Microbiology, for extending necessary facilities.

I would like to thank Prof. G. N. Nayak, Head, Department of Marine Science & the Dean of Faculty of Life Sciences & Environment, Goa University.

I am thankful to Prof. D. J. Bhat, Head, Department of Botany, Dr. Nandkumar Kamat, Lecturer in Botany, Department of Botany, for their help in identification of fungal isolates.

I would like to thank Dr. C. G. Naik and Dr. Vijaya of Chemical Oceanography Department for carrying out FT-IR; and Mr. Khedekar, Mr. Arif of the Instrumentation Centre for SEM; of the National Institute of Oceanography.

I would like to thank Shri. Salim A. Veljee, Dy. Director of Food and Drugs Administration, Panaji, who introduced me to the World of Microbes in 1983. I would like to thank Shri. S. N. Tripathi, Ex-Director of Food and Drugs Administration, and Mr. Kambli, Ms. Tarcar and Ms. Dangui of FDA for helping me with the AAS and Flame Photometer studies.

I would like to thank Dr. Sarita Nazareth and Dr. Harsha P. Uskaikar.

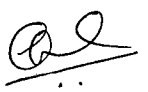
I would also like to thank the staff of PES's College of Arts and Science.

Paresh, Prema, Pooja, Pratibha, Naveen, Christine, Jenny, also helped me with various aspects of my thesis and I would like to thank them as well.

Mr. John de Melo deserves a special word of thanks.

I would like to thank my husband Paul for all his encouragement and for being so supportive of my goals; as well as my children-Annushka and Falan, my sister Tecla and the rest of my family for rendering a helping hand.

To everyone who helped me through this work I am truly grateful and you have all helped to make it a wonderful experience.



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Saline habitats / environments

Hypersaline environments are defined as those which have higher concentrations of salts than sea water (Edgerton and Brimblecombe, 1981). Classically, the hypersaline environment includes water and soil habitats (Ventosa, 1990). The former are classified into two categories: Thalassohaline, if the composition of ions is qualitatively similar to those found in sea water e.g. Great Salt Lake, Marine Solar Salterns from Spain, Yugoslavia, Italy; and Athalassohaline, if these amounts of salts are markedly different from those of sea water, e.g. Dead Sea, Lake Assal, Wadi Natrun or Texcoco (Ramos-Cormenzana, 1990) (Table 1).

Table 1 : Characteristics of some saline environments

Specific characteristics		Dead Sea	Great Salt Lake	Atacama Salar	Marine Saltern	La Mala Saltern
Sea level (m)		~400	1,280	2,400	10	700
pH		5.9-6.3	7.7-8.4	7.2-8.6	6.8-8.1	6.7-8.0
Temp (^{0}C)		21-36	-5-30	12-43	22-42	24-36
Total Salts (g/l)		299-332	113-332	137	140-260	170-230
Order of precipitation of ions Cations	1st	Mg (40.7)[a]	Na (105)	Na (38)	Na (87)	Na (57)
	2nd	Na (39)	Mg (11)	Ca (0.5)	Ca (0.5)	Ca (3.6)
	3rd	Ca (17)	K (6.7)	K (0.6)	K (0.6)	K (3.5)
Anions	1st	Cl (212)	Cl (181)	Cl (59)	Cl (169)	Cl (103)
	2nd	Br (5.1)	SO_4 (27)	SO_4 (2.4)	SO_4 (22)	SO_4 (4.9)
	3rd	SO_4 (0.5)	HCO_3(0.01)	HCO_3(0.06)	HCO_3	

( )[a] : concentration (g/l) (Ramos-Cormenzana, 1990).

i) Thalassohaline environments are characterized by the presence of sodium and chloride as the main ions and a neutral to slightly alkaline pH (Oren, 1994).

a) Solar Salterns present a man-made ecosystem (Madigan and Marrs, 1997) consisting of a discontinuous salinity gradient. A sequential precipitation of calcium carbonate,

calcium sulfate (gypsum) and sodium chloride (halite) occurs, while concentrated magnesium chloride brines (bitterns) remain in the course of evaporation of sea water (Oren, 1990, 1994). From a biological point of view, the ponds can be divided into three main categories. The first comprises those in which sea water is evaporated to about three times its original salinity, characterized by a microflora similar to that of seawater, with great species diversity and a low number of individuals of each species. At higher salinities, (3-7 times seawater) the brine becomes dark coloured, and supports dense algal populations (mainly biflagellated *Dunaliella*), on which the brine shrimp *Artemia salina* feeds. Bacteria in this brine are mainly moderate halophiles, present as a very heterogeneous community with many species (Rodriguez-Valera *et al*, 1981, 1985; Marquez *et al*, 1987). The third category comprises the hyper-saline ponds, from 6-7 times seawater salinity to saturation of sodium chloride-the crystallizer ponds. Concentrated brines of the halite crystallization ponds are often characterized by a bright red colour (Truper and Galinski, 1986) caused by dense microbial communities: *Halobacterium* species and also red *Dunaliella* cells (Oren, 1990, 1993, 1994; Tindall, 1991). Moderately and extreme halophilic bacteria overlap in the salinity range of 25-32% (Rodriguez-Valera *et al*, 1981, 1985), above which the bacterial community is dominated by red *Halobacterium* species. Halite crystallization leaves behind bitterns, although nutrient rich, donot support life as reported by Oren (1990).

b) Sabkhas are small evaporation ponds found near coastal areas, where seawater penetrates through seepage or via narrow inlets from the sea. Notable among these are Solar Lake, Gavish Sabkha, and Ras Muhammad Pool near the Red Sea Coast, Guerrero Negro on the Baja California coast, Lake Sivash near the Black Sea, and Sharks Bay in Western Australia. Hypersaline evaporation ponds have also been found in Antarctica

(e.g. Deep Lake, Organic Lake and Lake Suribati), several of which are stratified with respect to salinity (DasSarma and Arora, 2001). Deep Lake is a saline monomictic lake in the Vestfold Hills of Antarctica having a highest temperature of +11.5 ^{0}C and for eight months of the year, temperature is less than 0 ^{0}C. *Dunaliella* sp. and *Halobacterium lacusprofundi* sp. nov. are reported to grow in the lake (Franzmann *et al*, 1988).

c) Salt deposits/Evaporites include subterranean evaporite deposits consisting primarily of halite (NaCl), gypsum ($CaSO_4$. $2H_2O$) or anhydrite ($CaSO_4$) and containing bacterial and algal assemblages are well known in the fossil record and are still geographically wide spread (Grant *et al*, 1998; Rotthschild and Mancinelli, 2001). Deep-sea brines have been found in the Red Sea and Gulf of Mexico (DasSarma and Arora, 2001). Norton and Grant (1988) showed that microorganisms entrapped in fluid inclusions of growing NaCl crystals may be motile for three weeks, and may remain viable for up to six months. This phenomenon has been observed in solar salterns throughout the world. Preliminary studies have suggested that microbial activity occurs in some deep-sea hypersaline basins and viable microorganisms may be recoverable from brine inclusions in ancient salt deposits over 100 million years old (Oren, 1994; DasSarma and Arora, 2001).

ii) Athalassohaline environments are those in which the salts are of non marine proportion, i.e. after concentration of sea water leads to precipitation of NaCl, a high concentration of potassium and magnesium salts are left behind (DasSarma and Arora, 2001). Halophilic archaea are often present in high numbers in a number of alkaline hypersaline soda brines which include the Wadi Natrun lakes of Egypt (Oren, 1994), Lake Magadi in Kenya (Tindall *et al*, 1980) and the Great Basin lakes of the Western

United States (Mono Lake, Owens Lake, California (Oren, 1994), Searles Lake and Big Soda Lake), several of which are intermittently dry: Sambhar Salt Lake in India (Upsani and Desai, 1990) and different lakes in Tibet and Mongolia (Oren, 1994). These lakes have an extremely high salinity in combination with pH values of 9.5 and 11 and higher due to high content of carbonates (Grant and Ross, 1986). When the lakes are saturated with respect to carbonate, trona ($NaHCO_3.Na_2CO_3.2H_2O$) precipitates; as a result, concentrations of divalent cations such as Ca^{2+} and Mg^{2+} are very low, because of the low solubility of their hydroxides and carbonate salts.

Alkaline hypersaline lakes are often coloured red due to the presence of dense bacterial communities and anoxygenic photosynthetic bacteria of the genus *Ectothiorhodospira* (Oren, 1994). Neutrophilic halophilic archaea of the genera *Halobacterium, Haloferax, Haloarcula* and *Halococcus* are unable to grow at high pH values present. A specialized alkaliphilic archaea of the genera *Natronobacterium* and *Natronococcus* develop (Tindall *et al*, 1984; Mwatha, 1993) at high pH, high salinities and extremely low divalent cation concentrations. Optimal growth was observed at Mg^{2+} concentrations much below 1 mM while higher magnesium concentrations of 10 mM may be inhibitory (Tindall *et al*, 1980).

iii) Hypersaline soils harbour halophilic archaea which were isolated in USSR (Oren, 1994). Three halobacterial strains were isolated from rhizosphere of halophyte plants, growing in soil of 6.5% chloride content in Spain (Quesada *et al*, 1982,1983,1987).

2. Methods of study of Microbial Ecology

i) Conventional approaches

Enumerating microorganisms and estimating microbial biomass in the biosphere is complex. The diversity of the microorganisms and the habitats, in which they occur, requires the development and use of a variety of enumeration procedures (Atlas, 1982). There are no universal methods which can be applied to all microorganisms and all habitats. The successful enumeration of microbial components in the biosphere requires the ability to define carefully which populations are to be counted and from which habitats. Different approaches are required for enumerating different components of the total microbial population. Contamination with microorganisms is of serious concern when enumerating microorganisms, from environmental samples (Atlas, 1982). The diversity of microbial populations necessitates a variety of methodological approaches, for enumerating viral, bacterial, fungal, algal and protozoan populations. Direct weight measurements, cell volume measurements and microcalorimetry cannot be normally used for determining microbial biomass in environmental samples as it is impossible to separate the microbial biomass from their abiotic and biotic surroundings to permit accurate weighing of the recovered microorganisms (Jannasch and Jones, 1959).

Sample collection and handling

Sampling and handling procedures are an integral part of the enumeration procedure and must ensure that the numbers determined accurately reflect the numbers actually present in the biosphere (Atlas, 1982).

a) Counting procedures for enumeration and biomass determinations

Enumeration procedures involve counting by a) Direct b) Indirect c) Biochemical approaches.

Direct counts

Microorganisms can be counted and their biomass estimated using direct microscopic observation. A variety of counting chambers-haemocytometer/Petroff-Hauser chamber, may be used for relatively large microorganisms, such as protozoa and algae. Phase contrast microscopy is used as it eliminates the need for staining to increase contrast. Filamentous fungi can be enumerated by determining the length of hyphae using a micrometer. Various modifications of the agar film technique of Jones and Mollison (1948) are used for direct measurement of lengths of mycelia (Skinner *et al*, 1952). Microorganisms when present in low concentrations can be concentrated, stained and counted on filters of a suitable pore size. The use of fluorescence microscopy and fluorescence stains, such as Acridine Orange (AODC method) permits accurate counting of even small bacteria (Daley and Hobbie, 1975). Polycarbonate nucleopore filters retain microbes on a flat surface, while cellulose filters trap microbes in different planes of the filter making their observation difficult (Hobbie *et al*, 1977). Dark filters (black stained) are used (Jones and Simon, 1975) for increased contrast. Fluorescein diacetate is used to estimate the biomass of live fungi and bacteria. Only metabolically active hyphae and cells are stained, as fluorescence occurs only after enzymatic cleavage of the stain molecule. For estimation of both active and total biomass, fluorescein diacetate stain is used in conjunction with vital stain (Atlas, 1982). The presence of active microbial dehydrogenase can be detected and used to separate live from dead microorganisms (Patriquin and Doberiner, 1978; Zimmermann *et al*, 1978). When coupled with AODC microscopy method, use of 2-(*p*-iodophenyl) 3-(*p*-nitrophenyl) 5-phenyl tetrazolium chloride (INT), allows separation of metabolically active cells from dormant/dead microbes. Live microorganisms can also be detected by the use of autoradiography

combined with direct microscopic observation (Ramsay, 1974; Fliermans and Schmidt, 1975; Meyer-Reil, 1978). Either [^{3}H / [^{32}P]-labelled nucleotides are incorporated into DNA, in these procedures.

Another approach used for separation of living from dormant/dead microorganisms is based on inhibition of cell division (Kogure *et al*, 1979). Fluorescence staining and epifluorescence microscopy allows determination of numbers of elongated cells (actively growing) and the numbers of normal cells (dormant/dead microorganisms). Use of immunofluoresence permits determination of numbers of a specific microorganism even when other microorganisms are present in the sample. (Hill and Grey, 1967; Schmidt *et al*, 1968; Pugsley and Evison, 1975; Thacker, 1977; Strayer and Tiedje, 1978). An electron microscope can be used instead of a light microscope for the direct observation and enumeration of microorganisms (Harris *et al*, 1972; Pearl and Shimp, 1973; Bowden, 1977; Larsson *et al*, 1978). Electron microscopic observation can be coupled with nalidixic acid inhibition technique/autoradiographic procedures in order to separate active from inactive microorganisms. Coulter counter method (Kubitschek, 1969) is used for direct counting of microorganisms, allows discrimination between different particle sizes to give an estimate of total numbers of microorganisms.

Indirect Methods

Cultural Methods include a wide variety of methods involving culturing of microorganisms after necessary dilutions/concentrations of the sample so that a reasonable number of microorganisms grow on each plate (dilution plate) or in each tube. Basic assumption is that each propagule in the environment gives rise to one macroscopic colony which can be counted. Data from cultural studies indicate microbial

diversity. All sorts of diversity indices have been used- some based on colony characters, simple cell morphology & staining, others based on more complex, biochemical characterization of isolates. Such diversity indices give an idea of shifts/differences in community structure but are less rigorous than numerical taxonomy approach. Enrichment method is used where natural water/soil sample is enriched with a substrate that will favour the required microorganisms that they can be isolated in increased numbers.

Cultural methods are widely used in microbial ecology particularly for bacteria (Atlas, 1982).

Viable count procedures for enumerating microorganisms employ two basic approaches:

Plate count technique is most frequently used in microbial ecology for enumerating bacteria (Postgate, 1969) as it employs a variety of media and incubation conditions for enumeration of different microorganisms (Jannasch and Jones, 1959; Jones, 1970). Meeting the requirements of one physiological type of microorganisms, excludes other physiological types.

Most Probable Number (MPN) is used for determining viable numbers of microorganisms, which employs a statistical approach in which successive dilutions are performed to reach an extinction point. In MPN technique, replicates, usually 3-10, of each dilution are made and the pattern of positive and negative scores are recorded. A statistical table based on a Poisson distribution is used to determine MPN of viable microorganisms present in the original sample. MPN procedures employ different criteria for establishing positive and negative scores.

Microtitre plates, coupled with automated spectrophotometric readings to establish positive and negative scores has simplified the use of MPN techniques in some procedures (Rowe *et al*, 1977). MPN can be used for enumerating viable numbers of viruses, bacteria, fungi, algae, protozoa, by making use of various combinations of media and incubation conditions (Atlas, 1982).

b) Biochemical approaches to enumeration and biomass determination

This method depends on quantitative estimates of biochemical constituents of the cell. Various biochemicals have been used for estimating microbial biomass; but the biochemical used should be present in the microorganisms being enumerated and absent in other populations in the sample. It should be correlated with the biomass of the microorganisms being enumerated. Furthermore, all microorganisms being counted should have the same amount of the biochemical assayed. It should be possible to quantify accurately the biochemical in question in samples from various habitats without interference from other components present in the sample (Atlas, 1982).

Energy Components

ATP is the universal biochemical form of energy in biological systems, normally present in living cells in constant proportions relative to total cellular carbon. ATP rapidly degenerates following death of a cell and this can be used to estimate living biomass (Holm-Hansen, 1969; Holm-Hansen and Booth1969; Bancroft et al,1976). Concentrations of ATP can be determined using luciferin-luciferase assay.

Cell wall constituents

Cell wall components of bacteria and fungi have been proposed as biomass indicators. With few exceptions, bacterial cell walls contain a unique biochemical, murein, not

found in other microorganisms. The specific relationship between murein and bacteria makes quantification of this biochemical quite useful for estimating bacterial biomass (Millar and Casida, 1970; King and White, 1977). Conversion of muramic acid determinations to estimates of bacterial biomass is difficult when dealing with mixed microbial communities.

Estimation of fungal biomass

Chitin is a component of the cell wall of some fungi. Since all fungi do not contain chitin in their cell wall, hence the method is used only where similar groups of fungi are believed to be present. Different fungi contain different amounts of chitin relative to their biomass and the relative concentrations of chitin vary with the age and physiological state of the fungus. Chitin is relatively resistant to degradation and may remain intact following death of fungi, hence making the use of this biochemical for estimation of living fungal biomass questionable.

Cell envelope components

Lipopolysaccharide (LPS) method: The lipopolysaccharide complex associated with the cell envelope of Gram negative bacteria can be quantified and used to estimate bacterial biomass (Coates, 1977; Watson *et al*, 1977). Good correlation has been obtained between estimates of bacterial biomass based on direct epifluorescent microscopic counts, and those obtained by LPS method.

Nucleic Acids

Determination of concentrations of nucleic acids may be used as an estimation of microbial biomass (Holm-Hansen *et al*, 1968; Hobbie *et al*, 1972). Concentrations of

RNA vary greatly depending on the physiological state of a microorganism and thus RNA determinations are not reliable estimations of microbial biomass. However, concentrations of DNA are maintained in relatively constant proportions within microorganisms. DNA can be recovered from microbial cells, purified and quantified (Herbert *et al*, 1971). Concentrations of DNA can be estimated spectrophotometrically, with a chemical reagent, such as diaminobenzoic acid/ethidium bromide followed by spectrofluorometic analysis to determine the quantity of DNA (Jones and Simon, 1975).

Photosynthetic Pigments

Determination of concentrations of photosynthetic pigments can be utilised to estimate the biomass of photosynthetic microorganisms (Cohen *et al*, 1977). To quantify photosynthetic pigments spectrofluorometrically (Truper and Yentsch, 1967; Caldwell, 1977), which is more sensitive than spectrophotometric method, chlorophyll quantities can be determined *in vivo* i.e. without solvent extraction.

Protein

All microorganisms contain protein which can be readily quantified by Lowry method (Lowry *et al*, 1951). Protein determinations are used for estimating numbers of microorganisms and microbial biomass when dealing with pure cultures but there are extreme difficulties in applying protein determinations for estimating microbial biomass in environmental samples.

Physiological approaches as applicable to soil habitats

Soil fumigation approach has the advantage of directly assessing the amount of carbon, useful for estimating biomass. Microbial biomass can also be estimated by measuring respiration rates following substrate addition (Anderson and Domsch, 1975).

All these methods of assaying metabolic activity usually yield no information on the species present/their distribution in the environment. These general methods may sound rather depressing. Nevertheless, it is possible to get useful information by combining several methods, provided that their limitations are realized. Many valid comparisons can be made even if absolute values cannot always be put on microbial populations. If microbial ecologists had waited for the perfect technique nothing would have been learnt. The incentive to produce better methods is the need, to measure particular activities and to solve problems (Jannasch and Jones, 1959; Hungate, 1969; Postgate, 1969; Jones, 1970; Oslon, 1978; Atlas, 1982).

ii) Modern methods

a) Methods for DNA analysis

Polymerase Chain Reaction (PCR) can be applied to population ecology as limited/partially degraded samples, even if only a small fraction of the DNA is intact, target segments from unbroken DNA can be amplified completely in one bout (Brumlik *et al*, 2001; Buttner *et al*, 2001). **Southern blot and hybridization**-Southern blotting is a common technique for identifying polymorphic fragments of DNA that differ in size (measured in nucleotide base pairs) because of the gain/loss of restriction sites. Usually a radioactively labelled, single stranded DNA probe of a known genetic region is used to identify the allelic fragments. Probes that are isolated from different populations/even different species can be used to survey allelic diversity in DNA fragments. E. g. of universal probes are commonly known as DNA fingerprints (Jae-Chang and Tiedje, 2001). **Genomic library construction** - If probes are not available for a given application one can produce them from a sample of genomic DNA by creating a

recombinant DNA library. This term refers to a collection of bacteria containing plasmids/phage vectors into which DNA fragments from the study microorganism have been inserted. **Sequencing** - Nucleotide sequence differences exist among all individuals, even in the most homogeneous populations, yet DNA sequencing to identify such differences is very labor-intensive for most population level studies. DNA sequencing has become a routine procedure since the development of dideoxychain termination method. In combination with PCR, it provides a method of collecting precise data for short DNA sequences. **Classes of nucleic acid markers** - DNA markers provide a means for powerful, fine grained analysis of individual genotypes. Population biologists often need high resolution genetic markers. **Restriction Fragment Length Polymorphisms (RFLPs)** were the first DNA markers to be used by population biologists. An RFLP results when variation in restriction enzyme cleavage sites is detected by Southern blot hybridization. Southern blotting to study RFLPs is relatively time-consuming and expensive. A more efficient approach to RFLP analysis is to use PCR to amplify random fragments of the genome. For one/more clones of ~2 kb chosen from a DNA library, sequences are determined for 100-200 bases at each end of the clone (larger segments can be isolated with long PCR). PCR primers are constructed complementary to some of the flanking region, and these can then be used to amplify the same fragment in other individuals. The amplified product can then be treated with restriction enzymes and the fragments separated on an agarose gel and visualized by ethidium bromide staining to identify RFLPs. Although the use of PCR renders, this method more feasible than traditional RFLP analysis by Southern blot hybridizations, it still requires a great deal of labour, including the construction of a genomic DNA library, some initial DNA sequencing and fine tuning of PCR conditions. It has been reported

that many (upto 30%) of the primers do not yield adequate amplification under average conditions (Ticknor *et al*, 2001). **Random Amplified Polymorphic DNA (RAPDs) -** The RAPD process typically reveals several polymorphic genetic segments/primer within populations; other segments may appear as monomorphic bands within/across populations (Trebaol *et al*, 2001).

3. Microbiota of the Halo-econiches

Salt making was carried out since ancient times in solar evaporation ponds. The scanty bacteriological studies of strong brines up to 1925 were summarized briefly by Baas-Becking (Truper and Galinski, 1986). The first thorough study of a large natural highly saline lake was made by Volcani. He studied the microflora of the Dead Sea. Since then, numerous salt lakes and lake systems have been studied with respect to their microflora. Many of these natural salt lakes are also used as mineral sources for the chemical industry of the countries where they are situated. The chemical composition of brines depends on the minerals dissolved in the water before evaporation. Sea water has a rather constant composition world wide, brines in marine salterns and coastal lagoons confirm this uniformity (Truper and Galinski, 1986). The spectrum of species in saline biotopes is drastically restricted of eukaryotes.

i) Eukaryotic halophiles

a) Multicellular eukaryotes - Few organisms such as *Tilapia* species are found at about 5.9 % NaCl. A variety of obligate and facultative halophytic plants, e.g. *Atriplex halimus* and *Mesembryanthemum crystallinum*, can survive in moderately high saline soils. A number of invertebrates that can survive in hypersaline environments are rotifers

such as *Brachionus angularis* and *Keratella quadrata*, tubellarian worms such as *Macrostomum* species, copepods such as *Nitocra lacustris* and *Robertsonia salsa*, ostracods such as *Cypridis torosa*, *Paracyprideinae* spp., *Diacypris compacta*, and *Reticypris herbsti*; insects include brine flies *Ephedra hians* and *E. gracillis* and brine shrimp *Artemia franciscana*. Some hypersaline environments help to support birds like the pink flamingo (DasSarma and Arora, 2001).

b) Algae - Dense populations of obligately aerobic, photosynthetic, unicellular eukaryotic green algae are supported at moderately high salinities (5.9 %-20 %) NaCl). Some species produce large quantities of orange-coloured ß-carotene at high salinities. Green algae of the genus *Dunaliella*, e.g. *Dunaliella salina*, *D. parva*, and *D. viridis* (Post, 1977; DasSarma and Arora, 2001) are ubiquitous and are the main sources of food for brine shrimps and the larvae of brine flies. *Dunaliella salina* and *Asteromonas gracilis* (Ben-Amotz, and Avron, 1983; DasSarma and Arora, 2001) can grow even in saturated NaCl. Diatoms reported from hypersaline environments include *Amphora coffeaeformis* and *Nitzschia* sp. and *Navicula* sp. (DasSarma and Arora, 2001).

c) Protozoa - Identified species of protozoa from hypersaline environments include the moderate halophile *Fabrea salina* from a West Australian lake and the extreme halophile *Porodon utahensis* from the Great Salt Lake (DasSarma and Arora, 2001).

d) Fungi - *Debaromyces hansenii* is a halotolerant yeast, isolated from sea water, that can grow aerobically up to salinities of 26.5 % NaCl. A saprophytic hyphomycete, *Cladosporium glycolicum*, was found growing on submerged wood panels at a salinity exceeding 26.5 % NaCl in the Great Salt Lake. Halophilic fungi, e.g. *Polypaecilum pisce* and *Basipetospora halophila*, have also been isolated from salted fish (DasSarma and Arora, 2001). *Hortaea werneckii* and *Aureobasidium pullulans*, black yeast-like

fungi were isolated from hypersaline waters of salterns (Gunde-Cimerman *et al*, 2000; Kogej *et al*, 2005). Yeasts *Pichia guilliermondii, Debaryomyces hansenii, Yarrowia lipolytica* and *Candida parapsilosis* ; *Rhodosporidium sphaerocarpum, R. babjevae, Rhodotorula laryngis, Trichosporon mucoides*, and a new species resembling *C. glabrata* were isolated from hypersaline habitats like salterns as well as from the Dead Sea, Enriquillo Lake (Dominican Republic) and the Great Salt Lake (Utah). Ascomycetous yeast *Metschnikowia bicuspidata*, known to be a parasite of the brine shrimp, was isolated as a free-living form from the Great Salt Lake brine. In water rich in magnesium chloride (bitterns) from the La Trinitat salterns (Spain), two new species provisionally named *C. atmosphaerica*-like and *P. philogaea*-like were discovered (Butinar *et al*, 2005).

ii) Prokayotic halophiles

The strongly saline environment is mainly a domain of prokaryotes (Truper and Galinski, 1986).

a) Cyanobacteria dominate the planktonic biomass and form microbial mats in many hypersaline lakes. The top brown layer of microbial mats contains a common unicellular cyanobacterial species, *Aphanothece halophytica, A. halophytica* and similar unicellular cyanobacteria have been described from the Great Salt Lake, Dead Sea, Solar Lake and artificial solar ponds (Mackay *et al*, 1984; Reed *et al*, 1984). A planktonic cyanobacterium reported from the Great Salt Lake is *Dactylococcopsis salina* (Mackay *et al*, 1984; Reed *et al*, 1984). A variety of filamentous cyanobacteria, e.g. in the order Oscillatoriales, such as *Oscillatoria neglecta, O. limnetica, O. salina* and *Phormidium ambiguum*, have also been described that develop in the green second layer of mats in

hypersaline lakes. Another common species in the same family is *Microcoleus chthonoplastes* (DasSarma and Arora, 2001). The diversity of cyanobacteria occurring in hypersaline environments has not been studied extensively. Halophilic cyanobacteria. *Spirulina platensis*, *Synechococcus* and *Synechocystics* species are also reported (Mackay *et al*, 1984; Reed *et al*, 1984).

b) Other phototrophic bacteria occur beneath the cyanobacterial layers in anaerobic but lighted zones in hypersaline microbial mats. The green sulfur bacteria, such as the slight to moderately halophilic *Chlorobium limicola* and *C. phaeobacteriales*, moderately halophilic, filamentous green non-sulfur bacteria such as *Chloroflexus aurautiacus,* halophilic purple sulfur bacteria such as the Chromatiaceae, e.g. *Chromatium glycolicum*, *C. violescens* and *C. salexigens*. Moderate halophiles *Thiocapsa roseoparsarcina* and *T. halophila* from Guerrero Negro are reported (DasSarma and Arora, 2001). The moderately halophilic purple non-sulfur bacterium *Rhodospirillum salexigens* from evaporated seawater pools and *R. salinarum* from a saltern are also reported. The purple sulfur bacteria, *Ectothiorhodospira* species, dominate alkaline soda lakes in Egypt and Central Africa. *Ectothiorhodospira halochloris*, *E. halophila, E. abdelmalekii, and R. salinarum*, were isolated from Wadi Natrun (Truper and Galinski, 1986).

c) Sulfur-oxidizing bacteria - Below the cyanobacteria and the phototrophic bacteria in microbial mats, halophilic, filamentous, carbon dioxide-fixing bacteria like *Achromatium volutans* and *B. leptiformis* from Solar Lake, *Beggiatoa alba* from Guerrero Negro oxidize hydrogen sulfide (and elemental sulfur) to sulfate. A unicellular halophilic, chemoautotrophic sulfur-oxidizing bacterium, *Thiobacillus halophilus*, from a hypersaline western Australian lake, has also been described. Bacteria involved in the

sulfur cycle-sulfate reducers and sulfur oxidizers and in the nitrogen cycle-nitrate reducers and ammonia oxidizers are reported (DasSarma and Arora, 2001).

d) Anaerobic bacteria and archaea - A large variety of facultative and strictly anaerobic bacteria and archaea inhabit the bottom layers of microbial mat communities and sediment in hypersaline lakes which include fermentative bacteria, homoacetogenic bacteria, sulfate-reducing bacteria and methanogenic archaea. *Haloanaerobacter chitinovorans*, isolated from a saltern, is capable of fermenting chitin contained in brine shrimp and brine flies. Moderate halophilic isolates are *Haloanaerobacter saccharolytica*, which ferments carbohydrates, *Halobacterioides acetoethylicus*, from an oil well, and *Halocella cellulolytica*, which ferments carbohydrates including cellulose. *Sporohalobacter lorretii* and *S. marismortui* are sporogenous and ferment carbohydrates. Several homoacetogens, *Haloincola saccharolytica* ferments carbohydrates and *N*-acetylglucosamine and can grow at a wide range of NaCl concentrations. *Acetohalobium arabaticum*, isolated from Lake Sivash, which grows from 5.9 %-26.5 % NaCl, grows on glycine betaine and trimethylamine. *Desulfohalobium retbaense*, isolated from Lake Retba, Senegal, and *Desulfovibrio halophilus*, from Solar Lake, are two moderately halophilic sulfate-reducing species that have been described. Methanogens have been identified, including *Methanohalophilus halophilus* from a microbial mat, *M. muhii* from the Great Salt Lake, and *M. portucalensis* from a saltern. The slight halophile *Methanosalsus zhilinac* is also an alkaliphile and a slight thermophile. Methanogenesis has also been reported from deep-sea brine pools in the Gulf of Mexico that contain moderately high salinity.

e) Aerobic and facultative anaerobic Gram-negative bacteria belonging to the *Halomonas* and *Chromohalobacter* genera have been described. Other genera with

halophilic representatives include *Salinovibrio*, *Arhodomonas*, *Dichotomicrobium*, *Flavobacterium*, *Alcaligenes*, *Alteromonas*, *Acinetobacter*, and *Spirochaeta*. Most of these are heterotrophs, and include *Arhodomonas aqueoli*, isolated from a subterranean brine associated with an oil field and capable of nitrate reduction; *Chromohalobacter marismortui* from the Dead Sea, also capable of nitrate reduction; *Pseudomonas beijerinckii* from salted beans preserved in brine; *Pseudomonas halophila* from the Great Salt Lake; and *Salinovibrio costicola*, originally isolated from Australian bacon. Several *Halomonas* species are capable of nitrate reduction, including *H. elongata* (Vreeland *et al*, 1980), isolated from a solar saltern, and *H. halodenitrificans*, isolated from meat-curing brines. Others include *H. eurihalina*, isolated from saline soil, which produces an extracellular polysaccharide; *H. halodurans*, from estuarine waters, *H. halophila*, from saline soil; *H. panteleriense*, from alkaline saline soil, which grows at a pH optimum of 9; *H. salina*, from saline soil; and *H. subglaciescola*, from beneath the ice of Organic Lake in Antarctica. Among spirochaetes, the moderate halophile *Spirochaeta halophila*, found in Solar Lake, is a chemolithotroph capable of iron and manganese oxidization. The flavobacteria *Flavobacterium gondwanese* and *F. salegens*, are psychrotolerant halophiles isolated from Antarctic Lakes (DasSarma and Arora, 2001). A number of extremely halophilic and extremely halotolerant eubacteria are described and some of them are thoroughly studied e.g. *Vibrio costicola* (Kushner, 1968,1985), *Paracoccus halodenitrificans, Micrococcus halobius, Pseudomonas* (Ventosa *et al*, 1982; Imhoff and Truper, 1977;Imhoff and Rodriguez-Valera, 1984). *Beneckea* (Truper and Galinski, 1986) and *Pediococcus halophilus* (Villar *et al*, 1985) and *Halomonas elongata* (Vreeland *et al*, 1980). Strictly anaerobic heterotrophic eubacteria have also been described from extremely saline habitats, e.g. *Clostridium lortetii* (Truper and Galinski,

1986), *Haloanaerobium praevalens* (Truper and Galinski, 1986) and *Halobacteroides halobius* (Oren *et al*, 1994).

f) Gram-positive bacteria of the genera *Halobacillus*, *Bacillus* (Weisser and Truper, 1985), *Marinococcus*, *Salinococcus*, *Nesterenkonia*, and *Tetragenococcus* have been reported. They include cocci such as *Nesterenkonia halobia*, isolated from salterns, which produce yellow-red carotenoid pigments ; *Tetragenococcus halophilus*, *Salinococcus* species from salterns ; *B. haloalkaliphilus*, from Wadi Natrun ; and *B. halodenitrificans*, from a solar saltern in southern France. *Halobacillus litoralis* and *H. trueperi* are found in the Great Salt Lake. Actinomycetes from saline soils as *Actinopolyspora halophila* (Gochnauer *et al*, 1975) and *Norcardopsis halophila.*

Number of microbiota have been reported during recent years from various hypersaline environments (Table 2).

Table 2: Microbiota of various saline econiches

Salterns	Microbiota	References
Xinjiang salt lake, China	*Haloarcula aidinensis* sp. nov.	Zhou *et al*, 1994
Salterns and hypersaline soils of Spain	*Bacillus salexigens sp. nov.*	Garabito *et al*, 1997
Cabo Rojo, Puerto Rico;	*Halobacterium salinarum*	Montalvo-Rodriguez *et al*, 1997
Caribbean	*Haloferax volcanii Haloarcula japonica*	
Ljubljana, Slovenia	*Aspergillus fumigatus*	Tepsic *et al*, 1997
Salterns and hypersaline soils of Spain	*Bacillus pantothenticus* *B. firmus* *B. alcalophilus* *B. megaterium* *B. laterosporus*	Garabito *et al*, 1998
Cabo Rojo, Puerto Rico	*Haloferax Halogeometricum borinquense gen. nov., sp. nov.*	Montalvo-Rodriguez *et al*, 1998
Sevilla, Spain	*Nesterenkonia halobia*	Ventosa *et al*, 1998
Solar salterns, Morocco	*New taxa of moderately halophilic bacteria*	Bouchotroch *et al*, 1999
Mediterranean salterns	*Haloanaerobacter salinarius sp. nov.*	Mounes *et al*, 1999
Slovenia	*Hortaea werneckii*	Petrovic *et al*, 1999
Permo-Triassic salt deposits, Austria	*Halococcus salifodinae*	Stan-Lotter *et al*, 1999
Solar salterns, France	*Thermohalobacter berrensis gen. nov. sp. nov.*	Cayol *et al*, 2000
San Francisco Bay, California, USA	*Artemia franciscana*	Clegg *et al*, 2000
Salterns, Ljubljana, Slovenia	*Hortaea werneckii* *Phaeotheca triangularis* *Trimmatostroma salinum* *Aureobasidium pullulans* *Cladosporium sp.*	Gunde-Cimerman *et al*, 2000
Salterns, U.S.A.	*Halomonas elongata* *Halobacterium salinarum*	Martin *et al*, 2000
Cabo Rojo, Puerto Rico	*Haloterrigena thermotolerans sp. nov.*	Montalvo-Rodriguez *et al*, 2000
Solar salterns, Salin-de-Giraud Camargue, France	*Orenia salinaria sp. nov.*	Moune *et al*, 2000
Guerrero Negro, Mexico	*Chloroflexus sp.*	Nuebel *et al*, 2001
Santa Pola near Alicante Balearic island of Mallorca, Spain	*Salinibacter, Dunaliella*	Oren and Rodriguez-Valera, 2001

Table 2: Microbiota of various saline econiches

Salterns	Microbiota	References
Alexandria, Egypt	*Haloferax alexandrinus sp. nov.*	Asker and Ohta, 2002
Puerto Rico, Dominican Republic	*Artemia sp.*	Mayer, 2002
Camargue, France	*Crustaceans- Moina salina* *Brine shrimp – Artemia parthenogenetica* *Branchinella spinosa* *Copepods-Cletocamptus retrogressus* *Eurytemora velox*	Thiery and Peunte, 2002 Campos-Ramos *et al*, 2003
Great Salt Lake, San Francisco Bay salterns, USA	*Artemia spp.*	
Salin-de-Giraud Camargue, France	*Halorhodospira neutriphila sp. nov.*	Hirschler-Rea *et al*, 2003
Mediterranean salterns of Salin-de-Giraud , France	*Cytophaga-Flavobacterium-Bacteroides*	Mouné *et al*, 2003
Salterns, USA	*Cyanobacteria*	Bebout *et al*, 2004
Shandong Province, China	*Artemia franciscana*	Zheng-Bo *et al*, 2004
Teguidda-n-Tessoumt, West Africa, Sahara	*Methanogens, γ-proteobacteria*	Alvey *et al*, 2005
Portuguese Salterns, French Mediternanean coast, Cadiz bay (Spain)	*Artemia sp.*	Amat *et al*, 2005
Bhavnagar, India	*Halobacterium salinarum* *Halobacterium sp. 1,* *Halobacterium sp. 2,* *Halobaculum sp. Halococcus saccharolyticus, Halorubrum saccharovorum,* *Halogeometricum sp.* *Haloterrigena turkmenica* *Natrialba sp.*	Anshuman and Dave, 2005
Ljubljana, Slovenia	*Eurotium amstelodami,* *E. repens,* *E. herbariorum* *E. rubrum,* *E. chevalieri,* *E. halotolerans*	Butinar *et al*, 2005a,b,c
Adriatic salterns	*Melanized halophilic fungi*	

Table 2: Microbiota of various saline econiches

Salterns	Microbiota	References
Dead Sea, Enriquillo Lake, (Dominican Republic)	***Pichia guilliermondii, Debaryomyces hansenii, Yarrowia lipolytica, Candida parapsilosis, Rhodosporidium sphaerocarpum, R. babjevae, R. layngis, Trichosporon mucoides C. glabrata***	**Butinar *et al*, 2005a,b,c**
Great Salt Lake, Utah	***Metschinkowia biscuspidata***	
Cabo Rojo, Solar Salterns Puerto Rico	***Hortaea werneckii***	**Diaz-Munoz and Montalvo-Rodriguez, 2005**
Salterns, Spain	***Hortaea werneckii, Aureobasidium pullulans***	**Kogej *et al*, 2005**
Tunisia (Sfax)	***Zooplankton ciliate Fabrea, Artemia***	**Toumi *et al*, 2005**
Adriatic Secovlje, Ljubljana, Slovenia	***Haloquadratum, Halorubrum***	**Pasic *et al*, 2005**
Ayakekum Lake, China	***Natrinema***	**Xu *et al*, 2005**
Taean-Gun, Chungnam, Province, Korea	***Salinivibrio costicola Halobacillus treperi Vibrionaceae, Pseudoalteromonadaceae Halomonadaceae Alteromonadaceae Idiomarinaceae***	**Yeon *et al*, 2005**
Ljubljana, Slovenia	***Wallemia sp.***	**Zalar *et al*, 2005**

4. Salt and its importance

Salt is physiologically absolutely necessary for human life. Prior to the Industrial Revolution, the known mineral salt sources were limited so much so, that its supply was a critical demographic power factor for most communities, until industrial means of extraction from brines were devised. It was only available as visible and exposed rock outcrops in arid regions, or as dried out salt cakes on the shores of some seas and salt lakes. In areas with wet climates, the protruding salt, dissolved, making it almost

impossible to discover. Hence, many of the great civilizations first developed near deserts and desert climates. Every day, each of the Earth's 5.9 billion inhabitants uses salt. Annual salt production has increased over the past century from 10 million tonnes to over 200 million tonnes today. Nearly 100 nations have salt producing facilities ranging from primitive solar evaporation to advanced, multi-stage evaporation in salt refineries (Bloch, 1996).

i) World salt production totalled 210 million tonnes in 2001 (Bloch, 1996). Major salt producing countries are U.S., Mexico, Italy, China, France, Sicily, Germany, United Kingdom, Austria, Canada, Brazil, Switzerland, India, Bolivia, Mediterranean, Australia, Mali.

ii) Three Techniques of salt manufacture around the world are - 1) Solar evaporation of sea water or saline lake water, 2) Solution mining and vacuum pan evaporation and 3) Conventional deep-shaft (rock salt) mining (http:// www.saltinstitute.org/36.html).

Five ancient methods adopted for the **manufacture of salt** at a global level include : 1) by the spontaneous (natural) evaporation of sea-water / solar evaporation in large shallow basins, called "salt gardens" or "salines" or wide flat coastal areas (Bloch, 1996; Le Conte, 1999). Natural, shallow depressions, lagoons or man-made salterns about 15-20 cms deep were positioned at mid-tide level to facilitate filling (without industrial pumps) of the evaporation pans, in order to concentrate the brine, and later for harvesting and drying the precipitated crystallized salt (Bloch, 1996). The crystals first form on the surface of the brine. As they become soaked, the evaporating surface brine reaches saturation point before the cooler lower layers. The specific gravity of a sodium chloride crystal is 2.16, and the saturated brine at 25 ^{0}C contains 26.7% salt, and has a specific gravity of 1.2004. At 15^{0}C, a saturated solution contains 26.5% salt, and has a specific

gravity of 1.203. Hence, a solution saturated at a higher temperature is specifically lighter, even though it contains a greater quantiity of salt. This explains how salt makers could crystallize "blocks" or briquettes of salt on the surfaces of ponds, using floating elements such as sticks and straws to form the crusts of salt. With most other substances, crystallization cannot occur at the solution surface because their solubility increases more rapidly than their specific gravity decreases (Bloch, 1996). Chinese technology, included drilling deep into a salt deposit, by using bamboo pipes, with atleast two holes, one to feed and flood fresh water into the salt diaper, and the second hole to allow the water to "well" up after dissolving the salt, into the evaporation pans, where it could be again concentrated by evaporation brought about by solar heat or by manual boiling using convenient fuel for burning (Bloch, 1996). 2) by "Salines" and artificial evaporation (boiling) combined, where solar energy could not be used, the salt maker was forced to use solid fuel like wood, peat, coal, seaweed (Japan), to 'boil' brines (Bloch, 1996; Le Conte, 1999). 3) by spontaneous evaporation in graduation houses and boiling, combined, operations are independent of rainy weather. 4) by lixiviating saline sand and then boiling the brine. This method is employed in Lower Normandy, France (Le Conte, 1999). 5) by boiling sea-water, method employed in countries like Scotland, where fuel is very cheap (Bloch, 1996; Le Conte, 1999).

It takes approximately 50,000 cubic/m of sea water spread over 10,00,000 sq/m, of flat solar evaporation area, to produce 1000 tonnes of salt a year, provided conditions of equable climate with a warm breeze and hot sun; and a reasonably steady sea-level are met. Such areas were abundant when the sea was one or two metres below its present level. Erratic sea level variations, either seasonal, short lived or long term may have been

caused by different events or a combination of them, like changes in atmospheric pressure, changes in ocean currents, wind driven waves, storm surges, heavy rainfall increasing the run-off from rivers, global warming or cooling at the Antarctic pole. These were responsible for changing coastlines and catastrophically destroyed salt evaporation ponds at the sea shores. The establishment of new pond levels was very difficult even in Delta areas of some rivers, like the Nile, Rhone, and Euphrates. Cycling of brines from one level of concentration to the next could take months and years. A change of climate conditions, or a minor ocean fluctuation could have had serious effect for ancient coastal salt making. Hence, manual rock mining or inland salt springs or lakes like the Dead Sea suddenly became the only alternative (Bloch, 1996).

5. Family Halobacteriaceae are salt-loving microorganisms that grow optimally at about 4.5 M NaCl-ten times the salinity of sea water (Robb *et al*, 1995). **The main characteristics of the family Halobacteriaceae are-**i) rods, cocci, a multitude of involution forms (discs, triangles,etc.), ii) requirement for atleast 1.5 M (approximately 8%) NaCl for growth, iii) red pigmentation due to the presence of bactorio-ruberins (C_{50} carotenoids) in the cell, iv) lack of muramic acid containing peptidoglycan in the cell envelope, v) membrane lipids composed of ether-linked isoprenyl phosphoglycerides, vi) insensitivity towards many antibiotics (especially cell wall and protein synthesis inhibitors), vii) occur in habitats with high salt concentration (salt lakes, salterns, etc) and viii) DNA composed of a major component (mol % G+C = 61-71) and a minor component (mol % G+C = 51-59) (Elazari-Volcani,1972; Gibbons, 1974; Larsen, 1984; Ventosa, 1990).

Haloarchaea are known by the trivial name halobacteria. They occur as rods, cocci, and multitude of pleiomorphic forms including flat discs, triangles and squares. Resting stages are not known, although there are reports of structures referred to as halocysts in some strains. They may be non-motile or motile by tufts of flagella. They are Gram-negative or Gram-positive after fixation in 2% (w/v) acetic acid followed by staining with crystal violet, Gram's iodine, decolourizer and safranine, all prepared in 20 % NaCl. Hypersaline lakes are highly productive microbial environments that provide many advantages for microbial ecologists, including stable communities of relatively low diversity (mainly haloarchaea) (Dyall-Smith *et al*, 2003). Water bodies with NaCl concentrations approaching saturation are often populated by dense microbial communities. Red halophilic archaea of the family Halobacteriaceae dominate in such environments. The application of molecular biological techniques, in particular the use of approaches based on the characterization of ribosomal RNA sequences, has greatly contributed to our understanding of the community structure of halophilic archaea in hypersaline ecosystems(Oren, 2002).

i) Bioenergetics

Halobacteria are aerobic chemo-organotrophic archaea that grow optimally between pH 8.0 and 9.0 using a wide range of carbon sources. These archaea have developed alternative processes of energy provision for conditions of high cell densities and reduced solubility of molecular oxygen in concentrated brines. The halobacteria can switch to anaerobic metabolism by using an alternative final acceptor in the respiratory chain or by fermentation or alternatively, they can employ photophosphorylation. Light energy is converted by several retinal-containing membrane proteins that, in addition to

generating a proton gradient across the cell membrane, also make phototaxis possible in order to approach optimal light conditions (Bickel-Sandkoetter *et al*, 1996).

Two fundamentally different strategies enable microorganisms to cope with the osmotic stress inherent to the presence of high salt concentrations are: i) cells may maintain high intracellular salt concentrations, osmotically atleast equivalent to the external concentrations-the 'salt-in' strategy, ii) cells may maintain low salt concentrations within their cytoplasm-the 'compatible-solute' strategy (Oren,1999a).

ii) Proteins

Proteins of halobacteria are either resistant to high salt concentrations or require salts for activity. As a group, they contain an excess ratio of acidic to basic aminoacids, a feature likely to be required for activity at high salinity. This characteristic is shared with proteins from some halophilic bacteria. Surface negative charges are thought to be important for solvation of halophilic proteins, and to prevent the denaturation, aggregation and precipitation that usually results when non-halophilic proteins are exposed to high salt concentrations.

iii) Metabolism

They are aerobic or facultatively anaerobic with or without nitrate. Some strains have a fermentative mode of growth on arginine. Most strains grow well in nutrient-rich complex media with yeast extract, peptone or casamino acids as carbon and energy sources. Difco's Bacto-Peptone, however, has been known to cause lysis of non-coccoid halobacteria due to the presence of high concentration of taurine conjugate of cholic acid (Kamekura and Kates, 1988). Some strains grow in defined media (Rodriguez–Valera *et*

al, 1980) with glucose or sucrose as carbon sources and ammonia or glutamate as nitrogen sources. Anaerobic growth occurs either by an uncharacterized fermentative mode, linked to arginine utilization (Hartmann *et al*, 1980) or by using alternative electron acceptors such as nitrate (Tomlinson *et al*, 1986), dimethyl sulfoxide or trimethylamine N-oxide (Oren and Truper, 1990) or fumarate (Oren, 1991). 3-phenyl propionic acid is degraded by *Haloferax* sp.(Fu and Oriel, 1999). *Halobacterium mediterranei* produces large amounts of poly-3-hydroxybutyrate (PHB) when grown on sugars. It was found that the key factors determining the amount of PHB were the concentration and nature of the carbon source and the concentration of the phosphorus source (phosphate). Neither the nature or concentration of the nitrogen source nor the oxygen concentration or any other factor assayed gave significant changes in the amount of polymer (Rodriguez-Valera and Lillo, 1992).

iv) Pigments

Bacteriorhodopsin (bR) is the only protein in the purple membrane of halobacteria (Betlach and Shand, 1991). Previous work has shown that the protein of *Halobacterium halobium*, molecular weight 26,000, consists of a single polypeptide chain of 248 amino acids and contains one molecule of retinaldehyde per protein linked as Schiff's base to the ε-amino group of a lysine residue (Dunn *et al*, 1981). bR is a transmembrane protein that aggregates to form crystalline patches in the membrane. The patches preserve their structure when isolated and are known as the purple membrane (pm). bR is the light-driven proton pump of halobacteria (Brown *et al*, 1995). When bR absorbs light, it ejects protons from the cell, generating an electro chemical gradient (Soppa *et al*, 1989) across the cell membrane that may reach 280-300 mV. This proton gradient directly drives

metabolic processes like ATP synthesis, locomotion. Most of the absorbed energy is first converted from a proton into Na^+ and K^+ electro chemical gradients having a high energy storage capacity, and regenerating the proton motive face (PMF) or directly driving other energy-requiring processes like uptake of amino acids, in presence of high salt concentrations. The crystalline arrangement of bR in purple membrane allows determination of its tertiary structure at ~7 A° resolution. The polypeptide chain crosses the membrane seven times, forming a tight crescent-shaped cluster of alpha-helical segments oriented with their long axis roughly normal to the plane of the membrane. The last 3 to 6 amino acids of the N-terminus are exposed on the exterior surface of the membrane, while the last 17-24 amino acids of the C-terminus are accessible from the aqueous phase on the cytoplasmic side.

Visible absorption peak of purple membrane slowly shifts from 570 to about 560 mm. Exposure to light, results in a shift back to 570 nm. This transition is not accompanied by a gain or loss of H^+. Light adaptation seems to consist entirely of isomerization of retinal: in the dark, purple membrane consists of 13-*cis* and all-*trans* retinal-containing bR conformers in equal amounts. Both components undergo cyclic photoreactions, but a branching pathway leads from the 13-*cis* into the all-*trans* cycle, and the back reaction is very slow, so that in the light, the 13-*cis* component rapidly disappears. Only the all-*trans* photoreaction cycle translocates protons across the membrane. Atleast four intermediates have been identified in the cycle; their rise and decay constants range from 10^{11} to 10^2 sec^{-1}. A transient deprotonation of the Schiff base and all-*trans* to 13-*cis* isomerization of the retinal occur during the cycle as well as protein conformational changes (Lanyi, 1978; Stoeckenius and Bogomolni, 1982). Bacteriorhodopsin (λ_{max}=568 nm) also appears to function as signal transducer for phototactic responses. bR together

with a second **blue-absorbing retinal pigment**, allows the bacteria to accumulate in light with a strong green component (Marwan and Oesterhelt, 2000).

P_{588} or halorhodopsin: is a third retinal pigment, which strongly resembles bR, but mediates a light-driven Na^+ ejection from the cells. It appears to function as a receptor pigment for phototaxis in the long wavelength region (λ_{max}=578nm) of the visible spectrum (Stoeckenius and Bogomolni, 1982; Marwan and Oesterhelt, 2000).

SR I and SR II (SR) are two **sensory rhodopsins** that mediate a simple form of colour vision during phototaxis (Kitajima *et al*, 1996; Marwan and Oesterhelt, 2000). **Phoborhodopsin (pR)** (λ_{max}=480 nm) is the fourth retinal-containing pigment (Tomioka *et al*, 1986). These four rhodopsins form the bR family in *Halobacterium salinarum* (Tateno *et al*, 1994; Mukohata, 1994). The lipids in the purple membrane consist of diether analog of phosphatidyl glycerol phosphate and a glycosulfolipid. Both lipids contain only dihydrophytanyl groups (Lanyi, 1978).

Ketocarotenoids as 3-hydroxy-echinenone and *trans*-astaxanthin, are present in high amounts in *Halobacterium salinarum*, *Haloarcula hispanica*, *Haloferax mediterranei.* (Calo *et al*, 1995).

Biosynthesis of purple membrane

The purple membrane serves only as an auxiliary energy conversion device, in addition to the respiratory chain. Hubbard and Rhinehart(1976) have shown that significant quantities of purple membranes are synthesized by the cells only when required, i.e. at low oxygenation and when light is available. Bacterio-opsin is the retinal-free protein, synthesis of which is accelerated in response to low oxygen tension; which in turn, regulates retinal synthesis. Bacterio-opsin in aerated or nicotine treated cells is found in a distinct membrane fraction called "brown membrane". These constitute patches on the

cell surface, as the purple membranes do, but do not show crystalline structure. The brown membrane contains cytochromes and other proteins besides bacterio-opsin and is the *in vivo* precursor of the purple membrane (Lanyi, 1978). *Halorubrum* is the host for archaerhodopsins(aR) (Mukohata, 1994). Bacterial rhodopsins of the cruxrhodopsin (cR) tribe-cruxrhodopsin-3 (cR-3) were found as the proton pumps, anion pumps (chR-3) and sensors (csR-3) in *Haloarcula vallismortis* (Kitajima *et al*, 1996). A retinal protein found in *Halobacterium* sp. aus-1 collected in Western Australia, named archaerhodopsin-1 (aR-1) is a 27-k Da H^+ pump, the amino acid sequence of which is only 60% identical to that of bR, but 85% identical to archaerhodopsin-2, an H^+ pump from another Australian isolate. H^+ pumps cruxrhodopsin (cRs) anion pumps, cruxhalorhodopsins (chRs) which pair with the H^+ pumps and also differ from the known anion pumps, are found in *Haloarcula* sp. arg-1.

The purple membrane of *H. halobium* contains identical protein molecules of molecular weight 26,000 (Blaurock and Stoeckenius, 1971) which make up 75% of the total mass, and lipid which makes up the remaining 25% retinal, covalently linked to each protein molecule in a 1:1 ratio, is responsible for the characteristic purple colour (Henderson and Unwin, 1975).

v) Lipids

The structure and composition of membrane lipids is characteristic of related organisms under their particular growth conditions : so that lipid structure and composition, or the lipid 'profile' can serve as a bacterial taxonomic marker (Kates, 1993). For the archaebacteria (archaea), membrane lipid patterns (Kates, 1993), along with their 16S ribosomal RNA sequences and their cell wall structures are particularly useful

taxonomically to distinguish between the three groups of archaebacteria (extreme halophiles, methanogens and thermoacidophiles) and to separate the archaea from all other microorganisms. Archaeal membrane lipids are unique in structure consisting of derivatives of a C_{20}-C_{20}-isoprenyl glycerol diether, *sn*-2,3-diphytanyl-glycerol (archaeol) and its dimer, dibiphytanyldiglycerol tetraether (caldarchaeol). These core ether lipids are completely absent in eubacteria and eukarya, which contain predominantly diacylglycerol-derived lipids. Within the archaea, the extreme halophiles are distinguished by their containing only archaeol-derived lipids, while the methanogens contain both archaeol and caldarchaeol-derived lipids and the thermoacidophiles contain predominantly caldarchaeol-derived lipids. The haloarchaea can be distinguished from other extremely halophilic prokaryotes by the possession of ether-linked phosphoglycerides, which can be readily detectable by the procedures of Ross *et al* (1985), Torreblanca *et al* (1986) and Kamekura and Kates (1988). The lipids of all halobacteria contain phytanyl ether analogs of phosphatidyl glycerol (PG) and phosphatidyl glycerol phosphate methyl ester (PGP-Me). Many strains also contain phosphatidyl glycerol sulfate (PGS). One or more glycolipids and sulfated glycolipids are also present in most strains including a sulfated tetraglycosyl diether (S-TeGD), triglycosyl diethers (TGD), and diglycosyl diethers (DGD). All halobacteria have diphytanyl (C_{20} C_{20}) glycerol ether core lipids, but some strains have additional phytanyl-sesterterpanyl (C_{20} C_{25}) glycerol ether core lipids (De Rosa *et al*, 1982; Kamekura and Dyall-Smith, 1995), and certain strains of haloalkaliphiles have di-sesterterpanyl (C_{25} C_{25}) glycerol ether lipids (De Rosa *et al*, 1983). Bi-diphytanyl tetraethers (C_{40} C_{40}) have not been detected in any halobacteria to date. Isoprenoid quinones are of the menaquinone type (MK-8 and MK-8 (H_2); Collins *et al*, 1981), not the ubiquinone type.

vii) Taxonomy

The current classification of halophilic archaea is based on three kinds of data: Phenotypic data-such as cell morphology, growth properties (Tindall, 1992); Chemical data-patterns of polar lipids present in membranes (Torreblanca *et al*, 1986; Tindall, 1992); Genetic data (16S rRNA sequence information and DNA-DNA hybridization results (Oren *et al*, 1997). There are presently eighteen validly described genera of Halobacteria, fourteen of which are indicated in Table 3.

Table 3: Main differential features between the fourteen genera of the family Halobacteriaceae

Characteristics	*Halobacterium*	*Haloarcula*	*Halobaculum*	*Halococcus*	*Haloferax*	*Halogeometricum*	*Halorubrum*
Gram character cell Morphology	-ve rods	-ve pleiomorphic rods flat triangles, rectangular, irregular disks	-ve rods	-ve /+ve cocci	-ve pleiomorphic rods	-ve pleiomorphic squares, triangles, ovals	-ve rods
pH range for growth	5.0 - 8.0	5.0 - 8.0	5.0 - 8.0	6.0 - 9.5	5.0 - 8.0	6.0 - 8.0	5.0 - 8.0
Amino acid requirement	Arginine	-	Arginine	glutamine histidine proline valine	-	-	-
Mg^{2+} requirement (mM)	5.0 - 50.0	5.0 - 50.0	5.0 – 50	5.0 - 50.0	20.0 - 50.0	40.0 - 80.0	5.0 – 50
Optimum salt requirement	3.5 – 4.5	2.0 – 3.0	3.5 – 4.5	3.5 – 4.5	2 – 3	3.5 – 4	2.5 – 4.5
Polarlipids: PG	+	+	-	+	-	+	+
PGP-Me	+	+	-	+	-	-	+
PGS	+	+	-	-	-	-	+
DGD – 1	-	-	-	-	-	-	-
DGD – 2	-	+	-	-	-	-	-
S - DGD - 1	-	-	+	+	+	-	+
TGD – 1	+	-	-	-	-	-	-
TGD – 2	-	+	-	+	-	-	-
S- TGD – 1	+	-	-	-	-	-	-
S- TeGD	+	-	+	-	-	-	-
Unidentified non- sulfate containing glycolipids	-	-	-	-	-	+	-
Lysis in distilled water	+	+	+	-	+	+	+

Table 3: Main differential features between the fourteen genera of the family Halobacteriaceae

Characteristics	*Haloterrigena*	*Natrialba*	*Natrinema*	*Natronobacterium*	*Natronococcus*	*Natronomonas*	*Natronorubrum*
Gram character cell Morphology	-ve rods or coccoid	-ve rods	-ve rods	-ve rods	+ve cocci	-ve rods	-ve pleiomorphic rods
pH range for growth	6.0 - 8.5	5-10.0/ 6.0-8.0	5.0 - 8.5	8.5 - 11.0	8.5 - 11.0	8.5-11.0	8.0-10.0
Amino acid Requirement	Arginine	Casamino acids	+	-	-	Casaminoacids, glutamate	Casaminoacids
Mg^{2+}requirement (mM)	5.0 – 50.0	5.0 – 50.0	5.0 – 50.0	< 1	< 1	< 1	< 1
Optimum salt requirement	3 – 4	3.5 – 4.5	3.4 – 4.3	3.5 – 4.5	3 – 4	3.5 – 4.5	3.4 – 3.8
PG	+	+	+	+	+	+	+
PGP-Me	+	+	+	+	+	+	+
PGS	-	-	-	-	-	-	-
DGD – 1	-	-	-	-	-	-	-
DGD – 2	-	-	-	-	-	-	-
S – DGD – 1	-	-	-	-	-	-	-
TGD – 1	-	-	-	-	-	-	-
TGD – 2	-	-	-	-	-	-	-
S- TGD – 1	-	-	-	-	-	-	-
S- TeGD	-	-	-	-	-	-	-
Unidentified phospholipids	+	+	-	+	+	+	+
S_2DGD	+	+	-	-	-	-	-
Unidentified glycolipids	-	-	+	-	-	-	-
Lysis in distilled water	-	+	+	+	Show leakage of contents	-	+

Aims and objectives

Research on terrestrial marine ecosystems have largely been concentrated world wide. Econiches as natural salt pans have not only been neglected but are been recklessly converted for housing with the upcoming urbanization. However, there is a **need** to preserve such econiches, for they have a dual significance-in the production of crude organic salt and the beneficial 250 million-old halophilic archaea, which could be harnessed for agricultural and industrial benefits (Mc Genity *et al,* 2000).

In Goa, land at the mouth of these estuaries is low lying inter-tidal and annually undergoes three distinct phases: (i) draught, (ii) brine/salt, (iii) water-logged. The locals of these regions manoeuvre these lands particularly during November to May for production of crude solar salt, which are popularly known as **salt pans.** These are also devoid of vascular plant vegetation. Following heavy rains, they retain standing water for around four to five months, whilst in dry spells, the pans dry out leaving crystalline salt. (Sequeira, 1992).

Various microorganisms belonging to Bacteria (Kerkar,2004; Raghavan and Furtado, 2004), Cyanobacteria (Shaikh, 1996), Archaebacteria (Braganca and Furtado, 1999; Raghavan and Furtado, 2004) and Fungi (Nazareth, 2005) have been reported from one or two salt pans of Goa, during sporadic studies.

For the first time, a study was undertaken to evaluate culturable haloarchaea, associate microbial community, unveil the impact of climatic changes, determine the role of

haloarchaea in C and N_2 cycles and the occurrence of metal and faecal pollutants in salt pans of Goa.

CHAPTER II

MATERIALS & METHODS

MONITORING OF PHYSICO-CHEMICAL AND MICROBIAL STATUS OF SALT PANS OF GOA

I. Study of Geographical and Topographical location of salt pans of Goa

Three Talukas of Goa, India, viz. Pernem, Bardez and Tiswadi were surveyed for villages carrying on conventional salt farming activity. Salt pans in villages of Arambol(A), Agarwada(Ag), Arpora(Ar), Nerul(N), Ribandar(R), Batim(B) and Siridao(S) of three talukas were identified as sites for study. The geographic positions of the seven sites were marked on site, using a Global Positioning System GPS 12, GARMIN Olathe, KS, USA.

II. Collection of samples

On site, temperature was recorded with mercury thermometer at individual salt pans. Samples of water above salt crystals, called brine were collected into clean plastic bottles while sediment samples from a depth of 15 cms in a zig-zag manner, all along the rectangular pans, were collected with the help of a shovel, into plastic bags from salt pans of Arambol, Agarwada, Arpora, Nerul in 2003, while in case of Ribandar, Batim, Siridao during the sampling period of 2001, 2003, 2005. Salt flakes and crude solar salt, were also collected. Samples of water and of sediments of each salt pan were immediately transported to the Haloarchaeal Laboratory, Department of Microbiology, at Goa University (HLDMGU), Goa-India, and studied for: **a) Colour of sediment; b) Soil texture** by 'feel' method; **c) Moisture content :** One gram sediment in a petridish was dried overnight at 105-110^{0}C in the oven, cooled in desicator and weighed to constant weight.

III.Treatment of samples

Samples of brine from salt farming and shallow stages were used directly for obtaining microbial counts, while two and half litres of water sample collected during monsoon when

the salt pan land is submerged under water was centrifuged in C 24 Remi (India), cooling centrifuge at 10,000 rpm and 4^0C. The resulting pellets were collected and resuspended separately into 100 ml of supernatant and then used for analysis. Portions of each sediment sample weighing 6 g was suspended into 60 ml of sterile 25% NaCl solution, mixed vigorously, allowed to stand for 30 minutes. The overlying sediment-free suspension was then used for analysis of:

a) Hydrogen ion concentration (pH)

Aliquots of above sediment-free suspension was checked for pH using a digital pH meter (Lab India), with a double electrode, pre-calibrated with standard buffers.

b) Salinity

Five ml of sediment-free suspension was filtered through Whatman No.1 filter paper and filtrate was diluted with 10 ml distilled water and titrated against $AgNO_3$ using $K_2Cr_2O_7$ as indicator till endpoint of dull red/dirty orange, that persisted for at least 30 seconds. Salinity was calculated using Harvey's Table (Grasshoff, 1983).

c) Analysis of metal ions

Sediment-free suspension was taken for Na^+, K^+, Ca^{2+} estimation by Flame Photometer Systronics (India). 100 ppm solutions of NaCl and KCl, while 1000 ppm solution of $CaCO_3$ were used as standards.

For heavy metal ions of Cd^{2+}, Hg^{2+}, Cu^{2+}, Pb^{2+}, five grams of the well mixed sample was weighed on a Mettler AE 200 Toleido Monopan balance, Switzerland, accurately in a tared silica dish, then heated by a soft flame to volatilize as much organic matter as possible, transferred to a 300^0C controlled muffle furnace, until material was dry and charred, temperature then was increased to 450^0C, till no carbon remained and ash became white. Then 1-2 ml of concentrated nitric acid was added, evaporated to dryness, heated again in

muffle furnace till ashing was complete. To the cooled silica dish, 40 ml of HCl-water (1:1 v/v) was added, heated on a steam bath for 30 minutes with a cover, rinsed and heated again for another 30 minutes, to which 10 ml of $HCl:H_2O$ (1:1 v/v) was added to dissolve the salts. The solution was then filtered into 100ml volumetric flask using Whatman no. 44 filter paper, residue basin washed twice using dilute HCl, volume made with water (Toteja *et al*, 1990). Heavy metals Cd^{2+}, Hg^{2+}, Cu^{2+}, Pb^{2+} were analysed by Double Beam Atomic Absorption Spectrophotometer GBC 902 model. 1000 ppm solutions of $3CdSO_4.8H_2O$, $C_4H_6HgO_4$, $CuSO_4.5H_2O$ and $(CH_3COO)_2Pb.3H_2O$ were used as standards.

d) Iodine in crude and commercial salt

Fifty grams of iodised salt was taken in a 250 ml volumetric flask, volume made upto mark, solution filtered, of which 200 ml filtrate was transferred to a conical flask, made faintly acid with 6N HCl to methyl orange (3 drops), after which 1 ml of saturated bromine water was added, mixture boiled until salt separated and redissolved in minimum quantity of distilled water followed by addition of 1 M HCl and 0.2 g KI and titration with 5 mM $Na_2S_2O_3$ using starch as indicator. Amount of liberated iodine was calculated using the formula :

1 ml 5 mM $Na_2S_2O_3$ = 0.1058 mg I_2 = 0.1384 mg KI (Egan *et al*, 1981).

IV. Enumeration of microorganisms

A 100 ml aliquot of overlying sediment-free suspension was used as inoculum. 100 ml of brine sample was directly used as inoculum without any treatment. Enumeration was carried out using most probable number (MPN) method suitably modified for halophiles (Raghavan and Furtado, 2004) and using a set of 15 tubes. First five tubes containing 5ml of double strength NTYE (Steensland and Larsen,1968) broth, pH 5.0/7.0 OR *Natronobacterium pharaonis* broth (Soliman and Truper, 1982), pH 10.0 OR Nutrient Broth (Cruickshank,

1965), pH 7.0 were inoculated with 500 µl of brine/sediment sample, second set of 5 tubes containing 5ml of single strength respective media were inoculated with 50 µl of inoculum and the third set of 5 tubes containing 5ml of single strength respective media were inoculated with 5 µl of respective inoculum. Each set of tubes were incubated at room temperature (28±2^{0}C), under aerobic conditions. Growth within each set of tubes was monitored visually, from 2nd day to 20 days. The MPN of microorganisms was deduced as per the Mc Crady's Table (Cruickshank, 1965).

V. Isolation of microorganisms

Bacterial, Cyanobacterial, Fungal and Yeast growth from MPN tubes for each sample was plated on **Nutrient rich agar media**, namely :

a) Tryptone yeast extract containing 25% crude solar salt (NTYE) b) *Natronobacterium pharaonis* (NP) c) Purple membrane (PM) (Brown, 1963) d) *Halobacterium sodomense* (HS) (Oren, 1983) e) Nutrient agar (NA) (Cruickshank, 1965) f) Malt Extract agar with 20% crude solar salt (NMEA), pH 5.5. **Synthetic medium (NSM)** (Aguiar and Furtado,1996) containing 20% NaCl and 0.2% of glucose (NGSM)/0.5% Casein (NCSM)/0.5% Starch (NSSM)/Agar (NASM)/Synthetic medium without nitrogen source (NGSMN$^-$)/Synthetic medium with glucose and 2mM Fe^{2+}, as $FeSO_4.7H_2O$ (NG(Fe)SM)/2mM Mn^{2+}, as $MnCl_2.4H_2O$ (NG(Mn)SM).

VI. Purification of cultures

Bacterial, Cyanobacterial, Fungal and Yeast isolates were purified by streaking onto solid media having the same composition as that of isolation, checked for purity microscopically. Isolates obtained were given designation: **Bacteria** $GUFF_1$ through $GUFF_{234}$, **Fungi** $GUFFf_1$

through GUFFf$_{42}$, **Cyanobacteria** GUFFc$_{190}$ through GUFFc$_{192}$, Yeast GUFFf$_{41}$. The notation for bacterial isolates of GUFF for Goa University Fernandes C. and Furtado I., while for fungi GUFFf, f for fungi and c for cyanobacteria.

VII. Maintenance of cultures

Bacterial cultures were grown on NTYE/NP/PM/HS/NA/NGSM/NCSM/NSSM/NASM/ NGSMN^{-}/NG(Fe)SM/NG(Mn)SM slopes; fungal cultures were grown on NMEA medium slopes; cyanobacterial cultures on NSM medium slopes were maintained at room temperature (28±2^{0}C) and subcultured into respective medium after 30days.

VIII. Characterization of Bacterial, Cyanobacterial, Fungal and Yeast isolates

a) Colony characteristics

Each type of isolate was spot inoculated onto NA/NTYE/Sabouraud's Agar (SAB)/SAB with 20% crude solar salt (NSAB), Potato Dextrose Agar (PDA) (Cruickshank,1965) and MEA and NMEA, incubated at 28±2^{0}C. Colony size, texture, margin, colour on reverse were determined after 7, 10 and 23 days of incubation.

b) Wet mounts

Wet mounts of fungal, yeast, cyanobacterial cultures in lactophenol cotton blue/25% NaCl, type of mycelium & spores observed under bifocal light microscope (Olympus BX40 Magnus CH 20i Microscope (India), 100x magnification.

c) Scanning Electron Microscopy of selected isolates

Culture was grown in NTYE for 5 days under shaker conditions, centrifuged at 8000 rpm, pellet obtained was washed and suspended in NSM, to obtain O.D. of 0.8 at 600 nm. 100 µl

of this suspension was spread on coverslip. It was then treated with 2% glutaraldehyde in NSM and kept overnight. The next day, the coverslips were exposed to 30%, 50%, 70%, and 90% in acetone : water serially for ten minutes each, followed by 100% acetone for half an hour and then air dried. Specimen on coverslip was stuck to brass stubs using double sided tape with specimen upwards and coated with 15-20 nm gold on SPI-MODULE Sputter Coater instrument for 90 seconds and then subjected to Scanning Electron Microscopy (JEOL, Japan). One drop of silver paint was applied to a part between coverslip and stub, to maintain the continuity of flow of electrons when focusing the processed sample.

d) Gram character

A smear of bacterial cells was prepared by taking a loopful of culture, heat fixed/air dried and fixed with 2% acetic acid solution for 30 secs to 1', Gram stained after air drying, a smear was made from a loopful of culture and heat-fixed. 2% aqueous crystal violet stain for 1 minute. The excess stain was washed with water, treated with 1% aqueous Gram's iodine for a minute, rinsed with 70% ethanol, treated with 0.5% aqueous safranine for 2 minutes, washed, air dried and observed under oil immersion objective imaging microscope Olympus BX40 Magnus CH 20 i Microscope (India), 100x magnification.

Characterization of Cultures

a) Growth of isolates on different substrates

Yeast culture was characterized by spot inoculation on NMEA, pH 5.5, NA pH 7.0 and NTYE pH 5.0, 6.0, 7.0, 8.5 and 10.5 with 1, 50, 80, 5 and 0.07 mM Mg^{2+} respectively.

Individual Fungal/Bacterial isolates were spot inoculated on NSM with 0.2% glucose, 0.5% of each of the following, i.e. starch, casein, chitin, chitosan, cellulose, cellobiose,

pectin, gelatin and grease in a concentration of 0.2% as sole source of carbon. In the case of grease, NSM plates were overlayered with grease in petroleum ether (40^0-60^0C) and incubated at 28±2^0C. Growth in terms of zone size, colour of mycelium, spores, texture, margin, colour on reverse were recorded after 7, 10 and 23days of incubation. Detection of starch hydrolysis was carried out by flooding starch agar plates with aqueous Gram's iodine reagent. Clear colourless/straw yellow zone around fungal colony was indicated as a positive test. Gelatin hydrolysis was detected by flooding growth on plates with 1% aqueous mercuric chloride (Cruickshank, 1965). White zone around fungal colony indicated a positive test.

b) Growth of isolates at various temperatures

Individual Fungal isolates were spot inoculated onto a set of three plates containing NGSM, pH 5.5. Individual plates of the set were incubated either at 37^0C/42^0C and 50^0C and growth recorded for a period of one month.

Bacterial isolates

The various **bacterial isolates** were separately spot inoculated onto TYE, pH7 containing 0%/25% crude solar salt, pH 5.0/7.0 and NTYE with 500 U/ml of penicillin/20µg of Kanamycin/20% crude solar salt, MacConkey's agar, pH7/NP, pH 8.5/10.5. Plates were incubated at 42^0C for 2-3 days and checked for growth, colony characteristics, visual pigmentation, periodically in individual cultures.

IX. Biochemical Characterization

i) Catalase

Bacterial culture was inoculated onto a nutrient agar slant, incubated at room temperature, for 24 hours, 3% v/v H_2O_2 was added directly and checked for effervescence.

ii) Oxidase

A loopful of culture was rubbed on a filter paper strip soaked in freshly prepared oxidase reagent (tetra methyl paraphenylene) placed in a petridish. Paper was then checked for purple colouration.

iii) H_2S and motility

Culture was stab inoculated into H_2S and motility medium. A lead acetate paper strip was then inserted at the mouth of the test tube, incubated at room temperature, for 24 hours, and blackening of the lead acetate paper strip was then checked.

iv) Sugar fermentation

A loopful of culture was added to 5 ml of peptone water medium, with 0.1% phenol red indicator and 0.5 ml of 10% sugars-glucose/sucrose/fructose/maltose, tubes incubated at room temperature for 24 hours, colour change and gas production if any, were recorded.

v) Hugh-Leifson's (HL)

Culture was inoculated into a set of two tubes containing HL media. A layer of sterile paraffin oil 10 mm thick was added to one tube. Tubes were incubated at room temperature for 48 hours and growth recorded.

vi) Christensen's urea

Culture was inoculated into liquid medium and incubated at room temperature for 48 hours, pink colouration to the medium indicates +ve test.

vii) Gelatin

To 5 ml of gelatin hydrolysis medium, a loopful of culture was inoculated and incubated at room temperature for 24 hours.

viii) Triple Sugar Iron (TSI)

Culture was inoculated onto TSI slant, incubated at room temperature for 48h and then observed for colour and growth.

ix) Nitrate

To 5 ml of nitrate reduction medium, a loopful of culture was inoculated and incubated at room temperature for 24 hours, 3-4 drops of Greiss-Illosway's reagent was added.

x) Indole

A loopful of culture was inoculated into peptone medium, the tube incubated at room temperature for 24 hours. After incubation, xylol was added, mixed thoroughly, after which 2-3 drops of Kovac's reagent was added. Pink colour to xylol layer indicates a +ve test.

xi) Methyl Red Vogus Proskauer (MRVP)

A tube containing glucose peptone water was inoculated with the culture, incubated at room temperature for 48 hours.To half the growth, 3-4 drops of methyl red indicator was added. Development of red colour is a +ve test. To remaining half, 4 drops of Kovac's reagent was added. The tube was then kept at 37^0C for 4h. A +ve test is indicated by a pink colour.

All media used for various tests were same as used for eubacterial cultures except that instead of peptone base for sugars, NSM containing 20% crude solar salt was used.

X. Chemotaxonomic characterization of Cultures

a) Response of isolates to Mg^{2+}

Cultures were spot inoculated on to a plate containing 0.07, 10 and 790 mM Mg^{2+} as $MgSO_4.7H_2O/MgCl_2.6H_2O$ and incubated at 42 ^{0}C for 2-3 days and checked for growth.

b) Production of Polyhydroxyalkanoate (PHA)

Cultures were spot inoculated on a plate containing NSSM/NGSM, pH 7.0, plate incubated at 42 ^{0}C for 2-3 days. Plate was then flooded with 0.02% Nile Blue in methanol (Shirokitamura and Doi, 1994), kept for 10 minutes, excess drained, allowed to evaporate and viewed under UV transilluminator.

c) Response to distilled water

A loopful of 5 day old culture was dispersed in 5 ml of 25% NTYE broth, O.D. made to 1.0 at 600 nm. Centrifuged at 5000 rpm for 10', followed by addition of 5 ml of D/W, O.D. taken at 600 nm after 30' and after keeping at room temperature for 24 hours.

d) Pigment characteristics

Culture was spot inoculated onto a nutrient agar plate, incubated at room temperature for 48 hours. Visual pigmentation was noted down. Cellular pigment was extracted in chloroform-methanol (1:1 by volume) using sonicator (B. Braun Biotech International, U.S.A.) at 0.7 cycles/second. The pigment scans were taken using UV-VIS spectrophotometer.

e) Demonstration of Glycerol Diether Moiety (GDEM)

Cell pellet of 7d old cultures grown in NTYE was obtained by centrifugation at 3000rpm for 10 minutes. Methanol (3 ml), toluene (3 ml) and concentrated H_2SO_4 (0.1 ml) was added and kept at 50^0C for 15-18 hours for hydrolysis (Ross *et al*, 1981). Long chain components were extracted from this mixture by adding 1.5ml hexane. Detection of hydrolysis products was done by TLC using silica gel H, using solvent system petroleum ether (60-80^0C):diethyl ether (85:15 v/v) and visualized with 10% dodecaphosphomolybdic acid in absolute ethanol and heating at 150^0C for 15 minutes (Torreblanca *et al*, 1986).

f) Polar lipids

Cell pellet was suspended in 2 ml of 4 M NaCl and extracted with 3.75 ml of chloroform: methanol (1:2 v/v) for 12 hours. The extract was collected by centrifugation and pellet was re-extracted with 4.75 ml methanol:chloroform:water (2:1:0.8 v/v) (Oren and Gurevich, 1993). With the help of separating funnel, chloroform layer was collected and concentrated at room temperature. Individual concentrates were applied individually to Silica gel G plates and developed in the solvent systems chloroform:methanol:acetic acid:water (85:22.5:10:4 by volume). Lipid spots were visualized by exposure to iodine vapours, while glycolipids were

visualized by spraying the developed chromatogram with 0.5% α-naphthol in 50% methanol-water followed by 5% H_2SO_4 in ethanol and heating the plates at 100^0C (Siakotos and Rosser, 1965). Phospholipids were visualized using ammonium molybdate-sulphuric acid spray (Dittmer and Lester, 1964).

g) Fluorescence Transmission Infra Red (FT-IR) spectroscopy of cultures

Bacterial strains were streaked on agar plates containing NTYE medium and incubated for 7 to 10 days. Loopful of each of the cultures $GUFF_5$, $GUFF_{10}$, $GUFF_{120}$, $GUFF_{124}$, $GUFF_{169}$, $GUFF_{195}$ dried in aluminium foil at 42^0C. Following drying of samples, the bacteria were subjected to diffused reflectance spectral analysis between 400-4000 nm in FT-IR spectrometer.

XI. Biosorption of Mn^{2+} metal by haloarchaeon GUSF (MTCC 3265)

Aliquots of 5 µl, 50 µl, 500 µl, 1000 µl of GUSF cells suspended in 15% NaCl to an absorbance of 2 at 600 nm, were mixed with 3ml of 4M (Mn^{2+}) $MnCl_2$ solution, at room temperature, at 150 rpm for 15 minutes after which absorbance was read. The mixture was centrifuged at 3000 rpm for 5 minutes. The cell-free supernatants were then scanned on a UV-VIS spectrophotometer at 190-700 nm against a control of 4 M $MnCl_2.4H_2O$.

CHAPTER III

RESULTS

SALT PAN ECOLOGY HALOARCHAEA AND THEIR ROLE

SECTION A

I. SALT FARMING IN GOA

1. Salt pans and their location

Field surveys conducted during 2001, 2003, 2005 years, indicated that villages of Arambol (A) and Agarwada (Ag) of **Pernem Taluka;** Arpora (Ar), Nerul (N) and Pilerne (P) of **Bardez Taluka**; St. Cruz (C), Batim (B), Goa Velha (GV), Ribandar (R), Siridao (S) of **Tiswadi Taluka**; Cavelossim (Ca), Orlim (O) and Chinchinim (Ch) in **Salcete Taluka,** carry on the conventional salt farming activity; these sites are depicted in **Fig. 1.**

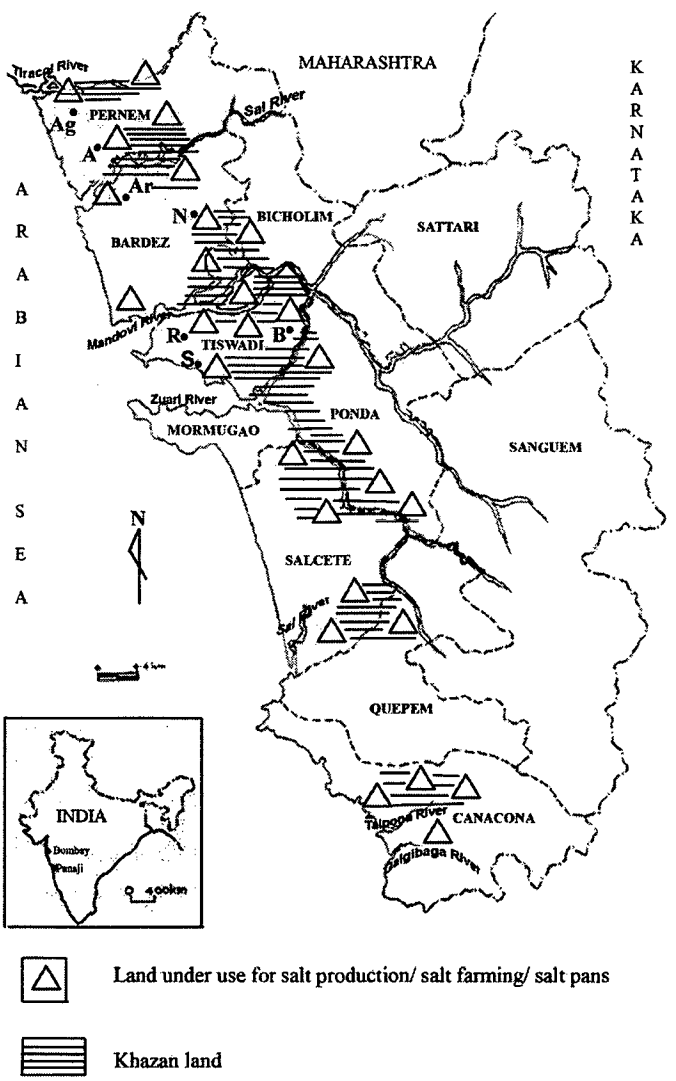

Fig. 1: Map of Goa (Furtado, 1994) indicating salt pans and sampling sites

Individual (main and sub)-salt farming facilities, strategically selected for this study are recorded, by a colloquial name, along with geographic locations in **Table 4.**

Table 4: Evaluation of Salt Farming Facilities/Salt Pans in Goa-India for geographical location and salt yield*

Name of Salt Farming facility	Village	Taluka	Area (in hectare)	Geographic location	No. of salt producer	Salt yield/ annum (in tonnes)
Ghoddgecho Agor	Arambol (A)	Pernem	7.0	N 15^{0}41'03.7" E 073^{0}42'40.0"	14	420.0
Devacho Agor (AgD)	Agarwada (Ag)			N 15^{0}38'34.4" E 073^{0}45'56.1"		
Bappacho Agor (AgB)						
Sabhacho Agor (AgS)						
Pednekar Agor (AgP)						
Cansau Agor (AgC)						
Telgancho Agor (Ar_1T)	Arpora (Ar)	Bardez	11.13	N 15^{0}34' 06.6" E 073^{0}45'39.6"	12	1,750.0
Telgancho Agor (Ar_2T)						
Bhatim	Nerul (N) (N*)		9.00	N 15^{0}31'06.9" E 073^{0}47'20.7"	10	1,140.0
	Pilerne (P)		23.21	N 15^{0}31'00.9" E 073^{0}48'26.9"	6	2,000.0
	St. Cruz (C)	Tiswadi	Exact area is not available	Nd	1	48.0
	Batim (B)			N 15^{0}27'15.5" E 073^{0}52'55.6"	8	1,594.5
Patto	Ribandar (R)			N 15^{0}29'57.1" E 073^{0}50'23.3"	3	40.0
Zuarer	Siridao (S)			N 15^{0}26'28.5" E 073^{0}52'01.4"	1	9.0
	Goa Velha (GV)			Nd	3	1,342.0
	Cavelossim (Ca)	Salcete	0.012	Nd	1	20.0
	Orlim (O)		0.02	Nd	2	260.0
	Chinchinim (Ch)		1.18	Nd	5	276.0

N*-Non Conventional; Nd-not done; * Data collected for the year 2003

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Agarwada & Arambol salt pans are situated in Pernem Taluka, on the bank of Tiracol river. The salt pan area was at N $15^0$38'34.4" & E $073^0$45'56.1" and N $15^0$41'03.7" & E $073^0$42'40.0" respectively. Human habitations are in the close vicinity of this salt pan. **Arpora & Nerul salt pans** are located at N $15^0$34'06.6" & E $073^0$45'39.6" and N $15^0$31'06.9" and E $073^0$47'20.7" respectively. They are located in Bardez Taluka on the bank of Chapora river. **Ribandar salt pan** is situated along the Mandovi river at N $15^0$29'57.1" and E $073^0$50'23.3", used for the transportation of iron ore. **Siridao and Batim salt pans** are at N $15^0$26'28.5" & E $073^0$52'01.4" and N $15^0$27'15.5" & E $073^0$52'55.6" respectively of the Zuari river, in Tiswadi Taluka. The surrounding marshy land supports mangrove vegetation. In the monsoon, these salt pans are used for rice cultivation and pisciculture. Human habitations are distant from these salt pans. Salt pans at Siridao and Batim are along the National Highway 17-A.

2. Salt Farming Process in Goa

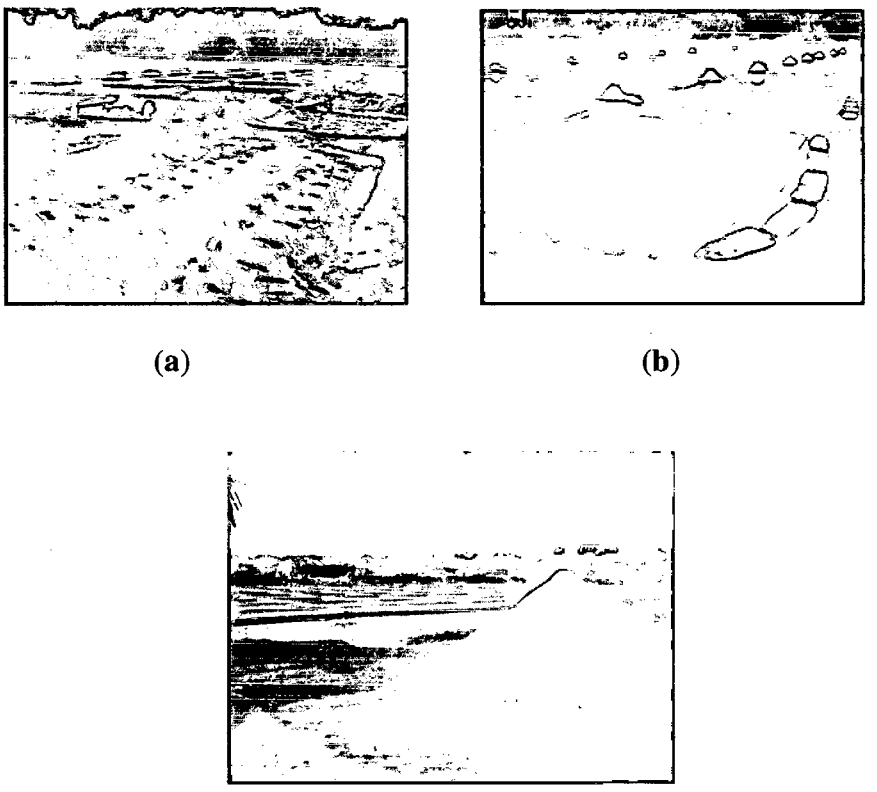
The salt farming process as studied by us is summarized as simple, highly economical and requires but little apparatus of any kind.

The land used for salt farming is prepared during December, while the salt farming process is operated in **summer season** (January to May).

These lands being exposed to vagaries of nature, no longer remain as salt pans for rest part of the year (as salt farming process has to be suspended) as with the onset of **Monsoon Season**, they get filled up with the rain water to give the **submerged/water-logged stage** (water level being 1-2 metres above soil, depending on the amount of rainfall) during the months of June to September. Some of these lands are used for pisciculture of fish, mollusks and crustaceans. After the monsoon, the waters are drained in the month of October (hundreds of piscivores birds crowd these areas during this period), after which there is shallow water stage (water

level to a depth of 15-20 cm) for a period of two months until November called **Shallow Water stage/Post-Monsoon Season.**

In Goa, conventional salt farming is carried out on large, low lying, khazan shallow land, located on the banks of estuaries, protected from the action of tides: natural man-made salt pans. The **reservoir**/heater/large primary basin called **Tapovanim** receives saline water from the nearest creek at high tides, by means of flood-gates/sluice gate. The bottom of this land is laid out perfectly even, and the soil is mostly clayey, to retain the water, which is beaten hard and smooth during preparation of land for salt farming as seen in **Plate 1**.



(a) (b)

(c)

Plate 1: Evaporation and salting out by solar exposure: Salt farming stage– (a) preparation of land (b) extensive salt precipitation (c) harvest of crude salt as heaps.

The water in the reservoir/tapovanim, gets warmed by the sun's rays, and begins to evaporate, causing formation of brine or water saturated with salts. From this reservoir, the partially concentrated brine is led by a canal/brine circulation channels, to a series of rectangular basins, from ten to sixteen inches in depth. These basins are divided into a series of compartments, by means of little cross banks, through which the brine flows successively, in a slow current, which is regulated as desired, so that larger area is available for evaporation and crystallization. All these basins are carefully prepared like the reservoir, so as to retain water. Here, by the action of the sun and wind, the water is rapidly evaporated, and deposits a portion of its lime, in the form of sulfate, until the whole of the lime is deposited on further concentration of brine. From this basin, the brine passes to another series of similar rectangular basins, larger and shallower, where remainder of the sulfate of lime is deposited. The evaporation is carried out to such a point that the water becomes saturated brine. At this time, the volume of the concentrated brine is greatly diminished, and is ready to be transferred to a third and last series of similar rectangular basins called salting pans, which are smaller and shallower basins, carefully constructed and divided into compartments, communicating with each other, where the layer of water is not more than 3 inches in depth. The brine is introduced into the salting pans after it has reached a sufficient degree of concentration.

In the salting pans, the brine soon begins to deposit salt, in the form of crystalline crusts, which are either collected with special wooden rakes as soon as they form, or allowed to accumulate at the bottom, until they form masses of several inches in thickness, which are then broken up and piled as small mounds at the intersection of the pans, which is then followed by purification of salt. The method used consists in simply washing the crude salt with concentrated brine, which removes impurities of soil. The crude salt thus obtained, is collected in bamboo baskets and dumped as heaps on the bandhs, and then graded: i) market

grade, for sale for human consumption, used as food seasoning; ii) preservative grade, a lesser impure salt used for salting of fish, on account of its tendency to keep them moist; iii) fertilizer grade, for fruit-yielding trees, as coconuts, mango, breadfruit, etc.

The brine on the pans is renewed daily, or every two days, according to the evaporation; whilst the reservoir as well as basins are constantly supplied with fresh brine. The concentration of the brines (mother liquors) in the salting tables is carefully watched, and their density never allowed to exceed as otherwise a deposit of sulfate of magnesia (Epsom salt) would be formed, rendering the salt impure. In case, this happens, then the mother liquors are run-off. It is usually necessary to draw them off, three or four times during the season.

Bhobe salt facility is the only modern salt crystallization facility in Nerul-Goa, wherein sea water is concentrated in reinforced concrete cemented flat tanks using solar energy during summer.

II. PHYSICO-CHEMICAL STATUS OF SALT FARMING ECONICHE OF GOA

Colour of the soil ranged from brownish grey to brownish black. Texture of the soil was mostly clay loam. Moisture content of the soil varied from 0.258 to 0.275 per gram of soil. The sediments had 8.0 to 8.33 pH, while brine was neutral. As in **Table 5**, at salt farming stage, salinity range was found between 39.0 and 41.0%. Estimation of elements – Na^+, K^+, Ca^{2+}, gave 269 ppm and 594 ppm of Na^+ per ml of brine of Batim and per gram of sediment of Siridao, respectively. 32 ppm were found in one ml of brine of Batim while one gram of sediment of Siridao gave 28 ppm of K^+. Brine of Batim showed the presence of 246 ppm per ml, while one gram of Siridao sediment showed 346 ppm of Ca^{2+}. 2.5 ppm of iodine were present in one gram of crude solar salt of Arambol, Agarwada, Ribandar, Siridao salt pans;

while crude salt from salt pans of Arpora & Nerul did not show the presence of iodine. One gram of refined salt (Captain Cook brand) revealed the presence of 40 ppm of iodine.

Table 5 : Physico-chemical parameters of salt farming econiche

Salt pans from Goan village	Sediment				Brine		
	Colour	Texture	Moisture content (g)	pH	Temp (^{0}C)	pH	Salinity (%)
Arambol (A)	Brownish grey	Clay loam	0.27	8.2	35	7.15	40.50
Agarwada (Ag)	Brownish grey	Clay loam	0.275	8.3	32	7.1	39.50
Arpora (Ar)	Brownish grey	Muddy	0.270	8.1	38	7.0	40.0
Nerul (N)	Brown	Muddy	0.265	8.33	37	7.2	39.20
Pilerne (P)	Brownish grey	Muddy	0.270	8.0	35	7.5	39.15
Batim (B)	Brownish black	Clay loam	0.258	8.15	35	7.2	39.0
Ribandar (R)	Brownish black	Clay loam	0.26	8.09	35	6.92	39.74
Siridao (S)	Brownish black	Clay loam	0.27	8.33	37	7.3	41.0

Na^+, K^+, Ca^{2+} values for Batim and Siridao salt pans were 269, 32, 246 and 594, 28, 346 ppm per gram of sample, respectively. All saltpans showed 2.5 ppm of iodine per gram of crude salt except for Arpora and Nerul.

III. MICROBIAL STATUS OF SALT FARMING ECONICHE

Enumeration of Microorganisms

1. Most probable number of bacteria in brine of salt pans

During summers of alternate years of 2001, 2003, 2005, brine and sediment samples of salt pans of Arambol, Agarwada of Pernem Taluka; Arpora , Nerul of Bardez Taluka; Ribandar and Siridao of Tiswadi Taluka, inoculated for MPN counts, showed growth that could be

visualized as in **Plate 2**: i) Pellicle formers (P); ii) Growing throughout the column of media (C) and iii) Growing submerged at the bottom of the tube (B).

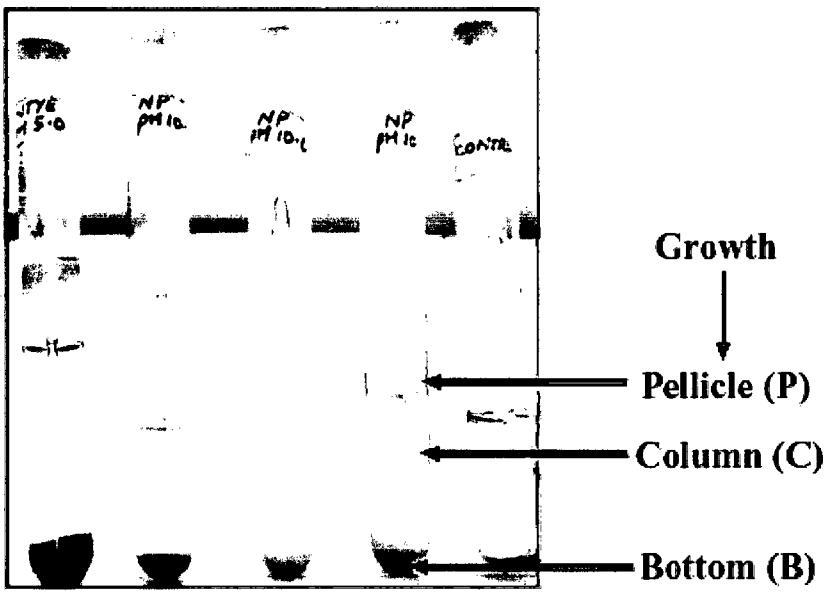


Plate 2: Growth in MPN tubes showing pellicle, column & bottom

As in **Table 6a** and **Table 6b,** cultures growing in Nutrient Broth (NB), *Halobacterium sodomense* (HS) medium and Tryptone yeast extract medium containing 25% crude solar salt (NTYE) gave counts of non-halophiles, moderate halophiles and extreme halophiles respectively; cfu/ml for brine and cfu/g for sediment.

Non-halophiles: Total counts for S salt pan were $3.7x10^4$ colony forming units per ml (cfu/ml) than that for R salt pan i.e. $9.2x10^3$ cfu/ml in 2001. In 2003, cfu/ml obtained for S salt pan were highest of $8.0x10^4$ cfu/ml, the least being $7.3x10^4$ cfu/ml for N salt pan while counts of $4.6x10^4$ cfu/ml were obtained for R salt pan in 2005. **Moderate halophiles:** Total counts for S and R salt pans in 2001 were about the same i.e. from $3.8x10^4$ cfu/ml to $4.0x10^4$cfu/ml, while counts in 2003, were highest in brines of A salt pan of $4.5x10^4$ cfu/ml and lowest in A & S salt pans of $4.2x10^4$cfu/ml. Higher counts of $5.0x10^4$ cfu/ml were obtained for S salt pan in 2005 as compared to those of R salt pan.

Table 6a: Most probable number of ***bacteria*** in ***brine*** of salt pans

ıka	Salt Pan	Counts of bacteria (cfu/ml)																	
		Non-halophiles						Moderate halophiles						Extreme halophiles					
		Culture Media	Nutrient broth with 0% NaCl						*Halobacterium sodomense* with 12.5% crude solar salt						TYE with 25% crude solar salt					
		Year	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC
ıem	A	P			$3.5x10^3$						$7.5x10^2$						$3.6x10^4$			
		C	-	-	$3.6x10^4$	$7.6x10^4$	-	-	-	-	$5.5x10^3$	$4.2x10^4$	-	-	-	-	$3.6x10^4$	$1.1x10^5$	-	-
		B			$3.6x10^4$						$3.6x10^4$						$3.6x10^4$			
	AgD	P			$3.8x10^3$						$8.0x10^2$						$3.6x10^4$			
		C	-	-	$3.6x10^4$	$7.6x10^4$	-	-	-	-	$6.0x10^3$	$4.3x10^4$	-	-	-	-	$3.6x10^4$	$1.1x10^5$	-	-
		B			$3.6x10^4$						$3.6x10^4$						$3.6x10^4$			
dez	Ar_1T	P			$4.5x10^3$						$9.0x10^2$						$3.6x10^4$			
		C	-	-	$3.6x10^4$	$7.5x10^4$	-	-	-	-	$8.5x10^3$	$4.5x10^4$	-	-	-	-	$3.6x10^4$	$1.1x10^5$	-	-
		B			$3.6x10^4$						$3.6x10^4$						$3.6x10^4$			
	N	P			$8.0x10^2$						$9.0x10^2$						$3.6x10^4$			
		C	-	-	$3.6x10^4$	$7.3x10^4$	-	-	-	-	$7.0x10^3$	$4.4x10^4$	-	-	-	-	$3.6x10^4$	$1.1x10^5$	-	-
		B			$3.6x10^4$						$3.6x10^4$						$3.6x10^4$			
ʻadi	R	P	$1.4x10^3$		$7.0x10^3$		$2.2x10^3$		$4.4x10^2$		$8.0x10^2$		$5.0x10^2$		$2.5x10^3$		$3.6x10^4$		$3.2x10^3$	
		C	$1.8x10^3$	$9.2x10^3$	$3.6x10^4$	$7.9x10^4$	$3.4x10^3$	$4.6x10^4$	$3.4x10^3$	$4.0x10^4$	$7.3x10^3$	$4.4x10^4$	$3.2x10^3$	$3.2x10^4$	$6.5x10^4$	$8.6x10^4$	$3.6x10^4$	$1.1x10^5$	$3.6x10^4$	$7.5x10^4$
		B	$6.0x10^3$		$3.6x10^4$		$4.0x10^4$		$3.6x10^4$		$3.6x10^4$		$2.8x10^4$		$1.8x10^4$		$3.6x10^4$		$3.6x10^4$	
	S	P	$9.5x10^3$		$7.5x10^3$		$3.3x10^3$		$2.8x10^2$		$4.4x10^2$		$6.0x10^2$		$1.8x10^3$		$3.6x10^4$		$3.5x10^4$	
		C	$2.8x10^4$	$3.7x10^4$	$3.6x10^4$	$8.0x10^4$	$3.6x10^3$	$4.3x10^4$	$3.1x10^3$	$3.8x10^4$	$5.2x10^3$	$4.2x10^4$	$4.5x10^3$	$5.0x10^4$	$1.8x10^3$	$5.4x10^3$	$3.6x10^4$	$1.1x10^5$	$3.6x10^4$	$1.1x10^5$
		B	$2.5x10^4$		$3.6x10^4$		$3.6x10^4$		$3.2x10^4$		$3.6x10^4$		$4.5x10^4$		$1.8x10^3$		$3.6x10^4$		$3.6x10^4$	

TC-Total Count; --not done

Extreme halophiles: Total MPN counts for R salt pan were $8.6x10^4$ cfu/ml in 2001. In 2003, all salt pans gave same number of extreme halophiles of $1.1x10^5$ cfu/ml. S salt pan showed higher counts of $1.1x10^5$ cfu/ml than those obtained for R salt pan in 2005.

2. Most probable number of *bacteria* in *sediments* of salt pans

Non-halophiles were nearly the same for R and S salt pans ranging from $4.3x10^5$ cfu/g to $4.4x10^5$ cfu/g in 2001. R salt pan showed highest counts of $8.1x10^5$ cfu/g and Ar_1T salt pan showed lowest counts of $4.9x10^5$ cfu/g in 2003. In 2005, S salt pan showed higher counts of $7.9x10^5$ cfu/g than R salt pan. **Moderate halophiles** were higher for R salt pan of $4.5x10^5$ cfu/g than S salt pan in 2001; compared to that of Ar_1 T salt pan had the highest of $6.5x10^5$ cfu/g to that of $4.3x10^5$ cfu/g of A salt pan in 2003; while in 2005, S salt pan gave highest counts of $4.6x10^5$ cfu/g than R salt pan. Total counts of **extreme halophiles** were $5.4x10^5$ cfu/g for R and S salt pans in 2001. Counts of $1.1x10^6$ cfu/g were obtained for A, AgD, R and S salt pans and counts of $7.3x10^5$ cfu/g for N salt pan in 2003, while counts obtained for R and S salt pans were $1.1x10^6$ cfu/g in 2005.

3. Most probable number of *haloarchaea* in *brines* of salt pans

Brine samples inoculated into **nutrient rich media** showed good growth of haloarchaea with NTYE and PM media as compared to that of HS medium, counts obtained in NP medium were in the range of $3.4x10^2$ cfu/ml to $1.8x10^3$ cfu/ml as in **Table 7a.** In **NTYE,** S salt pan showed $4.6x10^5$ cfu/ml of haloarchaea, and R salt pan gave $4.4x10^5$ cfu/ml in brine sample of 2001. In 2003, A, AgD, R and S salt pans showed same counts of $1.1x10^6$ cfu/ml while brines of N salt pan gave $7.3x10^5$ cfu/ml and Ar_1T gave $9.0x10^5$ cfu/ml. Counts of R and S salt pans were $1.1x10^6$cfu/ml in 2005. **HS medium** gave total counts of haloarchaea in R salt pan of $4.0x10^4$cfu/ml and S gave counts of $3.8x10^4$ cfu/ml in 2001; highest were $4.5x10^4$ cfu/ml of

Table 6b: Most probable number of *bacteria* in *sediments* of salt pans

Salt Pan	Culture Media	Counts of bacteria (cfu/g)																	
		Non Halophiles						Moderate Halophiles						Extreme Halophiles					
		Nutrient broth with 0% NaCl						*Halobacterium sodomense* with 12.5% crude solar salt						TYE with 25% crude solar salt					
	Year	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC
A	P	-	-	$3.2x10^3$	$7.2x10^5$	-	-	-	-	$4.1x10^4$	$4.3x10^5$	-	-	-	-	$3.6x10^5$	$1.1x10^6$	-	-
	C			$3.6x10^5$						$3.2x10^4$						$3.6x10^5$			
	B			$3.6x10^5$						$3.6x10^5$						$3.6x10^5$			
AgD	P	-	-	$3.5x10^4$	$7.6x10^5$	-	-	-	-	$3.2x10^4$	$4.4x10^5$	-	-	-	-	$3.6x10^5$	$1.1x10^6$	-	-
	C			$3.6x10^5$						$5.0x10^4$						$3.6x10^5$			
	B			$3.6x10^5$						$3.6x10^5$						$3.6x10^5$			
Ar_1T	P	-	-	$1.7x10^4$	$4.9x10^5$	-	-	-	-	$4.8x10^4$	$6.5x10^5$	-	-	-	-	$1.8x10^5$	$7.4x10^5$	-	-
	C			$1.1x10^5$						$2.4x10^5$						$3.6x10^5$			
	B			$3.6x10^5$						$3.6x10^5$						$3.6x10^5$			
N	P	-	-	$1.6x10^3$	$6.8x10^5$	-	-	-	-	$5.0x10^4$	$4.5x10^5$	-	-	-	-	$1.1x10^4$	$7.3x10^5$	-	-
	C			$3.2x10^5$						$4.0x10^4$						$3.6x10^5$			
	B			$3.6x10^5$						$3.6x10^5$						$3.6x10^5$			
R	P	$2.5x10^4$	$4.3x10^5$	$9.0x10^4$	$8.1x10^5$	$3.6x10^4$	$7.6x10^5$	$3.6x10^4$	$4.5x10^5$	$2.9x10^4$	$4.5x10^5$	$4.0x10^4$	$4.4x10^5$	$1.8x10^5$	$5.4x10^5$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$
	C	$9.0x10^4$		$3.6x10^5$		$3.6x10^5$		$5.0x10^4$		$6.4x10^4$		$4.4x10^4$		$1.8x10^5$		$3.6x10^5$		$3.6x10^5$	
	B	$3.1x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$1.8x10^5$		$3.6x10^5$		$3.6x10^5$	
S	P	$4.0x10^4$	$4.4x10^5$	$5.0x10^4$	$7.7x10^5$	$7.0x10^4$	$7.9x10^5$	$5.0x10^4$	$1.8x10^5$	$6.5x10^4$	$5.0x10^5$	$4.5x10^4$	$4.6x10^5$	$1.8x10^5$	$5.4x10^5$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$
	C	$1.6x10^4$		$3.6x10^5$		$3.6x10^5$		$5.3x10^4$		$7.1x10^4$		$5.3x10^4$		$1.8x10^5$		$3.6x10^5$		$3.6x10^5$	
	B	$3.8x10^5$		$3.6x10^5$		$3.6x10^5$		$8.0x10^4$		$3.6x10^5$		$3.6x10^5$		$1.8x10^5$		$3.6x10^5$		$3.6x10^5$	

TC-Total Count;--not done

Table 7a: Most probable counts of *haloarchaea* in *brine* of salt pans on nutrient rich haloarchaea specific media

ka	Salt pan	Culture media	Counts of haloarchaea (cfu/ml)																	
			NTYE						HS						PM					
		Year	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC
em	A	P			$3.6x10^5$						$7.5x10^2$						$3.6x10^5$			
		C	-	-	$3.6x10^5$	$1.1x10^6$	-	-	-	-	$5.5x10^3$	$4.2x10^4$	-	-	-	-	$3.6x10^5$	$1.1x10^6$	-	-
		B			$3.6x10^5$						$3.6x10^4$						$3.6x10^5$			
	AgD	P			$3.6x10^5$						$8.0x10^2$						$3.6x10^5$			
		C	-	-	$3.6x10^5$	$1.1x10^6$	-	-	-	-	$6.0x10^3$	$4.3x10^4$	-	-	-	-	$3.6x10^5$	$1.1x10^6$	-	-
		B			$3.6x10^5$						$3.6x10^4$						$3.6x10^5$			
lez	Ar_1T	P			$1.8x10^5$						$9.0x10^2$						$3.6x10^4$			
		C	-	-	$3.6x10^5$	$9.0x10^5$	-	-	-	-	$8.5x10^3$	$4.5x10^4$	-	-	-	-	$3.6x10^5$	$7.6x10^5$	-	-
		B			$3.6x10^5$						$3.6x10^4$						$3.6x10^5$			
	N	P			$1.1x10^4$						$9.0x10^2$						$1.5x10^4$			
		C	-	-	$3.6x10^5$	$7.3x10^5$	-	-	-	-	$7.0x10^3$	$4.4x10^4$	-	-	-	-	$3.6x10^5$	$7.4x10^5$	-	-
		B			$5.6x10^5$						$3.6x10^4$						$3.6x10^5$			
adi	R	P	$4.0x10^4$		$3.6x10^5$		$3.6x10^5$		$4.4x10^2$		$8.0x10^2$		$5.0x10^2$		$3.5x10^4$		$3.6x10^5$		$3.6x10^5$	
		C	$4.4x10^4$	$4.4x10^5$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$	$3.4x10^3$	$4.0x10^4$	$7.3x10^3$	$4.4x10^4$	$3.5x10^3$	$3.2x10^4$	$3.0x10^4$	$4.3x10^5$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$
		B	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^4$		$3.6x10^4$		$2.8x10^4$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
	S	P	$4.5x10^4$		$3.6x10^5$		$3.6x10^5$		$2.8x10^3$		$4.4x10^2$		$6.0x10^2$		$2.0x10^4$		$3.6x10^5$		$3.6x10^4$	
		C	$5.3x10^4$	$4.6x10^5$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$	$3.1x10^3$	$3.8x10^4$	$5.2x10^3$	$4.2x10^4$	$4.5x10^3$	$5.0x10^4$	$2.0x10^4$	$4.0x10^5$	$3.6x10^5$	$1.1x10^6$	$3.6x10^4$	$4.3x10^5$
		B	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.2x10^4$		$3.6x10^4$		$4.5x10^4$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	

TC-Total Count; --not done; Only column growth corresponding to $1.8x10^3$ cfu/ml and $3.4x10^2$ cfu/ml was obtained in NP medium for brine samples from Ar_1T and N salt pans.

Ar_1T salt pan and the lowest of $4.2x10^4$ cfu/ml in A and S salt pans in 2003. S salt pan gave higher counts of $5.0x10^4$cfu/ml than R salt pan in 2005. R salt pan showed $4.3x10^5$ cfu/ml counts of haloarchaea than S salt pan brine samples of 2001 in **PM medium**, while counts of $1.1x10^6$ cfu/ml were obtained for A, AgD, R and S salt pans in 2003; and R salt pan showed higher counts of $1.1x10^6$ cfu/ml than S salt pan in 2005. In **NP medium,** no haloarchaeal counts were obtained for R and S salt pans in 2001; A, AgD, R and S salt pans in 2003; while Ar_1T salt pan gave counts of $1.8x10^3$ cfu/ml and N salt pan gave $3.4x10^2$ cfu/ml in 2003. Also, no counts were obtained for R and S salt pans in 2005.

4. Most probable number of *haloarchaea* in *sediments* of salt pans

As seen in **Table 7b**, sediment samples inoculated into **nutrient rich media** showed luxuriant growth of haloarchaea in NTYE and PM than in HS and NP media. **NTYE** gave a count of $1.1x10^6$ cfu/g in 2001, 2003 and 2005 for R and S salt pans and for A, AgD which was monitored only in 2003. A lowest count of $7.3x10^5$ cfu/g was obtained for N salt pan in 2003. In **HS medium** haloarchaeal total MPN counts for R and S salt pans were nearly the same i.e. $4.3x10^5$ cfu/g to $4.5x10^5$ cfu/g in 2001. In 2003, counts obtained were highest for Ar_1T of $6.5x10^5$ cfu/g while lowest of $4.3x10^5$ cfu/g for A salt pan. R and S salt pans showed similar counts in 2005. Counts of haloarchaea obtained in **PM medium** were $1.1x10^6$cfu/g in all the salt pans through the years of 2001, 2003, 2005. Pellicle & bottom growers were absent for **NP medium**; haloarchaea were seen growing throughout the column of media, counts of which were $3.6x10^5$ cfu/g for Ar_1T and $3.2x10^5$ cfu/g for N salt pans in 2003.

Table 7b: Most probable number of *haloarchaea* in *sediments* of salt pans on nutrient rich haloarchaea specific media

			Counts of haloarchaea (cfu/g)																	
			Media																	
	Salt Pan	Culture Media	NTYE						HS						PM					
		Year	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC
	A	P			$3.6x10^5$						$4.1x10^4$						$3.6x10^3$			
		C	-	-	$3.6x10^5$	$1.1x10^6$	-	-	-	-	$3.2x10^4$	$4.3x10^5$	-	-	-	-	$3.6x10^5$	$1.1x10^6$	-	-
		B			$3.6x10^5$						$3.6x10^5$						$3.6x10^5$			
	AgD	P			$3.6x10^5$						$3.2x10^4$						$3.6x10^5$			
		C	-	-	$3.6x10^5$	$1.1x10^6$	-	-	-	-	$5.0x10^4$	$4.4x10^5$	-	-	-	-	$3.6x10^5$	$1.1x10^6$	-	-
		B			$3.6x10^5$						$3.6x10^5$						$3.6x10^5$			
	Ar_1T	P			$1.8x10^5$						$4.8x10^4$						$3.6x10^5$			
		C	-	-	$3.6x10^5$	$9.0x10^5$	-	-	-	-	$2.4x10^5$	$6.5x10^5$	-	-	-	-	$3.6x10^5$	$1.1x10^6$	-	-
		B			$3.6x10^5$						$3.6x10^5$						$3.6x10^5$			
	N	P			$1.1x10^4$						$5.0x10^4$						$3.6x10^5$			
		C	-	-	$3.6x10^5$	$7.3x10^5$	-	-	-	-	$4.0x10^4$	$4.5x10^5$	-	-	-	-	$3.6x10^5$	$1.1x10^6$	-	-
		B			$3.6x10^5$						$3.6x10^5$						$3.6x10^5$			
	R	P	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^4$		$2.9x10^4$		$4.0x10^4$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
		C	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$	$5.0x10^4$	$4.5x10^5$	$6.4x10^4$	$4.5x10^5$	$4.4x10^4$	$4.4x10^5$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$
		B	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
	S	P	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$5.0x10^4$		$6.5x10^4$		$4.5x10^4$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
		C	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$	$5.3x10^4$	$4.3x10^5$	$7.1x10^4$	$5.0x10^5$	$5.3x10^4$	$4.6x10^5$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$	$3.6x10^5$	$1.1x10^6$
		B	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$8.0x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	

TC-Total Count; -- Not done; Only column growth corresponding to $3.6x10^5$ cfu/g and $3.2x10^5$ cfu/g was obtained in NP medium for brine samples from Ar_1T and N salt pans.

5. Total viable counts of *halobacteria* in *brine* and *sediment* samples of salt pans on different media

Pigmented colonies were obtained on nutrient rich media -**NTYE**, pH 5; **PM**; **HS**, both at pH 7 and NP, pH 10.5 and synthetic media containing casein as a single carbon source **(Table 8)**. In **PM medium,** total viable counts of $4.6x10^9$ cfu/ml of red colonies in brine and $3.3x10^9$ cfu/g of colourless, white, cream and yellow colonies in sediment of Arambol salt pan were obtained. Sediment of AgB sub-salt pan of Ag gave $1.3x10^9$ cfu/g of orange colonies and $3.9x10^9$ cfu/ml of white, cream and orange colonies in brine. AgC sub-salt pan of Ag gave $2.9x10^{10}$ cfu/ml of white, orange colonies in brine, while counts of $4.9x10^{10}$ cfu/g of cream, yellow, peach and red colonies were obtained in sediment. AgD sub-salt pan yielded $3.6x10^{10}$ cfu/ml of peach, orange, red colonies in brine, while, $1.6x10^{10}$ cfu/g of cream and orange colonies in sediment. Counts of $3.4x10^{10}$ cfu/ml of white, cream, orange and red colonies in brine; $3.0x10^{10}$ cfu/g of white, cream, yellow, peach and orange colonies in sediment of AgP sub-salt pan of Ag were obtained. Brine of AgS sub-salt pan of Ag gave counts of $1.3x10^9$ cfu/ml of orange, and $3.2x10^9$ cfu/g of peach coloured colonies in sediment. Ar_1T sub-salt pan of Ar gave $7.7x10^9$ cfu/ml counts of white, peach, orange colonies in brine and $5.6x10^9$ cfu/g of white, cream, peach and orange colonies in sediment. Counts of $6.2x10^9$ cfu/ml of cream, yellow, orange colonies in brine, while $8.3x10^9$ cfu/g of peach colonies were obtained in sediment of Ar_2T sub-salt pan of Ar. N salt pan gave $1.4x10^{10}$ cfu/ml of white, yellow, peach colonies in brine and $8.9x10^{10}$cfu/g of cream, orange colonies in sediment. Counts of $2.9x10^{10}$ cfu/ml of white, orange colonies in brine, while $1.2x10^{10}$ cfu/g of white, cream, yellow, peach and orange colonies in sediment of R salt pan were obtained. Brine of S salt pan gave $8.0x10^6$ cfu/ml of yellow, peach colonies, while sediment gave $1.6x10^{10}$ cfu/g of white, peach and red colonies and on **HS agar,** viable counts of $1.1x10^9$ cfu/ml of peach

Table 8: Total viable counts of *haloarchaea* in *brine* and *sediment* of salt pans cultured on different nutrient rich media

Taluka	Samples from salt pan			Counts of bacteria in PM media (cfu/ml OR cfu/g)								
				Colorless	White	Cream	Yellow	Peach	Orange	Red	Brown	TC
Pernem	A		b	-	-	-	-	-	-	$4.6x10^{9}$		$4.6x10^{9}$
			s	$5.0x10^{8}$	$6.0x10^{8}$	$1.1x10^{9}$	$1.1x10^{9}$	-	-	-		$3.3x10^{9}$
	Ag	AgB	b	-	$2.4x10^{9}$	$3.0x10^{8}$	-	-	$3.6x10^{10}$	-		$3.9x10^{10}$
			s	-	-	-	-	-	$1.3x10^{9}$	-		$1.3x10^{9}$
		AgC	b	-	$4.6x10^{9}$	-	-	-	$2.4x10^{10}$	-		$2.9x10^{10}$
			s	-	-	$2.0x10^{8}$	$3.0x10^{8}$	$1.0x10^{8}$	-	$4.8x10^{10}$		$4.9x10^{10}$
		AgD	b	-	-	-	-	$6.2x10^{9}$	$1.9x10^{10}$	$1.1x10^{10}$		$3.6x10^{10}$
			s	-	-	$1.4x10^{10}$	-	-	$1.7x10^{9}$	-		$1.6x10^{10}$
		AgP	b	-	$2.6x10^{9}$	$1.9x10^{9}$	-	-	$2.8x10^{10}$	$1.8x10^{9}$		$3.4x10^{10}$
			s	-	$2.5x10^{10}$	$2.0x10^{8}$	$1.0x10^{9}$	$2.0x10^{8}$	$3.9x10^{9}$	-		$3.0x10^{10}$
		AgS	b	-	-	-	-	-	$1.3x10^{9}$	-		$1.3x10^{9}$
			s	-	-	-	-	$3.2x10^{9}$	-	-		$3.2x10^{9}$
Bardez	Ar	Ar_1T	b	-	$6.0x10^{8}$ $6.5x10^{7}$	-	-	$4.0x10^{8}$ $1.1x10^{9}$ $1.1x10^{8}$	$6.7x10^{9}$ $9.4x10^{7}$	-		$7.7x10^{9}$ $1.1x10^{9}$ $2.7x10^{8}$
			s	-	$1.7x10^{9}$ $7.6x10^{7}$ $6.8x10^{8}$	$1.0x10^{8}$	-	$6.0x10^{8}$ $6.5x10^{7}$	$3.2x10^{9}$ $1.0x10^{6}$ $7.0x10^{6}$	-		$5.6x10^{9}$ $7.7x10^{7}$ $7.5x10^{8}$
		Ar_2T	b	-	- $1.8x10^{7}$	$4.5x10^{9}$	$8.0x10^{8}$	- $7.2x10^{8}$	$9.0x10^{8}$ $2.8x10^{7}$	-		$6.2x10^{9}$ $7.2x10^{8}$ $4.6x10^{7}$
			s	-	-	-	-	$8.3x10^{9}$	- $6.0x10^{8}$ $2.5x10^{7}$	-		$8.3x10^{9}$ $6.0x10^{8}$ $2.5x10^{7}$
	N		b	-	$1.9x10^{9}$ $3.6x10^{7}$	-	$4.0x10^{8}$	$4.0x10^{9}$ $3.8x10^{8}$ $2.8x10^{8}$	-	-		$1.4x10^{10}$ $4.2x10^{8}$ $2.8x10^{8}$
			s	-	- $3.4x10^{8}$ $1.1x10^{8}$	$1.5x10^{9}$ $1.0x10^{6}$	-	- $4.7x10^{7}$	$8.7x10^{10}$ $3.4x10^{8}$ $3.2x10^{7}$	- $3.6x10^{7}$		$8.9x10^{10}$ $7.6x10^{8}$ $3.2x10^{7}$
Tiswadi	R		b	-	$2.0x10^{10}$	-	- $6.0x10^{6}$	- $8.0x10^{6}$	$9.3x10^{9}$ $1.8x10^{7}$ $4.0x10^{6}$	-		$2.9x10^{10}$ $2.4x10^{7}$ $1.2x10^{7}$
			s	-	$1.2x10^{9}$ $7.6x10^{7}$	$4.0x10^{9}$	$7.0x10^{8}$ $1.4x10^{7}$	$2.8x10^{9}$ $1.0x10^{6}$ $1.7x10^{8}$	$3.4x10^{9}$ $1.0x10^{6}$ $2.2x10^{7}$	-		$1.2x10^{10}$ $9.2x10^{7}$ $1.9x10^{8}$
	S		b	-	- $1.8x10^{7}$ $3.0x10^{9}$	- $1.8x10^{11}$	$7.0x10^{6}$ $3.4x10^{10}$	$1.0x10^{6}$ $1.1x10^{9}$ $1.1x10^{10}$	- $2.8x10^{7}$	-	- $7.1x10^{10}$	$8.0x10^{6}$ $1.1x10^{9}$ $4.6x10^{7}$ $7.5x10^{10}$ $3.7x10^{10}$
			s	-	$6.1x10^{9}$ $4.2x10^{7}$ $2.0x10^{10}$	- $4.0x10^{9}$	-	$2.9x10^{9}$ $1.0x10^{9}$ $1.6x10^{8}$	-	$7.2x10^{9}$ $1.0x10^{11}$ $1.0x10^{9}$	- $2.8x10^{11}$	$1.6x10^{10}$ $1.0x10^{9}$ $2.0x10^{8}$ $1.9x10^{11}$ $8.3x10^{9}$

Total viable counts on various media are indicated as: printed in blue-HS; red-NCSM; purple-NP, pH 10.5; green-NTYE, pH 5;
TC-Total Count; --No counts; b–brine; s–sediment

colonies in brine, while $7.7x10^6$ cfu/g of white and orange colonies in sediment of Ar_1T sub-salt pan and $7.2x10^8$ cfu/ml of peach colonies in brine and $6.0x10^8$ cfu/g of orange colonies in sediment of Ar_2T sub-salt pan of Ar were obtained. Brine of N salt pan brine gave $4.2x10^8$ cfu/ml of white and peach coloured colonies, and sediment gave counts of $7.6x10^8$ cfu/g of white, peach, orange and red colonies. Viable counts of $2.4x10^7$ cfu/ml of yellow, orange colonies in brine and $9.2x10^7$ cfu/g of white, yellow, peach and orange colonies were obtained in sediment of R salt pan. Counts obtained in brine and in sediment of S salt pan were nearly the same i.e. $1.0x10^9$ cfu/ml/cfu/g of peach colonies.

Viable counts on **NCSM** gave $2.7x10^8$ cfu/ml of white, peach and orange colonies in brine of Ar_1T sub-salt pan, while $4.6x10^7$ cfu/ml of white, orange colonies in brine of Ar_2T sub-salt pan of Ar; $2.8x10^8$ cfu/ml of peach colonies in brine of N salt pan. Brine gave $1.2x10^7$ cfu/ml of peach, orange colonies and sediment gave $1.9x10^8$ cfu/g of peach, orange colonies for R salt pan. S salt pan gave counts of $4.6x10^7$ cfu/ml of white, orange colonies in brine.

6. Total viable counts of fungi on different media

Total viable counts of fungi in brine & sediment of salt pan were obtained on nutrient rich media as NTYE, HS and NA media at pH 7.0 and synthetic media with glucose as a sole source of carbon. As seen in **Table 9**, **NTYE** yielded a total viable count of $1.5x10^3$ cfu/ml for A salt pan, $2.0x10^3$ cfu/g for AgD salt pan, $4.0x10^3$ cfu/ml for Ar_1T salt pan, $1.0x10^3$ cfu/ml for N salt pan, $5.0x10^3$ cfu/g for R salt pan and $3.0x10^3$ cfu/ml for S salt pan, while in **HS**, counts of $9.8x10^7$ cfu/ml were obtained for N salt pan. A, AgD, Ar_1T, R and S salt pans did not give any counts. On **NA**, counts of $3.0x10^3$ cfu/ml, $2.5x10^3$ cfu/g, $3.5x10^3$ cfu/ml, $5.0x10^3$ cfu/ml, $7.2x10^3$ cfu/g and $6.3x10^3$ cfu/ml were obtained for A, AgD, Ar_1T, N, R, S salt pans respectively. **NGSM** yielded counts of $1.7x10^4$ cfu/ml, $1.0x10^3$ cfu/g, $1.7x10^4$

cfu/ml, $2.4x10^4$ cfu/ml, $1.1x10^4$ cfu/g and $1.6x10^4$ cfu/ml for A, AgD , Ar_1T, R & S salt pans respectively.

Table 9: Total viable counts of *fungi* on different media at pH 7.0

Taluka	Salt pan		Counts of fungi in brine (cfu/ml) and sediment (cfu/g)			
			Culture media with 20 % crude solar salt			
			Nutrient rich media			Synthetic media
			NTYE	HS	NA*	NGSM
Pernem	A	b	$1.5x10^3$	0	$3.0x10^3$	$1.7x10^4$
	AgD	s	$2.0x10^3$	0	$2.5x10^3$	$1.0x10^4$
Bardez	Ar_1T	b	$4.0x10^3$	0	$3.5x10^3$	$1.7x10^4$
	N	b	$1.0x10^3$	$9.8x10^7$	$5.0x10^3$	$2.4x10^4$
Tiswadi	R	s	$5.0x10^3$	0	$7.2x10^3$	$1.1x10^4$
	S	b	$3.0x10^3$	0	$6.3x10^3$	$1.6x10^4$

*** Medium containing 0.5 % NaCl; b- brine s- sediment**

IV. ISOLATION & PURIFICATION OF MICROBES FROM SALT PANS

Two hundred and thirty-four pure isolates designated as $GUFF_1$ through $GUFF_{234}$ were obtained on various nutrient rich media pH 5/7/pH 10.5, in NTYE, HS, NP, PM and NA and also those obtained on synthetic media, i.e. NSM, NGSM, NCSM, NSSM, $NSMN^-$, NG(Fe)SM and NG(Mn)SM are maintained as pure cultures.

1. Bacterial cultures from different salt pans of Goa isolated on various media

Diverse bacterial isolates obtained from brine, sediment and water samples of different salt pans of Goa, have been compiled as **Table 10**. Three isolates from brine and 1 from sediment of A salt pan; 3 each from brine & sediment of Ag & Ar salt pans; 3 from brine and 2 from sediment of N salt pan; 3 from water, 2 from brine and 6 from sediment of R salt pan; 1 from

Table 10: Halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa isolated on different media, available at Haloarchaeal Repository (HRHLDMGU)

Isolation Media	pH	Salt pans (Habitat)					
		A	Ag	Ar	N	R	S
NTYE	7.0	$GUFF_{41}$, $GUFF_{42}$, $GUFF_{43}$, $GUFF_{44}$	$GUFF_{34}$, $GUFF_{35}$, $GUFF_{37}$, $GUFF_{38}$, $GUFF_{39}$, $GUFF_{40}$	$GUFF_{19}$, $GUFF_{20}$, $GUFF_{22}$, $GUFF_{23}$, $GUFF_{25}$, $GUFF_{26}$	$GUFF_{27}$, $GUFF_{28}$, $GUFF_{30}$, $GUFF_{32}$, $GUFF_{33}$	$GUFF_{1}$, $GUFF_{2}$, $GUFF_{3}$, $GUFF_{4}$, $GUFF_{5}$, $GUFF_{7}$, $GUFF_{8}$, $GUFF_{11}$, $GUFF_{15}$, $GUFF_{17}$, $GUFF_{21}$	$GUFF_{6}$, $GUFF_{9}$, $GUFF_{10}$, $GUFF_{12}$, $GUFF_{13}$, $GUFF_{14}$, $GUFF_{16}$, $GUFF_{18}$, $GUFF_{24}$, $GUFF_{29}$, $GUFF_{31}$, $GUFF_{36}$
	5.0	-	-	-	-	-	$GUFF_{224}$, $GUFF_{225}$, $GUFF_{226}$, $GUFF_{227}$, $GUFF_{234}$
NP	8.5	-	-	$GUFF_{67}$, $GUFF_{68}$	$GUFF_{69}$	-	-
	10.5	-	-	-	-	-	$GUFF_{228}$, $GUFF_{229}$, $GUFF_{230}$, $GUFF_{231}$, $GUFF_{232}$, $GUFF_{233}$,
HS	7.0	$GUFF_{54}$, $GUFF_{55}$	$GUFF_{58}$, $GUFF_{59}$	$GUFF_{51}$, $GUFF_{52}$, $GUFF_{53}$	$GUFF_{56}$, $GUFF_{57}$	$GUFF_{45}$, $GUFF_{46}$, $GUFF_{47}$, $GUFF_{60}$, $GUFF_{61}$	$GUFF_{48}$, $GUFF_{49}$, $GUFF_{50}$, $GUFF_{62}$, $GUFF_{63}$, $GUFF_{64}$, $GUFF_{65}$, $GUFF_{66}$
PM	7.0	$GUFF_{79}$, $GUFF_{94}$, $GUFF_{95}$, $GUFF_{96}$, $GUFF_{107}$	$GUFF_{86}$, $GUFF_{87}$, $GUFF_{88}$, $GUFF_{89}$, $GUFF_{90}$, $GUFF_{91}$, $GUFF_{92}$, $GUFF_{93}$	$GUFF_{80}$, $GUFF_{81}$, $GUFF_{97}$, $GUFF_{98}$, $GUFF_{99}$, $GUFF_{100}$, $GUFF_{101}$, $GUFF_{102}$, $GUFF_{103}$,	$GUFF_{82}$, $GUFF_{83}$, $GUFF_{84}$, $GUFF_{85}$, $GUFF_{104}$, $GUFF_{105}$, $GUFF_{106}$	$GUFF_{70}$, $GUFF_{71}$, $GUFF_{72}$, $GUFF_{73}$, $GUFF_{74}$, $GUFF_{110}$, $GUFF_{112}$	$GUFF_{75}$, $GUFF_{76}$, $GUFF_{77}$, $GUFF_{78}$, $GUFF_{108}$, $GUFF_{109}$, $GUFF_{111}$, $GUFF_{113}$
NSM	7.0	-	-	-	-	$GUFF_{C190}$	$GUFF_{C191}$, $GUFF_{C192}$

A-Arambol, Ag-Agarwada, Ar-Arpora, N-Nerul, R-Ribandar, S-Siridao
Habitats: Brine-b, Sediment-s, Water-w
Isolation season: salt farming; monsoon; post monsoon
*HRHLDMGU-Haloarchaeal Repository, Haloarchaeal Laboratory, Department of Microbiology, Goa University, Taleigao Plateau, Goa, India-403 203

Table 10: Halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa isolated on different media, available at Haloarchaeal Repository (HRHLDMGU)

Isolation Media	pH	Salt pans (Habitat)					
		A	Ag	Ar	N	R	S
NSAM	7.0	$GUFF_{180}$, $GUFF_{181}$	$GUFF_{186}$, $GUFF_{187}$, $GUFF_{188}$, $GUFF_{189}$	$GUFF_{177}$, $GUFF_{178}$, $GUFF_{179}$	$GUFF_{182}$, $GUFF_{184}$, $GUFF_{185}$	$GUFF_{168}$, $GUFF_{169}$, $GUFF_{170}$, $GUFF_{171}$, $GUFF_{172}$	$GUFF_{173}$, $GUFF_{174}$, $GUFF_{175}$, $GUFF_{176}$, $GUFF_{183}$
NGSMN	7.0	-	-	-	-	$GUFF_{193}$, $GUFF_{194}$, $GUFF_{195}$	-
NGSM	7.0	-	-	$GUFF_{114}$, $GUFF_{115}$	$GUFF_{116}$, $GUFF_{117}$, $GUFF_{118}$	-	-
NSSM	7.0	$GUFF_{161}$	$GUFF_{164}$, $GUFF_{165}$	$GUFF_{158}$, $GUFF_{160}$	$GUFF_{162}$, $GUFF_{166}$	$GUFF_{156}$, $GUFF_{159}$, $GUFF_{167}$	$GUFF_{157}$, $GUFF_{163}$
NCSM	7.0	$GUFF_{152}$, $GUFF_{153}$, $GUFF_{155}$	$GUFF_{146}$, $GUFF_{147}$, $GUFF_{148}$, $GUFF_{149}$	$GUFF_{134}$, $GUFF_{135}$, $GUFF_{136}$, $GUFF_{137}$, $GUFF_{138}$, $GUFF_{139}$	$GUFF_{140}$, $GUFF_{141}$, $GUFF_{142}$, $GUFF_{143}$, $GUFF_{144}$, $GUFF_{145}$	$GUFF_{119}$, $GUFF_{120}$, $GUFF_{121}$, $GUFF_{122}$, $GUFF_{124}$, $GUFF_{125}$, $GUFF_{127}$, $GUFF_{128}$, $GUFF_{150}$,	$GUFF_{123}$, $GUFF_{126}$, $GUFF_{129}$, $GUFF_{130}$, $GUFF_{131}$, $GUFF_{132}$, $GUFF_{133}$, $GUFF_{151}$, $GUFF_{154}$
NG(Fe)SM	7.0	-	-	$GUFF_{199}$, $GUFF_{200}$, $GUFF_{201}$	$GUFF_{202}$, $GUFF_{203}$	$GUFF_{196}$, $GUFF_{197}$	$GUFF_{198}$
NG(Mn)SM	7.0	-	-	$GUFF_{205}$	$GUFF_{206}$, $GUFF_{207}$	$GUFF_{204}$	$GUFF_{208}$, $GUFF_{209}$
NA	7.0	-	-	-	-	$GUEFF_{1}$, $GUEFF_{2}$, $GUEFF_{3}$	$GUEFF_{4}$, $GUEFF_{5}$, $GUEFF_{6}$
MacConkey's 0.5% NaCl, *20% NaCl	7.0	-	-	-	-	$GUEFF_{7}$, $GUEFF_{8}$, *$GUEFF_{10}$, *$GUEFF_{11}$	$GUEFF_{9}$, *$GUEFF_{12}$, *$GUEFF_{13}$, *$GUEFF_{14}$

A-Arambol, Ag-Agarwada, Ar-Arpora, N-Nerul, R-Ribandar, S-Siridao
Habitats: Brine-b, Sediment-s, Water-w
Isolation season: salt farming; monsoon; post monsoon
***HRHLDMGU-Haloarchaeal Repository, Haloarchaeal Laboratory, Department of Microbiology, Goa University, Taleigao Plateau, Goa, India-403 203**

water, 3 from brine and 8 from sediment of S salt pan, were obtained on NTYE media, pH 7.0. Five isolates were obtained from sediment of S salt pan on NTYE media, pH 5.0. Further, two isolates, one each from brine & sediment of Ar salt pan and 1 from brine of N salt pan were obtained on NP at pH 8.5; while 6 isolates were obtained from sediment of S salt pan at pH 10.5. The isolates obtained from various salt pans on different media of varying pH were studied for their colony characteristics as indicated in **Table 11**.

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_1$	Orange	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	+ve Pleiomorphic
$GUFF_2$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_3$	Yellow	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_4$	Orange	0.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_5$	Orange	Pinpoint	Circular	Smooth	Raised	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_6$	Orange	1	Circular	Smooth	Raised	Butyrous	Opaque	+ve Cocci
$GUFF_7$	Yellow	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_8$	Orange	1	Circular	Smooth	Raised	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_9$	Cream	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{10}$	Peach	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{11}$	Yellow	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{12}$	Cream	Pinpoint	Circular	Smooth	Raised	Butyrous	Opaque	-ve Cocci
$GUFF_{13}$	Orange	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	+ve Pleiomorphic
$GUFF_{14}$	Orange	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{15}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{16}$	Orange	1.0	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{17}$	Orange	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{18}$	Red	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{19}$	Yellow	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{20}$	Yellow	Pinpoint	Circular	Smooth	Raised	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{21}$	Cream	Pinpoint	Circular	Smooth	Raised	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{22}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{23}$	Red	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{24}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_{25}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{26}$	Yellow	1	Circular	Smooth	Convex	Butyrous	Opaque	+ve Pleiomorphic
$GUFF_{27}$	Red	1	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{28}$	Yellow	Pinpoint	Circular	Smooth	Raised	Butyrous	Opaque	-ve Cocci
$GUFF_{29}$	Yellow	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{30}$	Orange	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{31}$	Yellow	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{32}$	Yellow	1	Circular	Smooth	Raised	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{33}$	Yellow	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{34}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{35}$	Yellow	Pinpoint	Circular	Smooth	Convex	Dry	Opaque	-ve Rods
$GUFF_{36}$	Orange	1	Circular	Smooth	Raised	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{37}$	Orange	1.5	Circular	Smooth	Convex	Dry	Opaque	-ve Pleiomorphic
$GUFF_{38}$	Orange	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{39}$	Red	1	Circular	Smooth	Raised	Butyrous	Opaque	-ve Cocci
$GUFF_{40}$	Yellow	1	Circular	Smooth	Raised	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{41}$	Red	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	+ve Pleiomorphic
$GUFF_{42}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	+ve Pleiomorphic
$GUFF_{43}$	Orange	2	Circular	Smooth	Raised	Butyrous	Opaque	+ve Pleiomorphic
$GUFF_{44}$	Red	1.5	Circular	Smooth	Convex	Butyrous	Opaque	+ve Pleiomorphic
$GUFF_{45}$	Orange	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{46}$	Peach	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{47}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{48}$	Peach	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{49}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_{50}$	Peach	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{51}$	Peach	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{52}$	Orange	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{53}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{54}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{55}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{56}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{57}$	Red	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{58}$	Orange	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{59}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{60}$	Peach	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{61}$	Orange	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{62}$	Peach	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{63}$	Yellow	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{64}$	White	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{65}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{66}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{67}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{68}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{69}$	Orange	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{70}$	Pink	2	Circular	Smooth	Raised	Slimy	Opaque	+ve Cocci
$GUFF_{71}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	+ve Rods
$GUFF_{72}$	Red	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{73}$	Red	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{74}$	Red	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_{75}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{76}$	Cream	1	Circular	Smooth	Raised	Dry	Opaque	-ve Cocci
$GUFF_{77}$	Orange	1	Circular	Smooth	Raised	Dry	Opaque	-ve Cocci
$GUFF_{78}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{79}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{80}$	Orange	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{81}$	Cream	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{82}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{83}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{84}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{85}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{86}$	Yellow	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{87}$	Cream	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{88}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{89}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{90}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{91}$	Peach	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{92}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{93}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{94}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{95}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{96}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{97}$	Cream	2	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{98}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{99}$	Peach	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_{100}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{101}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{102}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{103}$	Yellow	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{104}$	Red	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{105}$	Pink	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{106}$	Cream	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{107}$	Peach	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{108}$	Cream	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{109}$	Cream	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{110}$	Cream	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{111}$	Peach	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{112}$	Pink	2	Circular	Smooth	Raised	V. Slimy	Opaque	-ve Cocci
$GUFF_{113}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{114}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{115}$	Cream	1.5	Circular	Smooth	Convex	Dry	Opaque	-ve Cocci
$GUFF_{116}$	Red	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{117}$	Red	1.5	Circular	Smooth	Convex	Dry	Opaque	-ve Cocci
$GUFF_{118}$	Peach	1	Circular	Smooth	Raised	Butyrous	Opaque	-ve Cocci
$GUFF_{119}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{120}$	Yellow	1	Circular	Smooth	Convex	Dry	Opaque	-ve Cocci
$GUFF_{121}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{122}$	Orange	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{123}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{124}$	Cream	1	Circular	Smooth	Convex	Dry	Opaque	-ve Cocci

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_{125}$	Orange	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{126}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{127}$	Orange	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{128}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{129}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{130}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{131}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{132}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{133}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{134}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{135}$	Peach	Pin point	Circular	Smooth	Convex	Dry	Opaque	-ve Cocci
$GUFF_{136}$	Orange	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{137}$	Orange	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{138}$	Orange	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{139}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{140}$	Peach	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{141}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{142}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{143}$	Cream	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{144}$	Peach	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{145}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{146}$	Red	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{147}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{148}$	Peach	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{149}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_{150}$	Peach	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{151}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{152}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{153}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{154}$	Peach	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{155}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{156}$	Peach	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{157}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{158}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{159}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{160}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{161}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{162}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{163}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{164}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{165}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{166}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{167}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{168}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{169}$	Peach	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{170}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{171}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{172}$	Yellow	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{173}$	Peach	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_{174}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{175}$	Pink	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{176}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{177}$	Red	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{178}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{179}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{180}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{181}$	Red	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{182}$	Peach	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{183}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{160}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{184}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{185}$	Peach	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{186}$	Peach	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{187}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{188}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{189}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Squares
$GUFF_{190}$	Green	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Spheres
$GUFF_{191}$	Green	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Spheres
$GUFF_{192}$	Green	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Spheres
$GUFF_{193}$	Pink	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{194}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{195}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{196}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{197}$	Cream	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_{198}$	Peach	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{199}$	White	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{200}$	Orange	Pin Point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{201}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{202}$	Orange	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{203}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{204}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{205}$	Orange	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{206}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Pleiomorphic
$GUFF_{207}$	Orange	1.5	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{208}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{209}$	White	2	Circular	Smooth	Raised	Mucoid	Opaque	-ve Cocci
$GUFF_{210}$	Orange	Pin point	Circular	Smooth	Convex	Dry	Opaque	+ve Cocci
$GUFF_{211}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{212}$	Yellow	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{213}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{214}$	Pale orange	Pin point	Circular	Smooth	Convex	Dry	Opaque	+ve Cocci
$GUFF_{215}$	White	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	+ve Cocci
$GUFF_{216}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{217}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{218}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{219}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{220}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{221}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods

Table 11: Colony characteristics of halophilic bacterial cultures (Haloarchaea & Eubacteria) from different salt pans of Goa

Isolate	Pigmentation	Size (mm)	Shape	Margin	Elevation	Consistency	Opacity	Gram character/ Morphology
$GUFF_{222}$	Cream	Pin point	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{223}$	Cream	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{224}$	Brown with metallic sheen	1	Circular	Smooth	Convex	Dry	Opaque	-ve Pleiomorphic
$GUFF_{225}$	White	2	Circular	Smooth	Flat	Butyrous	Opaque	-ve Rods
$GUFF_{226}$	Cream	2.5	Circular	Smooth	Flat	Butyrous	Opaque	-ve Rods
$GUFF_{227}$	White	4	Circular	Smooth	Raised	Mucoid	Opaque	-ve Pleiomorphic
$GUFF_{228}$	White	Pinpoint	Circular	Smooth	Flat	Dry	Opaque	-ve Rods
$GUFF_{229}$	Peach	4	Circular	Smooth	Raised	Mucoid	Opaque	-ve Rods
$GUFF_{230}$	Cream with brown centre	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{231}$	Cream	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Rods
$GUFF_{232}$	Light brown	1	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{233}$	Red	Pinpoint	Circular	Smooth	Convex	Butyrous	Opaque	-ve Cocci
$GUFF_{234}$	Red	Pinpoint	Circular	Smooth	Flat	Butyrous	Opaque	-ve Rods

Furthermore in **HS** medium, single isolate was obtained from brine and sediment of A, Ag and N salt pans; 2 from brine and 1 from sediment of Ar salt pan; 2 each from water & brine and 1 from sediment of R salt pan while 2 each from water & brine and 4 from sediment of S salt pan were obtained. **PM** gave two isolates from brine and 3 from sediment of A salt pan; 3 isolates from brine and 5 from sediment of Ag salt pan; 4 isolates from brine and 5 from sediment of Ar salt pan were obtained. N salt pan gave 4 isolates from brine and 3 isolates from sediment. 2 isolates from water, 3 from brine and 2 from sediment; while 1 isolate from water, 3 isolates from brine and 4 from sediment of R & S salt pans were obtained.

2. Fungal isolates

Fungal isolates growing on different media were isolated and purified by sub-culturing and designated as GUFFf$_1$ through GUFFf$_{42}$ and maintained on NMEA in HLDMGU laboratory repository and deposited in GUFCDB repository under the Accession numbers GFCC 14601 through 14640.

As seen in **Table 12**, NTYE medium gave two isolates–one from brine and sediment of A salt pan; one isolate from sediment of N salt pan and one from sediment of R salt pan were obtained. Only one isolate was obtained on **HS** from brine, sediment of Ar salt pan. No isolates were obtained either from brine or from sediment of A, Ag, N, R and S salt pans on HS ; while **NA** gave one isolate from brine sample of Ag salt pan ; 1 each from sediment of Ar salt pan and N salt pan ; 1 each from water, brine and sediment of R salt pan and 1 from sediment of S salt pan were obtained.

Brine samples inoculated on MEA containing 20% crude solar salt, under anaerobic conditions interestingly grew as white fluffy mat **(Plate 3)**.

Table 12: Halophilic fungal cultures from different salt pans of Goa isolated on different media

Isolation Media pH 7.0	Salt pans (Habitat)					
	A	Ag	Ar	N	R	S
NTYE	GUFFf$_{38}$ (GFCC14638), GUFFf$_{39}$ (GFCC14639)	-	-	GUFFf$_{37}$(GFCC14637)	GUFFf$_{40}$ (GFCC14640), GUFFf$_{41}$	-
HS	-	-	GUFFf$_{20}$ (GFCC14620)	-	-	-
NA	-	GUFFf$_{17}$(GFCC14617)	GUFFf$_{18}$ (GFCC14618)	GUFFf$_{19}$(GFCC14619)	GUFFf$_{13}$ (GFCC14613), GUFFf$_{14}$ (GFCC14614), GUFFf$_{16}$ (GFCC14616)	GUFFf$_{15}$(GFCC14615)
NGSM	-	GUFFf$_{35}$(GFCC14635)	GUFFf$_{36}$ (GFCC14636)	-	GUFFf$_{30}$ (GFCC14630), GUFFf$_{31}$(GFCC14631), GUFFf$_{33}$ (GFCC14633)	GUFFf$_{32}$(GFCC14632), GUFFf$_{34}$(GFCC14634)
NSSM	GUFFf$_{7}$ (GFCC14607)	GUFFf$_{6}$ (GFCC14606)	GUFFf$_{8}$ (GFCC14608)	GUFFf$_{9}$ (GFCC14609)	GUFFf$_{1}$(GFCC14601), GUFFf$_{2}$(GFCC14602), GUFFf$_{4}$(GFCC14604), GUFFf$_{10}$ (GFCC14610), GUFFf$_{11}$ (GFCC14611)	GUFFf$_{3}$(GFCC14603), GUFFf$_{5}$(GFCC14605), GUFFf$_{12}$(GFCC14612)
NCSM	-	-	GUFFf$_{21}$ (GFCC14621)	GUFFf$_{22}$(GFCC14622)	-	-
NG(Fe)SM	-	-	-	-	GUFFf$_{28}$ (GFCC14628)	GUFFf$_{29}$(GFCC14629)
NG(Mn)SM	-	-	GUFFf$_{25}$ (GFCC14625) GUFFf$_{26}$ (GFCC14626)	GUFFf$_{27}$(GFCC14627)	GUFFf$_{23}$ (GFCC14623)	GUFFf$_{24}$(GFCC14624) *GUFFf$_{42}$
Total isolates	3	3	7	5	15	9

Isolation season: salt farming; monsoon; post monsoon

[3] Fungal isolates obtained under anaerobic conditions of growth

[4] Fungal culture with single cell morphology

***NMEA, pH 5.5**

A-Arambol, Ag-Agarwada, Ar-Arpora, N-Nerul, R-Ribandar, S-Siridao

Habitats: Brine-b, Sediment-s, Water-w

GUFCDB – Goa University Fungal Collection Laboratory, Department of Botany

***HRHLDMGU-Haloarchaeal Repository, Haloarchaeal Laboratory, Department of Microbiology, Goa University, Taleigao Plateau, Goa, India-403203**

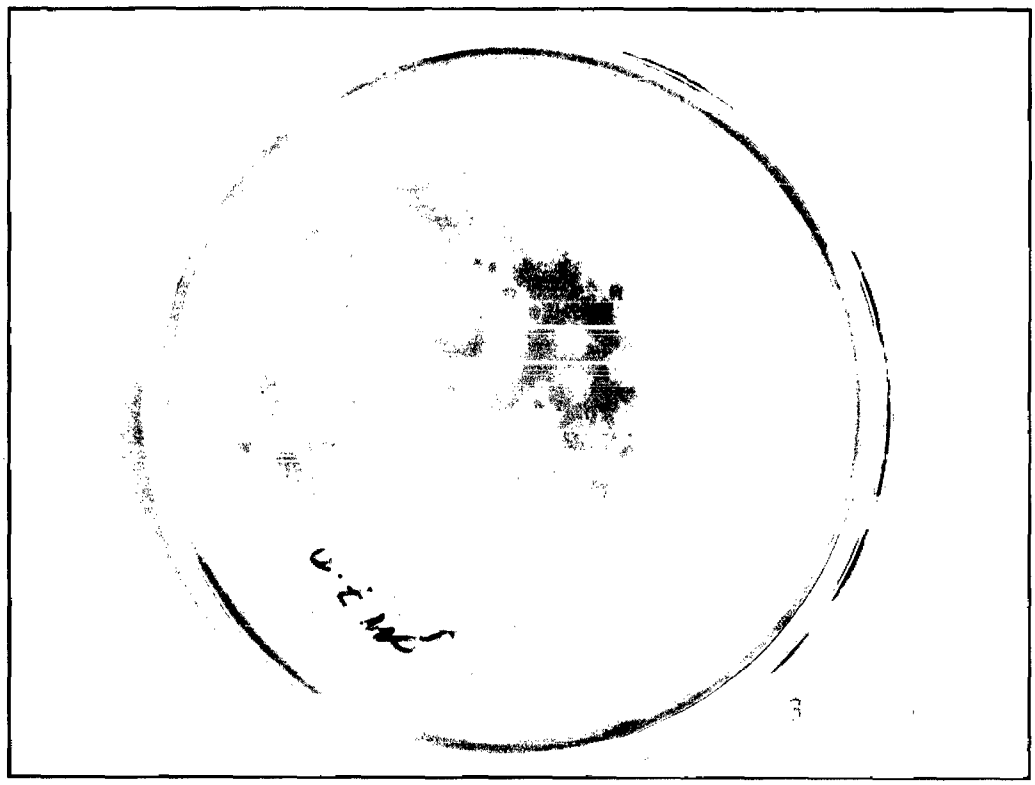


Plate 3: Halophilic fungi growing on NMEA under anaerobic conditions (Gas Pak anaerobic system, HiMedia)

V. IDENTIFICATION AND CHARACTERISATION OF ISOLATED FUNGAL CULTURES

i) Growth of fungal isolates with and without salt

Of the forty-two fungal isolates, listed in **Table 12,** 18 isolates namely, $GUFFf_2$, $GUFFf_3$, $GUFFf_4$, $GUFFf_5$, $GUFFf_6$, $GUFFf_7$, $GUFFf_{10}$, $GUFFf_{11}$, $GUFFf_{19}$, $GUFFf_{20}$, $GUFFf_{21}$, $GUFFf_{24}$, $GUFFf_{25}$, $GUFFf_{26}$, $GUFFf_{35}$, $GUFFf_{37}$, $GUFFf_{39}$, $GUFFf_{40}$ grew on Saboraud's agar containing 20% crude solar salt (NSAB) but failed to grow in SAB as recorded in **Table 13**.

Table 13: Colony characteristics of halophilic fungal isolates on nutrient rich media with 20% crude solar salt

Fungal Isolate	Colony size (mm)	Colour of mycelium	Texture	Margin	Elevation	Sporulation	Colour on reverse	Peripheral growth zone	Tentative Class of fungi
$GUFFf_1$	8	White	Carttilaginous, solid	Irregular	Convex	Non-sporulating	-	Erect aerial mycelium	Non-sporulating Basidiomycetes
	20	Off-white	Leathery, folded	Smooth	Flat with central depression	Non-sporulating	Dark brown		
	20	White	Powdery	Fringed	Flat	Dark grey spores	-		
	14	White	Leathery	Fringed	Flat	Non-sporulating	Dark brown		
$GUFFf_2$	0							Loose, filamentous	Basidiomycetes
	11	White	Cottony ball	Fringed	Flat with convex centre	Non-sporulating	-		
	14	White	Cottony	Fringed	Flat with convex centre	Non-sporulating	-		
	12	White	Cottony	Fringed	Flat	Non-sporulating	Light brown		
$GUFFf_3$	0							Loose, filamentous	Basidiomycetes
	13	White	Cottony ball	Fringed	Flat with convex centre	Non-sporulating	-		
	11	White	Cottony	Fringed	Flat with convex centre	Non-sporulating	-		
	13	White	Cottony	Fringed	Flat	Non-sporulating	Light brown		
$GUFFf_4$	0							Fringed	Hyphomycetes
	11	White	Fibrous	Like roots	Flat	Bottle green spores	-		
	25	White	Powdery	Like roots	Flat	Green spores	-		
	23	White	Cottony	Like roots	Flat with convex centre	Green spores	-		
$GUFFf_5$	0							Aerial mycelia forming a canopy over central part	Hyphomycetes
	12	White	Fibrous	Like roots	Flat	Pale green spores	-		
	28	White	Powdery	Like roots	Flat	Green spores	-		
	26	White	Cottony	Like roots	Flat with convex centre	Non-sporulating	Light brown		
$GUFFf_6$	0							Colony with heterogeneous pockets of loose fluffy mycelia	Basidiomycetes
	18	White	Fibrous	Fringed	Flat with convex centre	Non-sporulating	-		
	22	White	Cottony, fibrous	Fringed	Flat	Non-sporulating	Dark brown		
	22	Off-white	Fibrous	Fringed	Flat	Non-sporulating	-		

Agar media with 20 % crude solar salt, Navy Blue: NSAB-Sabouraud's, Light blue: SAB, Red: NMEA-Malt Extract, Purple: NPDA-Potato Dextrose

Table 13: Colony characteristics of halophilic fungal isolates on nutrient rich media with 20 % crude solar salt

Fungal Isolate	Colony size (mm)	Colour of mycelium	Texture	Margin	Elevation	Sporulation	Colour on reverse	Peripheral growth zone	TentativeClass of fungi
$GUFFf_7$	0							Aerial mycelia profuse	Basidiomycetes
	15	White	cottony	Fringed	Flat	Non-sporulating	-		
	24	White	Cottony	Fringed	Flat	Non-sporulating	-		
	13	White	Fibrous with granular centre	Fringed	Flat with convex centre	Non-sporulating	-		
$GUFFf_8$	6	White	Carttilaginous	Irregular	Convex	Non-sporulating	-	Slightly fringed	Actinomycetes
	15	White	leathery	Smooth	Flat with convex centre	Non-sporulating	Brown		
	27	White	Powdery	Fringed	Flat	Dark grey spores	Dark brown		
	13	White	Leathery	Fringed	Flat with convex centre	Brown spores	Dark brown		
$GUFFf_9$	8	White	Carttilaginous	Irregular	Convex	Non-sporulating	-	Slightly fringed	Actinomycetes
	13	White	leathery	Smooth	Flat with convex centre	Non-sporulating	Dark Brown		
	23	White	Powdery	Fringed	Flat	Dark grey spores	Dark brown		
	10	Light brown	Cartilaginous, leathery	Fringed	Flat with convex centre	Brown spores	Brown		
$GUFFf_{10}$	0							Colony with heterogeneous pockets of loose fluffy mycelia	Basidiomycetes
	26	White	Fibrous, centre cottony	Fringed	Flat with convex centre	Non-sporulating	-		
	30	White	Cottony, fibrous	Fringed	Flat	Non-sporulating	-		
	24	White	Fibrous, granular	Fringed	Flat	Non-sporulating	-		
$GUFFf_{11}$	0							Colony with heterogeneous pockets of loose fluffy mycelia	Basidiomycetes
	29	White	Fibrous, centre cottony	Fringed	Flat with convex centre	Non-sporulating	-		
	30	White	Cottony, fibrous	Fringed	Flat	Non-sporulating	-		
	23	White	Granular	Fringed, fibrous	Flat	Non-sporulating	-		
$GUFFf_{12}$	10	White	Carttilaginous solid	Irregular	Convex	Non-sporulating	-	Slightly fringed	Actinomycetes
	17	White	Leathery, folded	Smooth	Flat	Non-sporulating	Brown		
	24	White	Powdery	Fringed	Flat	Dark grey spores	Dark brown		
	17	White	Leathery	Fringed	Flat with convex centre	Non-sporulating	Brown		

Agar media with 20 % crude solar salt, Navy Blue: NSAB-Sabouraud's, Light blue: SAB, Red: NMEA-Malt Extract, Purple: NPDA-Potato Dextrose

Table 13: Colony characteristics of halophilic fungal isolates on nutrient rich media with 20 % crude solar salt

Fungal Isolate	Colony size (mm)	Colour of mycelium	Texture	Margin	Elevation	Sporulation	Colour on reverse	Peripheral growth zone	TentativeClass of fungi
$GUFFf_{13}$	13	White	Carttilaginous solid	Irregular	Convex	Non-sporulating	-	Slightly fringed	Actinomycetes
	15	White	Leathery, folded	Fringed	Flat with convex centre	Non-sporulating	Brown		
	20	White	Powdery	Fringed	Flat	Dark grey spores	Dark brown		
	10	White	Leathery	Fringed	Flat, folded	Non-sporulating	-		
$GUFFf_{14}$	8	White	Carttilaginous aolid	Irregular	Convex	Non-sporulating	-	Fringed, aerial mycelia present	Hyphomycetes
	13	White	Smooth	Fringed	Flat	Non-sporulating	Brown		
	20	White	Powdery	Fringed	Flat	Dark grey spores	Grey		
	10	White	Leathery	Fringed	Flat	Non-sporulating	Brown		
$GUFFf_{15}$	10	White	Carttilaginous solid	Irregular	Convex	Non-sporulating	-	Fringed	Hyphomycetes
	29	White	Fibrous	Like roots	Flat	Green spores	-		
	22	White	Fibrous	Like roots	Flat	Green spores	-		
	26	White	Fibrous, powdery	Like roots	Flat	Green spores	-		
$GUFFf_{16}$	8	White	Carttilaginous solid	Irregular	Convex	Non-sporulating	-	Slightly fringed	Actinomycetes
	21	Light brown	Leathery	Fringed, folded	Flat	Non-sporulating	Dark brown		
	23	White	Powdery	Fringed	Flat	Dark green spores	Dark brown		
	10	Brown	Leathery	Fringed	Flat, folded	Brown spores	Dark brown		
$GUFFf_{17}$	8	White	Carttilaginous solid	Irregular	Convex	Non-sporulating	-	Slightly fringed	Actinomycetes
	14	White	Leathery, folded	Fringed	Flat with convex centre	Non-sporulating	Brown		
	20	White	Powdery	Fringed	Flat	Dark grey spores	Brown		
	10	White	Leathery	Fringed	Flat, folded	Non-sporulating	Brown		
$GUFFf_{18}$	9	White	Carttilaginous solid	Irregular	Convex	Non-sporulating	-	Slightly fringed	Actinomycetes
	19	White	Leathery, folded	Fringed	Flat	Non-sporulating	Brown		
	22	White	Powdery	Fringed	Flat	Dark grey spores	Dark brown		
	17	White	Leathery	Fringed	Flat with convex centre	Light green spores	-		

Agar media with 20 % crude solar salt, Navy Blue: NSAB-Sabouraud's, Light blue: SAB, Red: NMEA-Malt Extract, Purple: NPDA-Potato Dextrose

Table 13: Colony characteristics of halophilic fungal isolates on nutrient rich media with 20 % crude solar salt

Fungal Isolate	Colony size (mm)	Colour of mycelium	Texture	Margin	Elevation	Sporulation	Colour on reverse	Peripheral growth zone	TentativeClass of fungi
$GUFFf_{19}$	0							Loose, filamentous	Basidiomycetes
	22	White	Fibrous, centre cottony	Fringed	Flat with convex centre	Non-sporulating	-		
	23	White	Cottony, smooth	Fringed	Flat	Non-sporulating	-		
	11	White	Fibrous	Fringed	Flat with convex centre	Non-sporulating	-		
$GUFFf_{20}$	0							tufts of colourless mycelia	Basidiomycetes
	16	White	Fibrous	Fringed	Flat with convex centre	Non-sporulating	-		
	19	White	Cottony	Fringed	Flat	Non-sporulating	-		
	13	Cream	Fibrous	Fringed	Flat	Non-sporulating	-		
$GUFFf_{21}$	0							tufts of colourless mycelia	Basidiomycetes
	22	White	Fibrous	Fringed	Flat with convex centre	Non-sporulating	-		
	26	White	Cottony	Fringed	Flat	Non-sporulating	-		
	18	White	Fibrous with granular centre	Fringed	Flat with convex centre	Non-sporulating	Brown		
$GUFFf_{22}$	7	White	Carttilaginous solid	Irregular	Convex	Non-sporulating	-	Loose, fluffy mycelia	Basidiomycetes
	18	White	Leathery, folded	Fringed	Flat	Non-sporulating	Brown		
	29	White	Powdery	Fringed	Flat with convex centre	Grey spores	Brown		
	13	White	Leathery	Fringed	Flat with convex centre	Non-sporulating	-		
$GUFFf_{23}$	17	White	Fibrous, waxy	Irregular	Convex	Green spores	-	Concentric tufts of mycelia	Basidiomycetes
	23	White	Fibrous	Fringed	Flat with convex centre	Non-sporulating	-		
	25	White	Cottony	Fringed	Flat	Non-sporulating	-		
	16	White	Fibrous, granular	Fringed	Flat with convex centre	Non-sporulating	-		
$GUFFf_{24}$	0							Profuse aerial mycelia	Basidiomycetes
	15	White	Cottony	Fringed	Flat with convex centre	Non-sporulating	-		
	24	White	Cottony. fibrous	Fringed	Flat	Non-sporulating	-		
	10	Cream	Leathery	Fringed	Flat with convex centre	Non-sporulating	-		

Agar media with 20 % crude solar salt, Navy Blue: NSAB-Sabouraud's, Light blue: SAB, Red: NMEA-Malt Extract, Purple: NPDA-Potato Dextrose

Table 13: Colony characteristics of halophilic fungal isolates on nutrient rich media with 20 % crude solar salt

Fungal Isolate	Colony size (mm)	Colour of mycelium	Texture	Margin	Elevation	Sporulation	Colour on reverse	Peripheral growth zone	TentativeClass of fungi
$GUFFf_{25}$	0							Aerial mycelia profuse	Basidiomycetes
	20	White	Fibrous	Fringed	Flat with convex centre	Non-sporulating	-		
	28	White	Cottony, smooth	Fringed	Flat	Non-sporulating	-		
	17	White	Fibrous. granular	Fringed	Flat with convex centre	Non-sporulating	-		
$GUFFf_{26}$	0							Colony with heterogeneous pockets of loose fluffy mycelia	Basidiomycetes
	18	White	Cottony	Fringed	Flat	Non-sporulating	-		
	23	White	Cottony	Fringed	Flat	Non-sporulating	-		
	10	Cream	Leathery	Fringed	Flat	Non-sporulating	Light brown		
$GUFFf_{27}$	18	White	Firbous, waxy	Irregular	Convex	Dark green spores	-	Loose, fluffy mycelia	Basidiomycetes
	14	White	Fibrous	Fringed	Flat	Non-sporulating	-		
	23	White	Cottony, smooth	Fringed	Flat	Non-sporulating	-		
	6	White	Cartilaginous	Fringed. irregular	Flat with convex centre	Non-sporulating	-		
$GUFFf_{28}$	5	White	Carttilaginous solid	Irregular	Convex	Non-sporulating	-	Slightly fringed	Actinomycetes
	22	Off-white	Leathery, folded	Fringed	Flat	Non-sporulating	Brown		
	27	White	Powdery	Fringed	Flat	Grey spores	Dark brown		
	10	Light brown	Leathery	Fringed, irregular	Flat with convex centre	Non-sporulating	Brown		
$GUFFf_{29}$	10	White with grey centre	Carttilaginous solid	Irregular	Convex	Sporulating	-	Slightly fringed	Actinomycetes
	17	Off-white	Leathery, folded	Fringed	Convex	Non-sporulating	Dark Brown		
	20	White	Powdery	Fringed	Flat	Grey spores	Dark brown		
	13	Creamish brown	Leathery	Fringed	Flat with convex centre	Non-sporulating	Dark brown		
$GUFFf_{30}$	20	White	Firbous, waxy	Irregular	Convex	Green spores	-	Fringed	Hyphomycetes
	34	White	Fibrous	Fringed	Flat	Green spores	-		
	40	White	Powdery	Like roots	Flat	Green spores	-		
	14	White	Leathery	Fringed	Flat with convex centre	Non-sporulating	-		

Agar media with 20 % crude solar salt, Navy Blue: NSAB-Sabouraud's, Light blue: SAB, Red: NMEA-Malt Extract, Purple: NPDA-Potato Dextrose

Table 13: Colony characteristics of halophilic fungal isolates on nutrient rich media with 20 % crude solar salt

Fungal Isolate	Colony size (mm)	Colour of mycelium	Texture	Margin	Elevation	Sporulation	Colour on reverse	Peripheral growth zone	TentativeClass of fungi
$GUFFf_{31}$	15	White	Fibrous, waxy	Irregular	Convex	Bottle green apores	-	Slightly fringed	Actinomycetes
	19	White	Leathery, folded	Fringed	Flat with convex centre	Non-sporulating	Light Brown		
	25	White	Powdery	Fringed	Flat with convex centre	Grey spores	Dark brown		
	16	Brown	Leathery	Fringed	Flat with convex centre	Brown spores	Dark brown		
$GUFFf_{32}$	22	White	Fibrous, waxy	Irregular	Convex	Bottle green spores	-	Fringed	Hyphomycetes
	19	White	Powdery	Fringed	Flat	Green spores	-		
	11	White	Cottony	Fringed	Flat	Non-sporulating	-		
	18	White	Fibrous	Fringed	Flat	Green spores	Brown		
$GUFFf_{33}$	12	White	Fibrous, waxy	Irregular	Convex	Non-sporulating	-	Filamentous	Basidiomycetes
	11	White	Cottony	Fringed	Flat	Non-sporulating	-		
	21	White	Cottony	Fringed	Flat	Non-sporulating	-		
	16	White	Fibrous	Fringed	Flat	Non-sporulating	-		
$GUFFf_{34}$	24	White	Firbous, waxy	Irregular	Convex	Bottle green spores	-	Fringed	Hyphomycetes
	24	White	Powdery	Fringed	Flat	Bottle green spores	-		
	26	White	Powdery	Like roots	Flat	Bottle green spores	-		
	9	Cream	Cartilaginous	Fringed	Flat	Non-sporulating	-		
$GUFFf_{35}$	0							Loose fluffy mycelia	Basidiomycetes
	11	White	Cottony	Fringed	Flat	Non-sporulating	-		
	11	White	Cottony	Fringed	Flat	Non-sporulating	-		
	9	Cream	Cartilaginous	Fringed	Flat	Non-sporulating	-		
$GUFFf_{36}$	22	White	Fibrous, waxy	Irregular	Convex	Bluish green spores	-	Fringed	Hyphomycetes
	19	White	Powdery	Fringed	Flat	Bottle green spores	-		
	19	White	Powdery	Fringed	Flat	Bottle green spores	-		
	17	White	Powdery	Fringed	Flat with convex centre	Green spores	Brown		

Agar media with 20 % crude solar salt, Navy Blue: NSAB-Sabouraud's, Light blue: SAB, Red: NMEA-Malt Extract, Purple: NPDA-Potato Dextrose

Table 13: Colony characteristics of halophilic fungal isolates on nutrient rich media with 20% crude solar salt

Fungal Isolate	Colony size (mm)	Colour of mycelium	Texture	Margin	Elevation	Sporulation	Colour on reverse	Peripheral growth zone	Tentative Class of fungi
$GUFFf_{37}$	0							Loose fluffy mycelia	Basidiomycetes
	12	White	Cottony	Fringed	Flat	Non-sporulating	-		
	21	White	Cottony	Fringed	Flat	Non-sporulating	-		
	10	White	Cartilaginous	Fringed	Flat with convex centre	Non-sporulating	-		
$GUFFf_{38}$	21	White	Fibrous, waxy	Irregular	Convex	Green spores	-	Loose, filamentous	Basidiomycetes
	40	White	Powdery	Fringed	Flat	Bottle green spores	-		
	23	White	Cottony	Fringed	Flat	Non-sporulating	-		
	15	White	Fibrous	Fringed	Flat with convex centre	Non-sporulating	-		
$GUFFf_{39}$	0							Abundant aerial mycelia	Basidiomycetes
	11	White	Cottony	Fringed	Flat	Non-sporulating	-		
	22	White	Cottony	Fringed	Flat with convex centre	Non-sporulating	-		
	20	White	Fibrous, granular	Fringed	Flat with convex centre	Non-sporulating	-		
$GUFFf_{40}$	0							Abundant aerial mycelia	Basidiomycetes
	12	White	Cottony	Fringed	Flat	Non-sporulating	-		
	17	White	Cottony	Fringed	Flat	Non-sporulating	-		
	18	White	Fibrous, granular	Fringed	Flat with convex centre	Non-sporulating	-		

Agar media with 20 % crude solar salt, Navy Blue: NSAB-Sabouraud's, Light blue: SAB, Red: NMEA-Malt Extract, Purple: NPDA-Potato Dextrose

Some of the cultures showed light brown, dark brown pigmentation in hyphae embedded in agar, although surface hyphae were colourless **(Plate 4).**

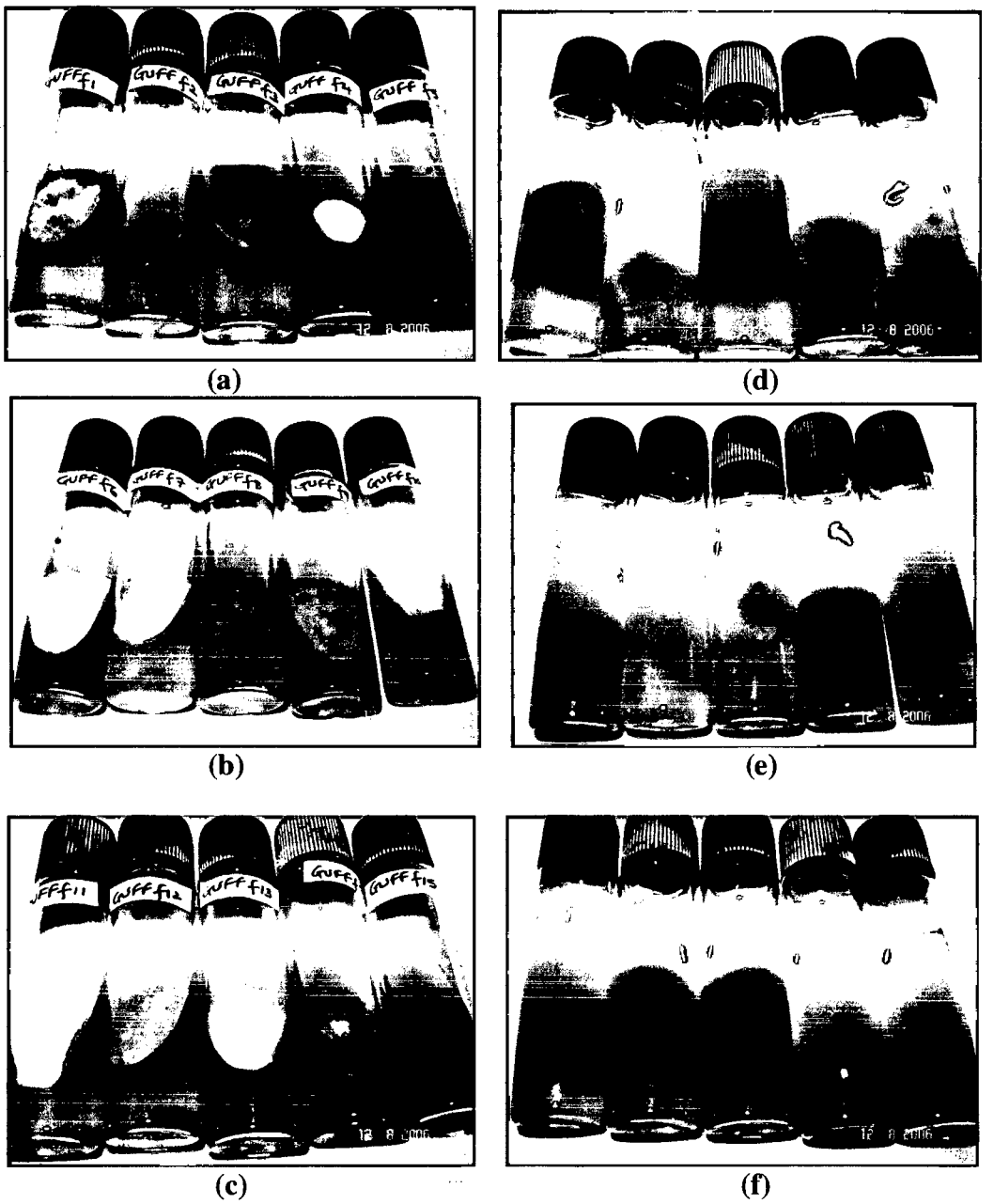


Plate 4: Six month old colonies of halophilic fungal isolates on MEA with 20% crude solar salt (left to right) (a) $GUFFf_1$-$GUFFf_5$ (b) $GUFFf_6$-$GUFFf_{10}$ (c) $GUFFf_{11}$-$GUFFf_{15}$ and the reverse of $GUFFf_1$-$GUFFf_5$ (d), $GUFFf_6$.$GUFFf_{10}$ (e), $GUFFf_{11}$-$GUFFf_{15}$ (f)

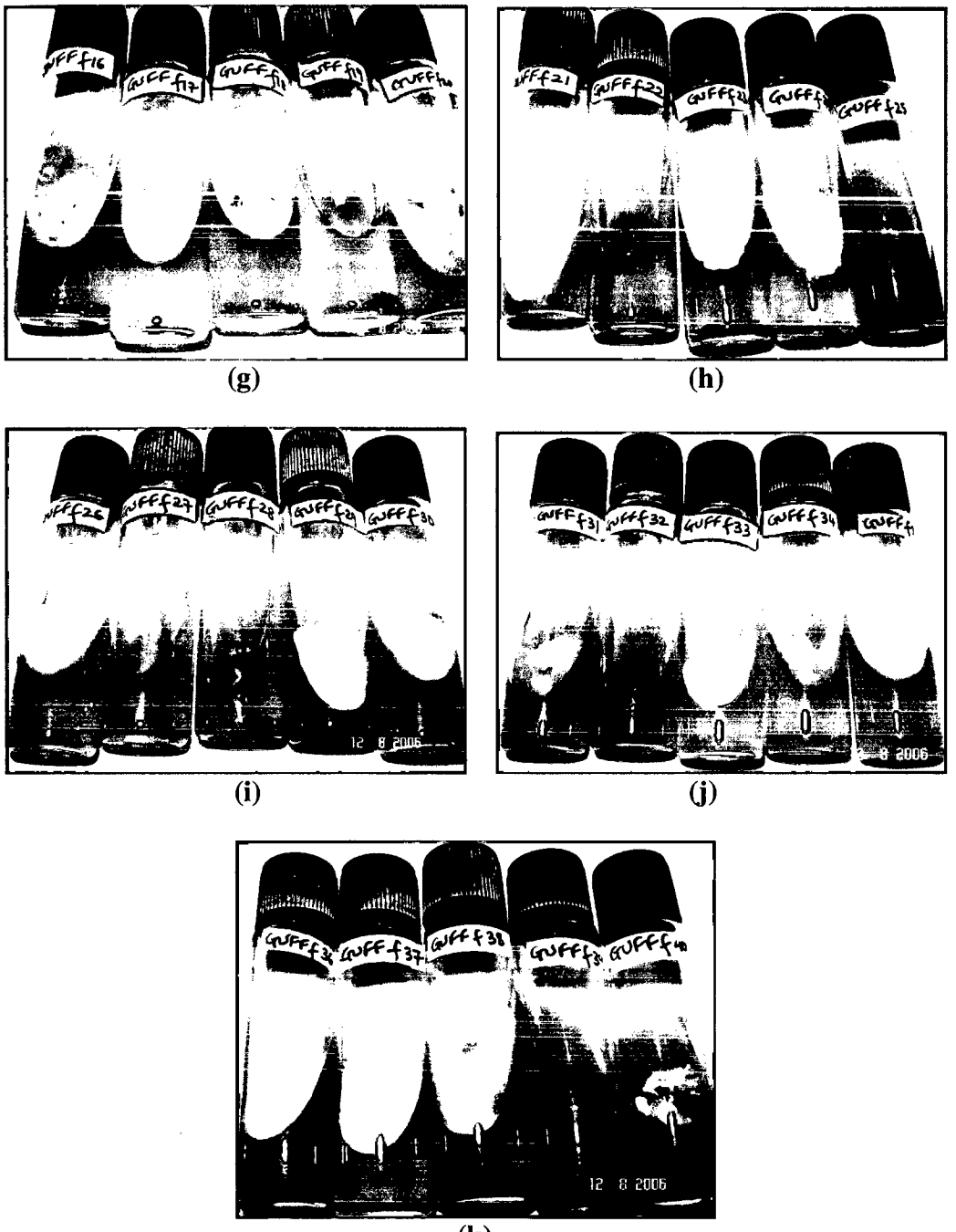


Plate 4: Six month old colonies of halophilic fungal isolates on MEA with 20% crude solar salt (left to right) (g) $GUFFf_{16}$-$GUFFf_{20}$ (h) $GUFFf_{21}$-$GUFFf_{25}$ (i) $GUFFf_{26}$-$GUFFf_{30}$ (j) $GUFFf_{31}$-$GUFFf_{35}$ (k) $GUFFf_{36}$-$GUFFf_{40}$

ii) Colony characteristics of fungal isolates on different media

Forty-two isolates showed different growth characteristics on different media each containing 20% crude solar salt i.e. NSAB, NMEA, NPDA (**Plate 5**). Maximum zone size of 40 mm was shown by $GUFFf_{38}$ and $GUFFf_{30}$ on NSAB and NMEA respectively, while 26 mm was shown by $GUFFf_5$, $GUFFf_{15}$ and $GUFFf_{32}$ on NPDA as seen in **Table 13** and **Fig. 2**.

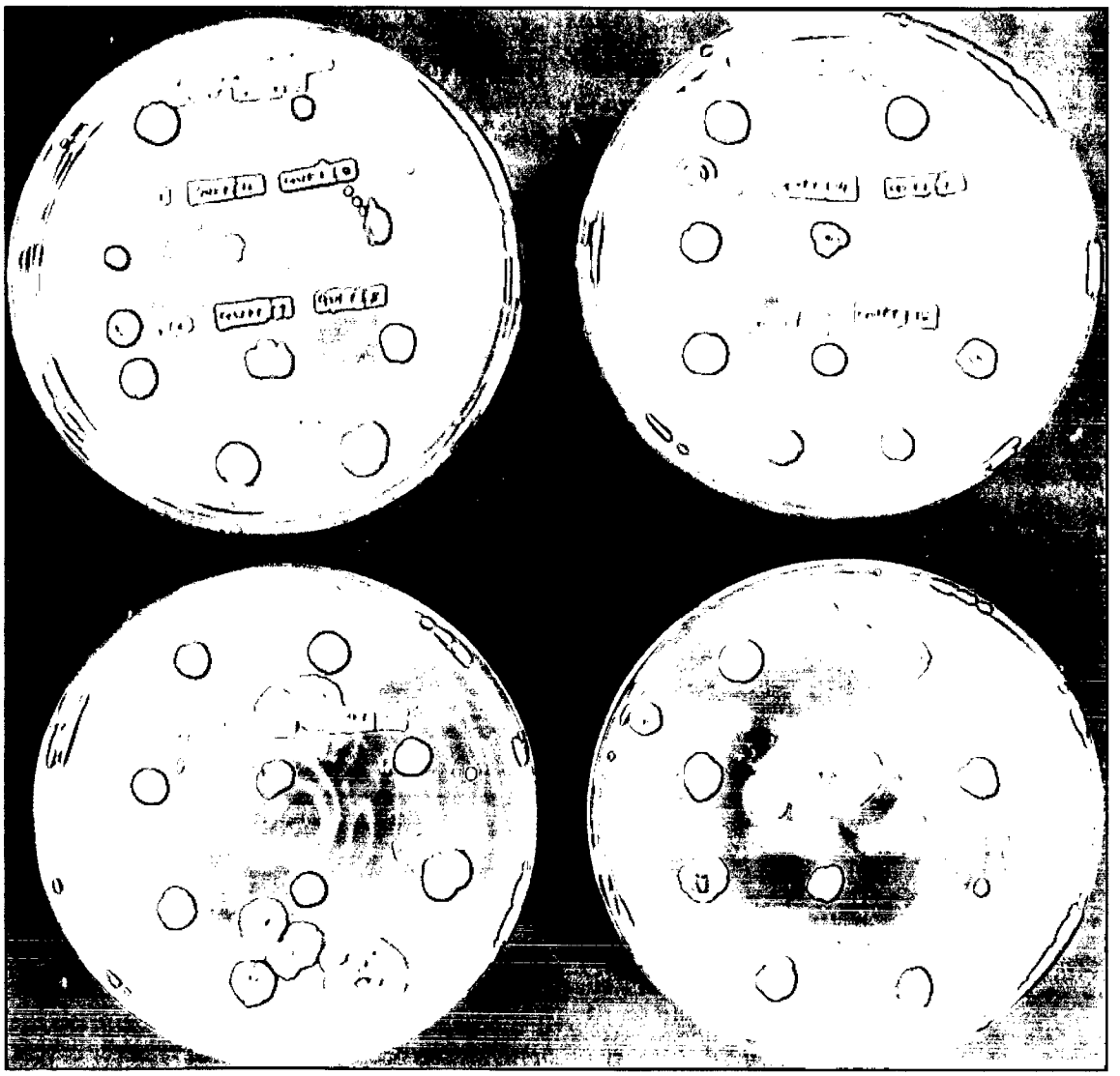


Plate 5: Growth of halophilic fungal isolates on nutrient rich medium with 20% crude solar salt-NSAB

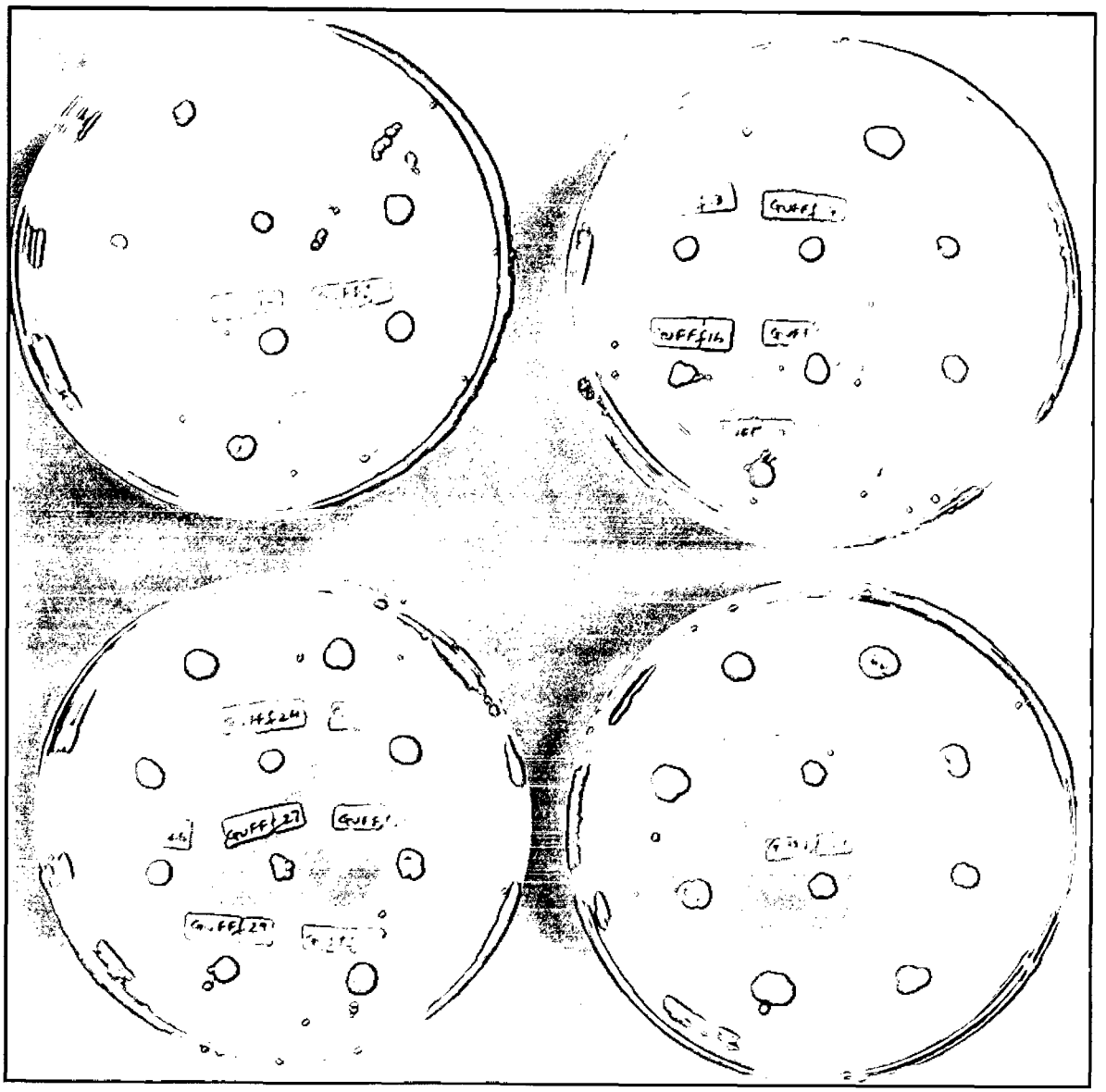


Plate 5: Growth of halophilic fungal isolates on nutrient rich medium with 20% crude solar salt - NPDA

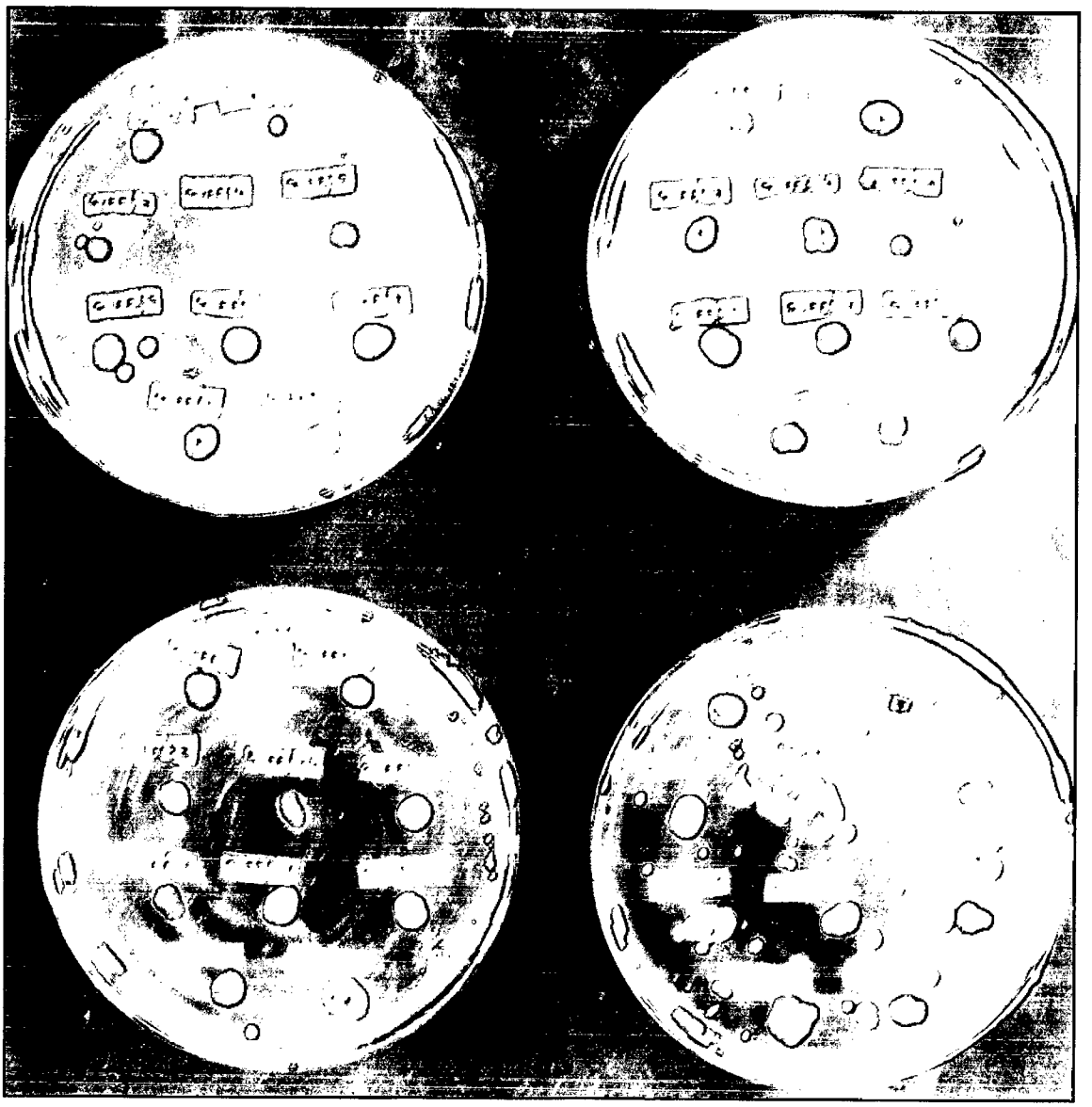


Plate 5: Growth of halophilic fungal isolates on nutrient rich medium with 20% crude solar salt-NMEA

Fig. 2: Growth of halophilic fungal isolates on nutrient rich culture media with 20% crude solar salt

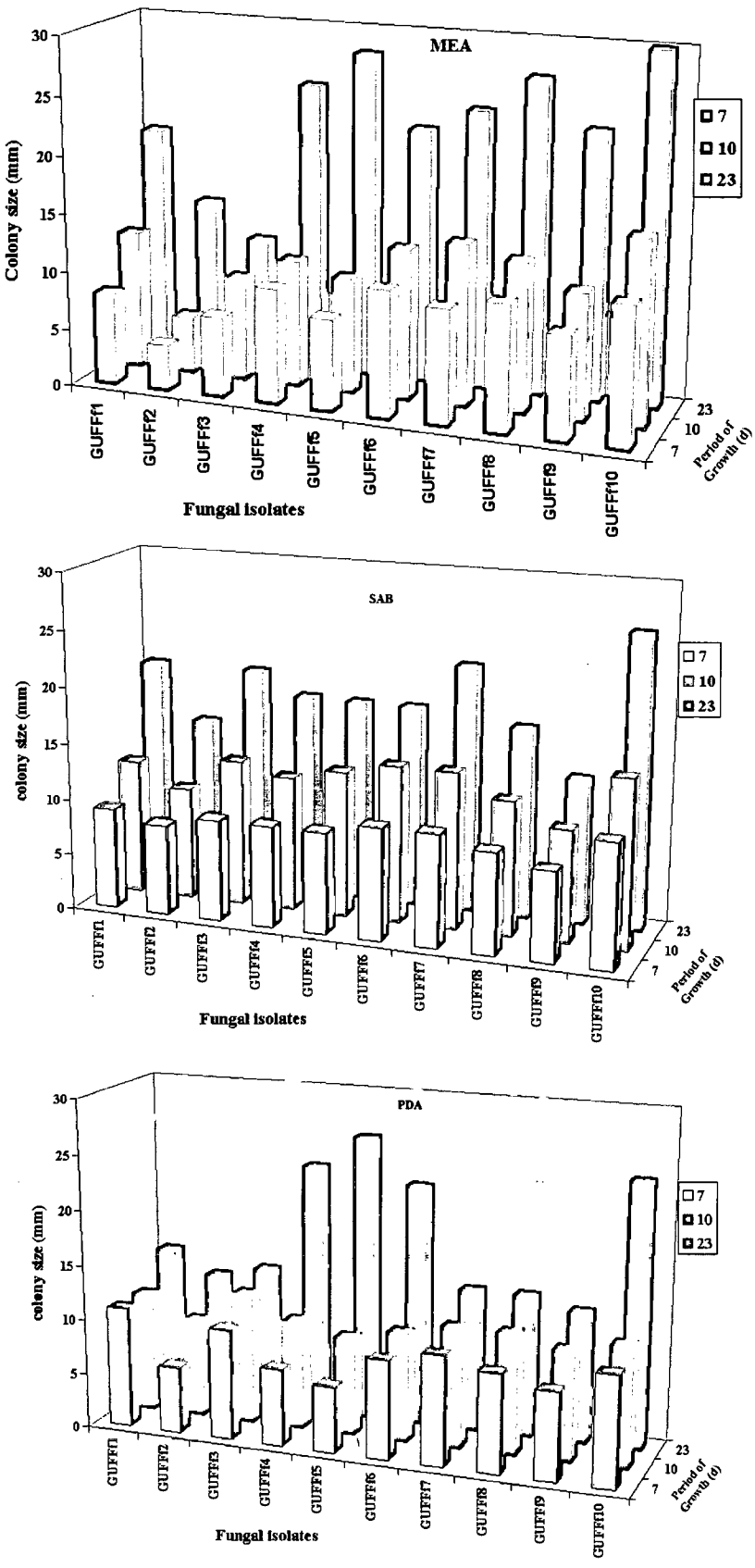


Fig. 2: Growth of halophilic fungal isolates on nutrient rich culture media with 20% crude solar salt

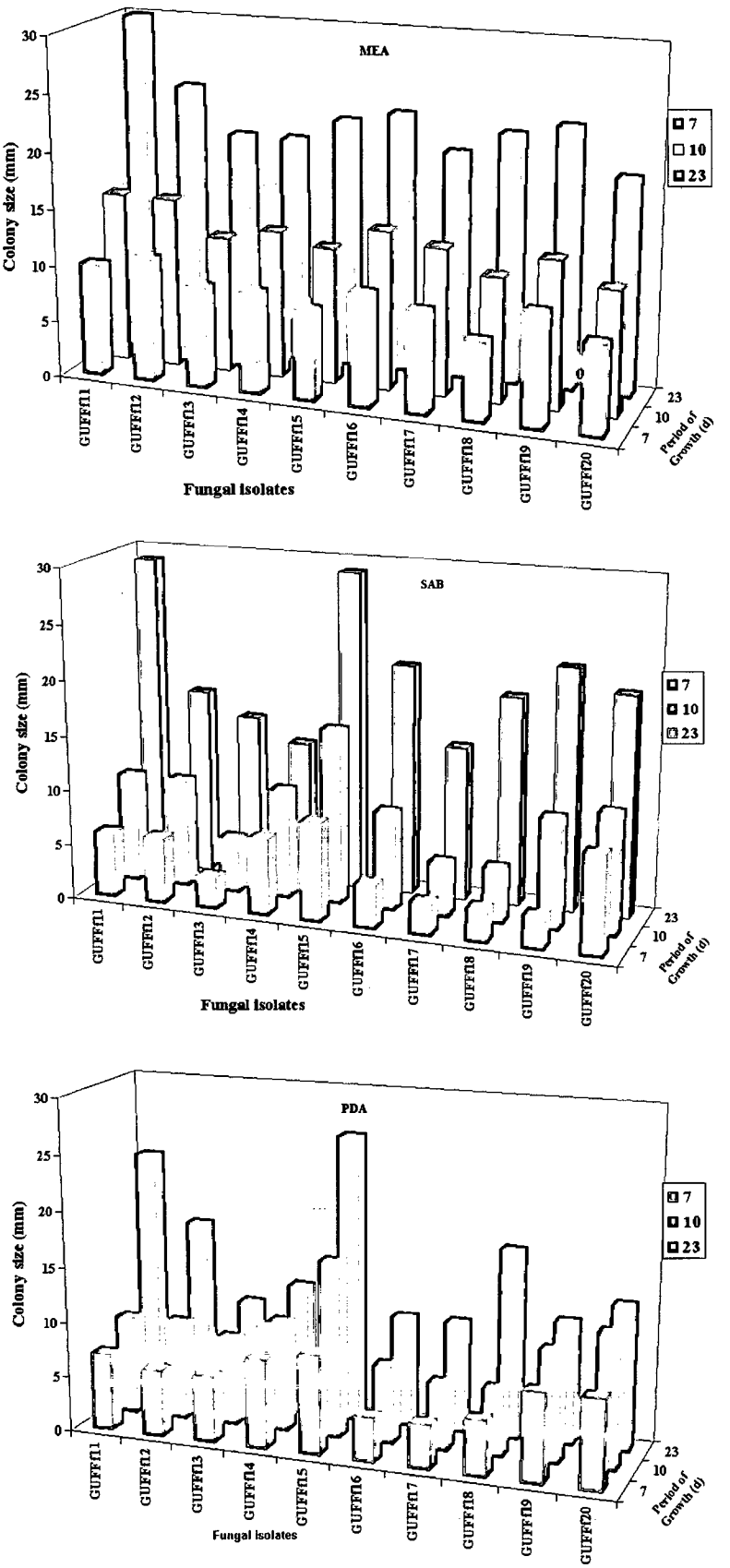


Fig. 2: Growth of halophilic fungal isolates on nutrient rich culture media with 20% crude solar salt

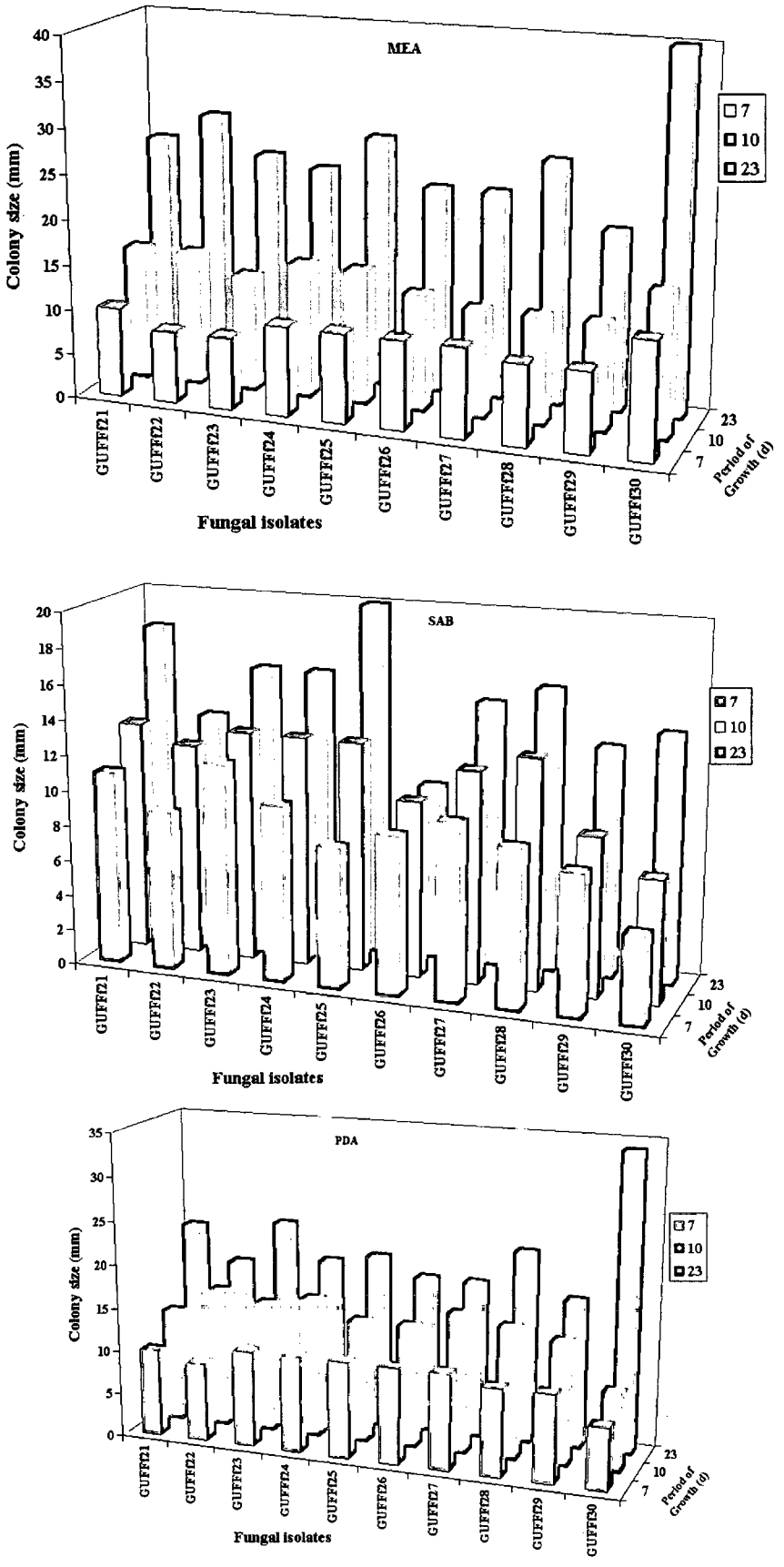
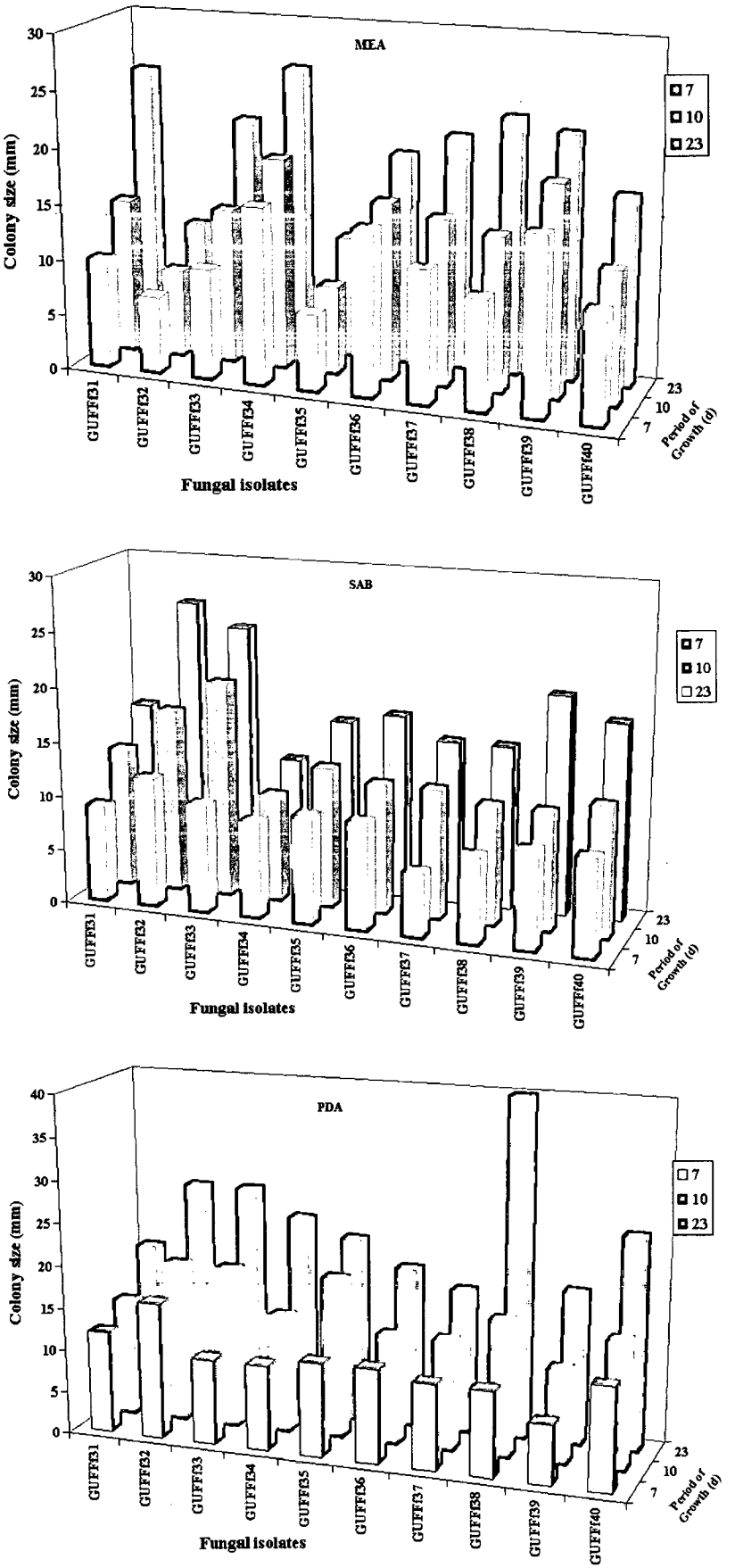


Fig. 2: Growth of halophilic fungal isolates on nutrient rich culture media with 20% crude solar salt



Nine of the fungal isolates as seen in **Table 14**; namely, $GUFFf_6$, $GUFFf_9$, $GUFFf_{12}$, $GUFFf_{21}$, $GUFFf_{22}$, $GUFFf_{24}$, $GUFFf_{28}$, $GUFFf_{30}$, $GUFFf_{32}$ had broad growth temperature of 28° C to 50° C.

Table 14: Growth of fungal isolates on 0.2% glucose in NSM at different temperature

Fungal Isolate	NGSM, pH 5.5											
	Incubation temperature											
	Ambient (28 ± 2°C)			37°C			42°C			50°C		
	Days of incubation											
	13	19	30	13	19	30	13	19	30	13	19	30
$GUFFf_1$	++	++	++	+	+	+	++	++	++	-	-	-
$GUFFf_2$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_3$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_4$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_5$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_6$	++	++	++	+	+	+	+	+	+	+	+	+
$GUFFf_7$	++	++	++	+	+	+	++	++	++	-	-	-
$GUFFf_8$	++	++	++	+	+	+	+	+	+	-	-	-
$GUFFf_9$	++	++	++	+	+	+	+	+	+	+	+	+
$GUFFf_{10}$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_{11}$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_{12}$	++	++	++	+	+	+	+	+	+	+	+	+
$GUFFf_{13}$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_{14}$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_{15}$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_{16}$	++	++	++	-	-	-	-	-	-	+	+	+
$GUFFf_{17}$	++	++	++	-	-	-	-	-	-	+	+	+
$GUFFf_{18}$	++	++	++	+	+	+	-	-	-	-	-	-
$GUFFf_{19}$	++	++	++	+	+	+	-	-	-	-	-	-
$GUFFf_{20}$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_{21}$	++	++	++	+	+	+	+	+	+	+	+	+
$GUFFf_{22}$	++	++	++	++	++	++	+	+	+	+	+	+

Table 14: Growth of fungal isolates on 0.2% glucose in NSM at different temperature

Fungal Isolate	NGSM, pH 5.5											
	Incubation temperature											
	Ambient (28 ± 2°C)			37°C			42°C			50°C		
	Days of incubation											
	13	19	30	13	19	30	13	19	30	13	19	30
$GUFFf_{23}$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_{24}$	++	++	++	+	+	+	+	+	+	+	+	+
$GUFFf_{25}$	++	++	++	+	+	+	+	+	+	-	-	-
$GUFFf_{26}$	++	++	++	+	+	+	-	-	-	-	-	-
$GUFFf_{27}$	++	++	++	+	+	+	+	+	+	-	-	-
$GUFFf_{28}$	++	++	++	+	+	+	++	++	++	+	+	+
$GUFFf_{29}$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_{30}$	++	++	++	-	-	-	+	+	++	-	-	-
$GUFFf_{31}$	++	++	++	+	+	+	++	++	++	+	+	+
$GUFFf_{32}$	++	++	++	+	+	+	+	+	+	+	+	+
$GUFFf_{33}$	++	++	++	-	-	-	-	+	+	-	-	-
$GUFFf_{34}$	++	++	++	+	+	+	+	+	+	-	-	-
$GUFFf_{35}$	++	++	++	-	-	-	++	++	++	-	-	-
$GUFFf_{36}$	++	++	++	+	+	+	+	+	+	-	-	-
$GUFFf_{37}$	++	++	++	-	-	-	+	+	+	-	-	-
$GUFFf_{38}$	++	++	++	+	+	+	-	-	-	-	-	-
$GUFFf_{39}$	++	++	++	-	-	-	+	+	+	-	-	-
$GUFFf_{40}$	++	++	++	-	-	-	-	-	-	-	-	-
$GUFFf_{41}$	++	++	++	+	+	+	+	+	+	-	-	-

iii) Stereomicroscopic characteristics of fungal growth on MEA

Growth of fungal colonies on MEA containing 20% crude solar salt showed slow to moderate growth, at the end of seven days. Direct observation of different colonies under stereomicroscope indicated that most colonies possessed mycelia which were mostly white, having wooly or granular texture. Margin was fringed/waxy/filamentous with convex elevation. The fungi seemed to be mostly non-sporulating when observed under the stereomicroscope. Peripheral growth zone mostly constituted of loose filamentous mycelia **(Table 13),** and hence 10/22/8 of the fungal isolates were tentatively identified as those belonging to Actinomycetes/Basidiomycetes/Hyphomycetes, respectively.

iv) Microscopic features and taxonomic placement

Out of 42 fungal isolates, twenty five were studied for morphological characteristics. Based on microscopic features recorded in **Table 15**, these fungal isolates were given taxonomic placement. Two/four/five/seven/seventeen fungal isolates were identified as each of *Acremonium/Humicola, Paecilomyces/Aspergillus/Talaromyces* /unidentified, respectively.

Table 15: Microscopic features and taxonomic placement of halophilic fungal isolates

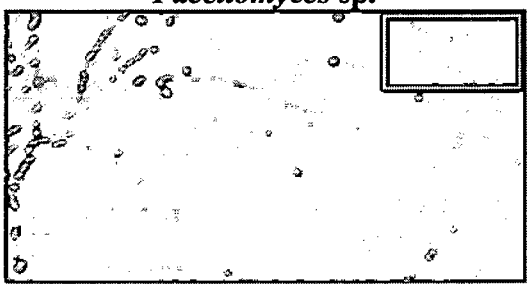
Isolates	Characteristics	Identification* to genus & species level
$GUFFf_3$, $GUFFf_{18}$, $GUFFf_{25}$	**Colony colour white, texture closely matted, conidia are ovate and borne in very long tangled chains. Phialides are narrow flask-shaped at the base and terminating in long slender spore-bearing tips, which are usually bent away from the main axis.**	***Paecilomyces* sp.** 

Table 15: Microscopic features and taxonomic placement of halophilic fungal isolates

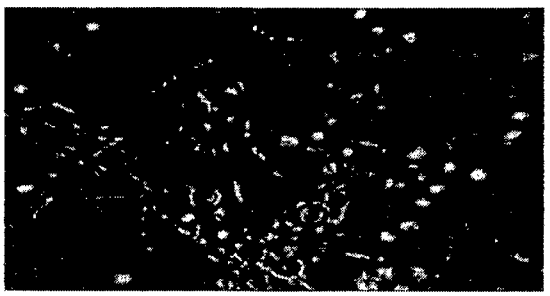
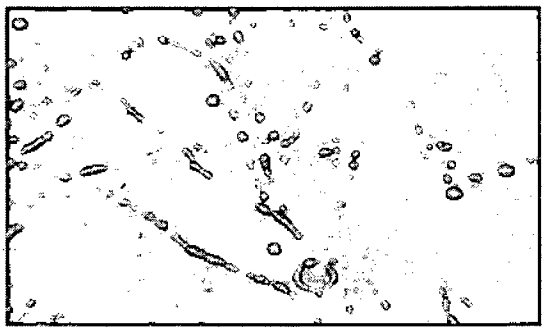
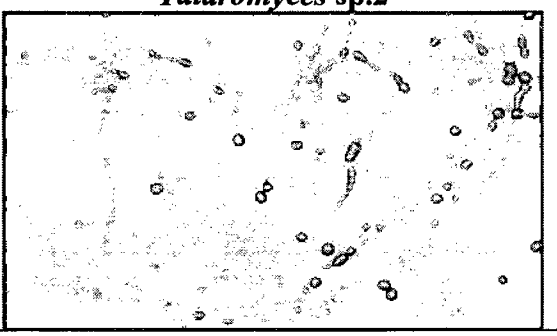
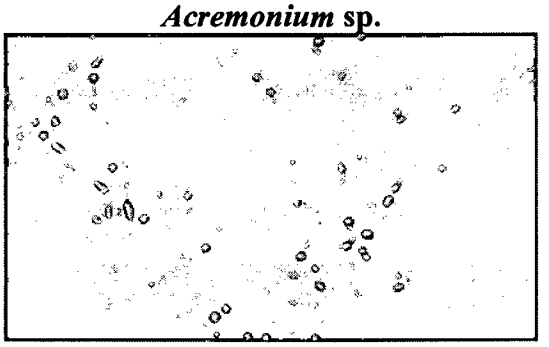
Isolates	Characteristics	Identification* to genus & species level
$GUFFf_4$, $GUFFf_{15}$	Mycelium white, dematiaceous, septate, partly submerged; fertile branches (conidiophores) arising from, specialized thick-walled enlarged mycelial cell (foot-cells), mostly non-septate, smooth, roughened, enlarging towards the apex, and terminating in a swelling (vesicle), hemispherical. Metulae bear clusters of phialides, which are produced simultaneously from surface of vesicles; conidia produced successively from tips of phialides, thus forming unbranched chains, conidial heads globose, radiate splitting in age, ochraceous.	*Aspergillus ochraceus* 
$GUFFf_5$	Mycelium white, dematiaceous, septate, partly submerged; fertile branches (conidiophores) arising from, specialized thick-walled enlarged mycelial cell (foot-cells), mostly non-septate, smooth, roughened, enlarging towards the apex, and terminating in a swelling (vesicle), hemispherical. Metulae bear clusters of phialides, which are produced simultaneously from surface of vesicles; conidia produced successively from tips of phialides, forming unbranched chains, conidial heads green, loosely columnar, cleistothecia present, stipes brownish.	*Aspergillus nidulans* 
$GUFFf_6$, $GUFFf_7$, $GUFFf_{10}$, $GUFFf_{23}$	Colonies effuse, cottony, at first white, later pale grey. Mycelium superficial and immersed. Stroma none. Setae and hyphopodia absent. Conidiophores micronematous, unbranched, straight, colorless, smooth. Conidiogenous cells monoblastic, integrated, terminal, determinate, cylindrical. Conidia solitary, dry, simple, spherical, smooth. It also has a phialidic state, phialides discrete, subulate, colorless, smooth.	*Humicola* sp. 

Table 15: Microscopic features and taxonomic placement of halophilic fungal isolates

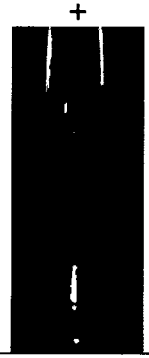
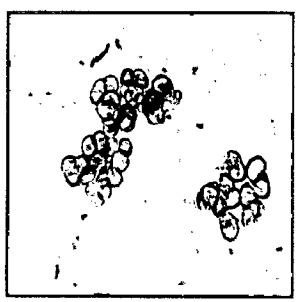
Isolates	Characteristics	Identification* to genus & species level
$GUFFf_8$, $GUFFf_{11}$, $GUFFf_{20}$, $GUFFf_{21}$, $GUFFf_{22}$	**Mesophilic, biverticillate-symmetrical, conidial state, with reduced conidial apparatus. Ascospores are ovate, without furrow, spinulose all over.**	***Talaromyces* sp. 1** 
$GUFFf_9$		***Talaromyces* sp.2** 
$GUFFf_{16}$, $GUFFf_{17}$	**Colonies white and brown, ropy, slightly wet, with single elongate phialides, bearing conidia in wet spore balls. Conidia cylindrical, there are no chlamydospores or synnemata.**	***Acremonium* sp.** 

*** Ellis, 1971; Onions *et al*, 1981**

v) Characterization and Identification of $GUFFf_{41}$

Isolate $GUFFf_{41}$ interestingly grew as dark black colonies which on microscopy were observed to be single-cellular, oval cells positive to Gram reaction. The cells changed from oval to spheres by the end of 5 days as depicted in **Table 16.**

Table 16: Characterization and identification of isolate GUFFf$_{41}$

Characteristics								Identification
Growth media	pH	NaCl conc. (%)	Mg^{2+} (mM)	Growth	Morphology			
					Gram character	Wet mount Age		
						2 days	5 days	
MEA	5.5	20.0	-	+ 	+ve 			*Hortaea* sp.
NA	7.0	0.5	-	+				
TYE	5.0	25.0	1.0	+				
	6.0		50.0	-				
	7.0		80.0	-				
	8.5		5.0	+				
	10.5		0.07	-				

vi) Retrievable Fungal community of salt pans

Fungi belonging to Hyphomycetes/Basidiomycetes/Actinomycetes were retrieved as culturable. Their occurrence in different salt pans and types of samples i.e. brine, sediment & water is depicted in **Fig. 3** inorder to reflect the possible retrievable fungal community. A salt pan harbours *Humicola* sp.; Ag salt pan contains *Humicola* sp. and *Acremonium* sp.; *Talaromyces* sp. 1, 2; *Paecilomyces* sp. were the fungi belonging to Ar salt pan; while *Talaromyces* sp. 2, and *Aspergillus* sp. constitute the fungal community of N salt pan. R salt pan had *Humicola*, *Paecilomyces* sp. 2 and *Talaromyces* sp. 2 together with a non-sporulating sp. while S salt pan had *Aspergillus ochraceus, Paecilomyces* sp.1 and *Hortaea* sp. Monsoon fungal community of R salt pan was *Aspergillus ochraceus, Humicola* sp. and *Acremonium* sp. 1, S salt pan had *Aspergillus* sp. During post monsoon, fungal community of R salt pan was *Talaromyces* sp. 1; while of S salt pan was *Aspergillus nidulans* and one non-sporulating sp.

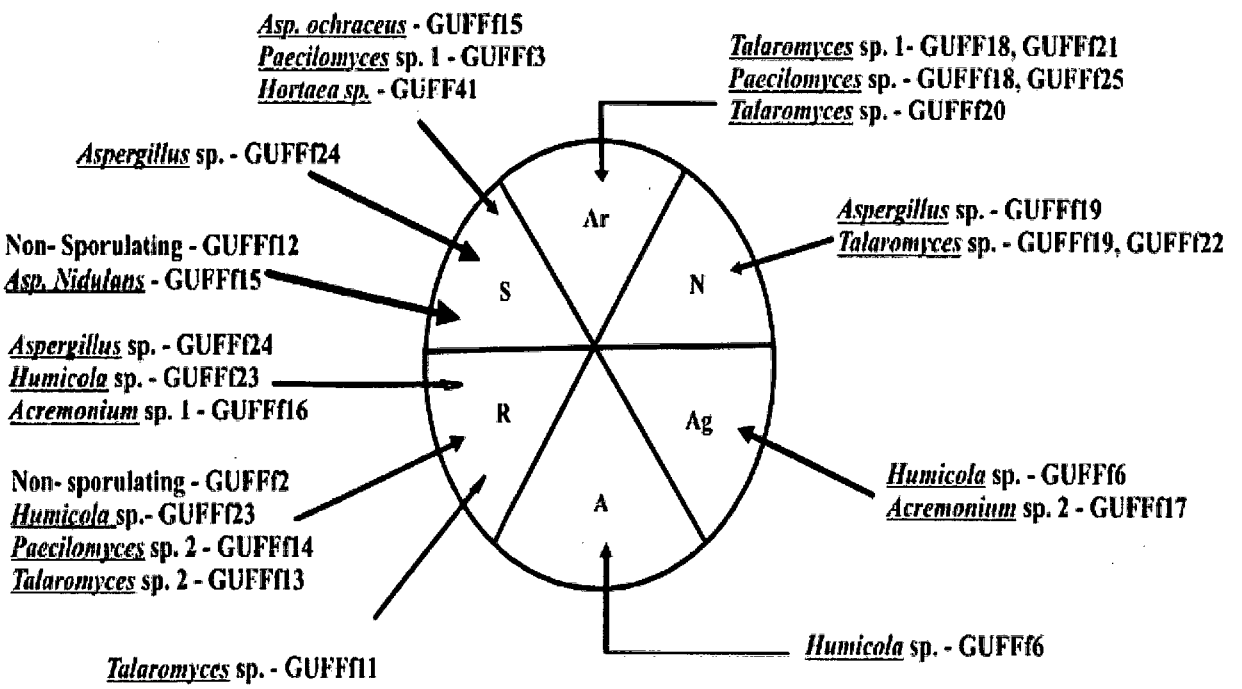


Fig 3: Retrievable fungal community of salt pans

Blue- monsoon, Red-post monsoon

2. BACTERIA

i) Domain affiliation of isolated bacteria

Bacteria can be referred to their domains on demonstration of presence of GDEM's, resistance to 500 U/ml of penicillin and sensitivity to bile salts, as Haloarchaea, the Eubacterial counterparts show sensitivity to penicillin and lack GDEM's, those having chlorophyll and growing in absence of carbon as Cyanobacteria. Domain affiliation of isolated bacteria is arrived at using chemotaxonomic markers listed in **Table 17, Fig. 4**. Of the 234 bacterial isolates, 56 and 178 were referred to domain eubacteria (i.e. 53 bacteria and 3 cyanobacteria) and haloarchaea, respectively.

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_1$	+	-	+	-	-	-	-	+	+	-	-	+		
$GUFF_2$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_3$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_4$	-	-	+	-	-	-	+	+	+	-	-	+		
* $GUFF_5$	+	-	+	-	-	-	-	+	+	-	-	+		
* $GUFF_6$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_7$	-	-	+	-	-	-	+	+	+	-	-	+		
* $GUFF_8$	+	-	+	-	-	-	-	+	+	-	-	+		
$GUFF_9$	-	-	+	-	+	-	+	+	+	-	-	+		
$GUFF_{10}$	-	-	+	-	-	-	+	+	+	-	-	+		
*$GUFF_{11}$	+	-	+	-	+	-	-	-	-	-	-		+	
$GUFF_{12}$	-	-	+	-	-	-	+	+	+	-	-	+		
*$GUFF_{13}$	+	-	+	-	-	-	-	+	+	-	-	+		
$GUFF_{14}$	-	-	+	-	-	-	+	+	+	-	-	+		
*$GUFF_{15}$	+	-	+	-	+	-	-	-	-	-	-		+	
*$GUFF_{16}$	-	-	+	-	-	-	+	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
*$GUFF_{17}$	+	-	+	-	-	-	-	+	+	-	-	+		
*$GUFF_{18}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{19}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{20}$	-	-	+	-	-	-	+	+	+	-	-	+		
*$GUFF_{21}$	+	-	+	-	-	-	-	+	+	-	-	+		
$GUFF_{22}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{23}$	-	-	+	-	-	-	+	+	+	-	-	+		
*$GUFF_{24}$	+	-	+	-	-	-	-	+	+	-	-	+		
$GUFF_{25}$	-	-	+	-	+	-	+	+	+	-	-	+		
$GUFF_{26}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{27}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{28}$	-	-	+	-	-	-	+	+	+	-	-	+		
*$GUFF_{29}$	+	-	+	-	+	-	-	-	-	-	-		+	
$GUFF_{30}$	+	-	+	-	-	-	-	+	+	-	-	+		
*$GUFF_{31}$	+	-	+	-	-	-	-	+	+	-	-	+		
$GUFF_{32}$	-	-	+	-	-	-	+	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{32}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{33}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{34}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{35}$	-	-	+	-	-	-	+	+	+	-	-	+		
*$GUFF_{36}$	+	-	+	-	-	-	-	+	+	-	-	+		
$GUFF_{37}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{38}$	-	-	+	-	+	-	+	+	+	-	-	+		
$GUFF_{39}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{40}$	+	-	+	-	+	-	-	-	-	-	-		+	
$GUFF_{41}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{42}$	+	-	+	-	+	-	_	-	+	-	-	+		
$GUFF_{43}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{44}$	-	-	+	-	+	-	+	+	+	-	-	+		
$GUFF_{45}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{46}$	-	-	+	-	-	-	+	+	+	-	-	+		
*$GUFF_{47}$	-	-	+	-	-	-	+	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{48}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{49}$	-	-	+	-	+	-	+	+	+	-	-	+		
*$GUFF_{50}$	-	-	+	-	-	-	+	+	+	-	-	+		
$GUFF_{51}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{52}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{53}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{54}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{55}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{56}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{57}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{58}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{59}$	-	-	+	-	-		+	+	+	-	-	+		
* $GUFF_{60}$	-	-	+	-	+		+	+	+	-	-	+		
* $GUFF_{61}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{62}$	-	-	+	-	-		+	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
* $GUFF_{63}$	-	-	+	-	-		+	+	+	-	-	+		
* $GUFF_{64}$	-	-	+	-	-		+	+	+	-	-	+		
* $GUFF_{65}$	-	-	+	-	-		+	+	+	-	-	+		
* $GUFF_{66}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{67}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{68}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{69}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{70}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{71}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{72}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{73}$	-	-	+	-	-		+	+	+	-	-	+		
* $GUFF_{74}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{75}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{76}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{77}$	+	-	+	-	-		-	+	+	-	-	+		
* $GUFF_{78}$	+	-	+	-	-		-	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{79}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{80}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{81}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{82}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{83}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{84}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{85}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{86}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{87}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{88}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{89}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{90}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{91}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{92}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{93}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{94}$	+	-	+	-	-		-	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{95}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{96}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{97}$	+	-	+	-	+		-	-	-	-	-	+		
$GUFF_{98}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{99}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{100}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{101}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{102}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{103}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{104}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{105}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{106}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{107}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{108}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{109}$	-	-	+	-	-		+	+	+	-	-	+		
*$GUFF_{110}$	+	-	+	-	-		-	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
*$GUFF_{111}$	-	-	+	-	-		+	+	+	-	-	+		
*$GUFF_{112}$	+	-	+	-	-		-	+	+	-	-	+		
*$GUFF_{113}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{114}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{115}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{116}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{117}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{118}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{119}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{120}$	-	-	+	-	+		+	+	+	-	-	+		
*$GUFF_{121}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{122}$	+	-	+	-	-		-	+	+	-	-	+		
*$GUFF_{123}$	+	-	+	-	+		-	-	-	-	-		+	
*$GUFF_{124}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{125}$	+	-	+	-	-		-	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
*$GUFF_{126}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{127}$	+	-	+	-	-		-	+	+	-	-	+		
*$GUFF_{128}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{129}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{130}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{131}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{132}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{133}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{134}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{135}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{136}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{137}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{138}$	+	-	-	-	+		-	-	-	-	-		+	
$GUFF_{139}$	-	-	+		-		+	+	+	-	-	+		
$GUFF_{140}$	+	-	-	-	+		-	-	-	-	-		+	

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{141}$	+	-	-	-	+		-	-	-	-	-		+	
$GUFF_{142}$	+	-	-	-	+		-	-	-	-	-		+	
$GUFF_{143}$	-	-	+	-	+		+	+	+	-	-		+	
$GUFF_{144}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{145}$	+	-	-	-	+		-	-	-	-	-		+	
$GUFF_{146}$	+	-	-	-	+		-	-	-	-	-		+	
$GUFF_{147}$	-	-	+	-	+		+	+	+	-	-		+	
$GUFF_{148}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{149}$	+	-	+	-	+		-	-	-	-	-		+	
$*GUFF_{150}$	+	-	+	-	+		-	-	-	-	-		+	
$*GUFF_{151}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{152}$	+	-	-	-	-		-	-	-	-	-		+	
$GUFF_{153}$	-	-	+	-	+		+	+	+	-	-		+	
$*GUFF_{154}$	+	-	+	-	+		-	-	-	-	-	-	+	
$GUFF_{155}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{156}$	+	-	+	-	-		-	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{157}$	-	-	+	-	+		+	+	+	-	-	+		
*$GUFF_{158}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{159}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{160}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{161}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{162}$	-	-	+	-	+		+	+	+	-	-	+		
*$GUFF_{163}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{164}$	+	-	+	-	+		-	-	-	-	-		+	
$GUFF_{165}$	+	-	+	-	+		-	-	-	-	-	+		
$GUFF_{166}$	-	-	+	-	-		+	+	+	-	-	+		
*$GUFF_{167}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{168}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{169}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{170}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{171}$	-	-	+	-	-		+	+	+	-	-	+		
*$GUFF_{172}$	-	-	+	-	-		+	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{173}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{174}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{175}$	-	-	+	-	-		+	+	+	-	-	+		
*$GUFF_{176}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{177}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{178}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{179}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{180}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{181}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{182}$	-	-	+	-	+		+	+	+	-	-	+		
*$GUFF_{183}$	+	-	+	-	+		-	-	-	-	-	+		
$GUFF_{184}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{185}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{186}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{187}$	-	-	+	-	+		+	+	+	-	-	+		
$GUFF_{188}$	-	-	+	-	-		+	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{189}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{190}$	-	-	-	-	-		-	-	-	+	-			+
$GUFF_{191}$	-	-	-	-	-		-	-	-	+	-			+
$GUFF_{192}$	-	-	-	-	-		-	-	-	+	-			+
$GUFF_{193}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{194}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{195}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{196}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{197}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{198}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{199}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{200}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{201}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{202}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{203}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{204}$	-	-	+	-	-		+	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{205}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{206}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{207}$	+	-	+	-	-		-	+	+	-	-	+		
$GUFF_{208}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{209}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{224}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{225}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{226}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{227}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{228}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{229}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{230}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{231}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{232}$	-	-	+	-	-		+	+	+	-	-	+		
$GUFF_{233}$	-	-	+	-	-		+	+	+	-	-	+		

Table 17: Domain affiliation of bacterial isolates

Bacterial isolate	TYE		NTYE		Mac + 20% NaCl	Light	Response to distilled water	GDEM	Type of pigment			Domain Identification		
	Penc +	Penc -	Penc +	Penc -					C_{40-50}	Chlor	Men	Haloarchaea	Eubacteria/Cyanobacteria	
$GUFF_{234}$	-	-	+	-	-		+	+	+	-	-	+		
$GUEFF_1$	-	+	-	-	-		-	-	-	-	+		+	
$GUEFF_2$	-	+	-	-	-		-	-	-	-	+		+	
$GUEFF_3$	-	+	-	-	-		-	-	-	-	+		+	
$GUEFF_4$	-	+	-	-	-		-	-	-	-	+		+	
$GUEFF_5$	-	+	-	-	-		-	-	-	-	+		+	
$GUEFF_6$	-	+	-	-	-		-	-	-	-	+		+	
*$GUEFF_7$	+	-	-	-	-		-	-	-	-	-		+	

***Bacterial isolates obtained from salt pan lands in monsoons and post monsoon,**
Others from salt crystallization stage

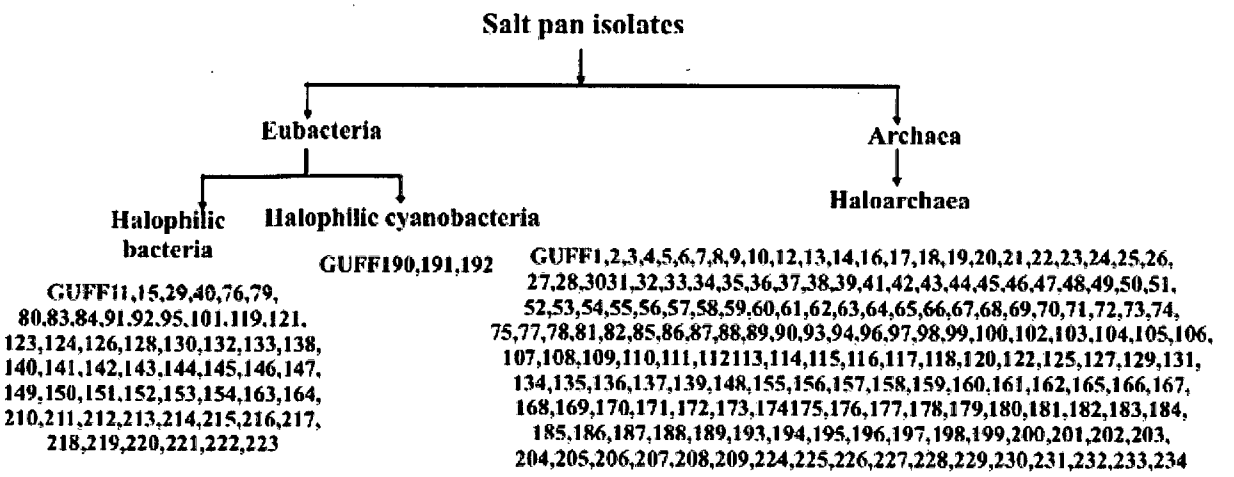


Fig. 4: Differentiation of bacterial isolates to their domains

Printed in green: Monsoon, Printed in pink: Shallow, Printed in black: Salt farming

Characterisation of isolated Cyanobacterial cultures

Salt pans showed coloured mat growth (**Plate 6**) spreading over metres, either on top of water or associated with salt crystals. Wet mount, Gram character and microscopic features (**Table 18**) indicated that they were *Spirulina sp.*, *Synechococcus sp.* and *Nodularia* sp.

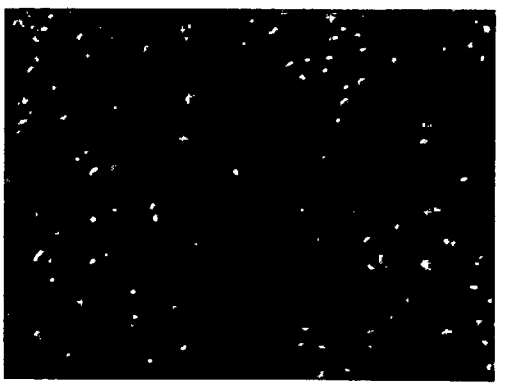


Plate 6: Cyanobacterial mat growth at Siridao salt pan

Table 18: Characterization of extremely halophilic Cyanobacterial isolates growing at 20% NaCl

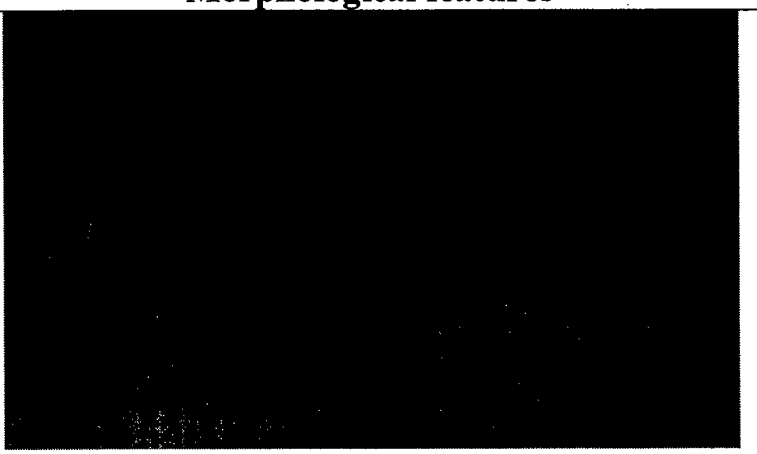
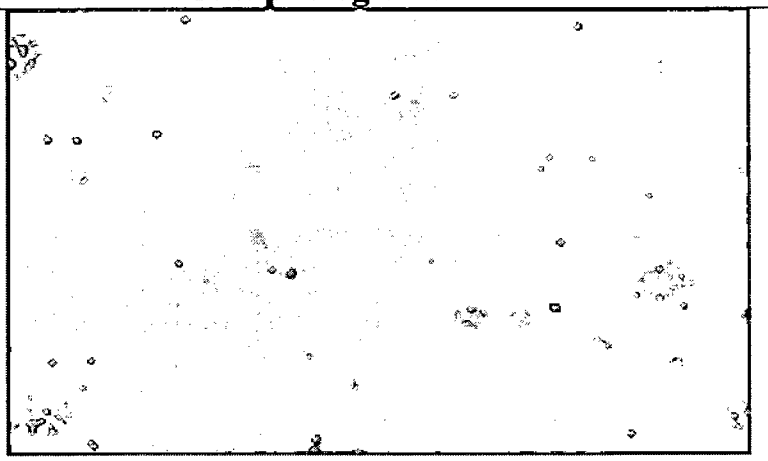
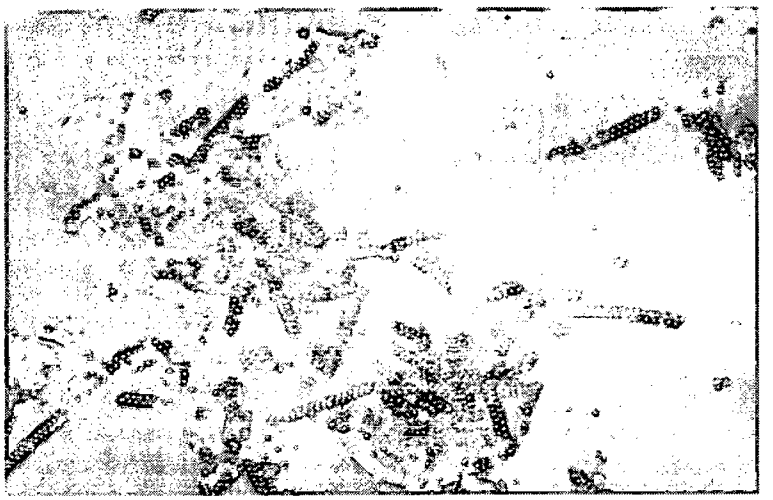
Isolate	Morphological features	Identification
$GUFF_{190}$	 Cells spherical to rod shaped, contain peripheral thylakoids; united into colonial aggregates by mucilage formation.	*Synechococcus* sp.

Table 18: Characterization of extremely halophilic Cyanobacterial isolates growing at 20% NaCl

Isolate	Morphological features	Identification
$GUFF_{191}$	 **Trichome straight, composed of discoid cells, surrounded by a thin sheath; immotile, heterocysts intercalary and terminal; akinetes in chains.**	***Nodularia* sp.**
$GUFF_{192}$		***Spirulina* sp.**

Characterisation of isolated Eubacterial cultures

Differentiation of isolated extremely halotolerant and euryhaline Eubacterial cultures

Gram character, morphological & biochemical features of eubacterial isolates **(Table 19)** were studied. The isolates were tentatively identified as per the Bergey's Manual upto the genus level. The Eubacterial cultures could be differentiated into three groups **(Fig. 5)**: i) Extremely halotolerant ii) euryhaline iii) bile salt and penicillin –ve eubacteria. The eubacterial community of salt pans is as shown in **Table 20**.

Table 19: Physiological and biochemical characteristics of eubacterial isolates

Bacteria	$GUFF_{11}$	$GUFF_{15}$	$GUFF_{29}$	$GUFF_{40}$	$GUFF_{76}$	$GUFF_{79}$	$GUFF_{80}$
Gram character	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Morphology	Pleio - morphic	Pleio - morphic	Pleio- morphic	Pleio - morphic	Cocci	Rods	Rods
Catalase	+	+	+	+	+	+	+
Oxidase	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Indole	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Nitratase	+	-	+	+	-	+	-
H_2S	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Gelatinase	+	+	+	+	-	+	+
PHA	-	-	-	-	-	+	+
Protease	+	+	+	+	-	+	+
Tween 80	-	-	-	-	-	-	
Amylase	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Citrate	+	+	+	+	-	+	+
Acetate	+	+	-	-	-	+	+
Malate	+	+	-	-	-	+	+
Pyruvate	+	+	+	+	+	+	+
Succinate	+	+	-	-	-	+	+
Lactate	+	-	-	-	-	+	+
Formate	+	+	-	-	-	+	+
Arginine	+	+	-	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+
pH 5	+	+	+	+	-	-	-
Tentative Identification	*Halomonas* sp.	*Halomonas* sp.	*Halomonas* sp.	*Pseudomonas* sp.	*Guconobacter* sp.	*Pseudomonas* sp.	*Halomaonas* sp.

Nd- not done

Table 19: Physiological and biochemical characteristics of eubacterial isolates

Bacteria	$GUFF_{83}$	$GUFF_{84}$	$GUFF_{91}$	$GUFF_{92}$	$GUFF_{95}$	$GUFF_{101}$	$GUFF_{119}$
Gram character	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Morphology	Cocci	Cocci	Rods	Rods	Cocci	Cocci	Cocci
Catalase	+	+	+	+	+	+	+
Oxidase	Nd	Nd	Nd	Nd	Nd	Nd	-
Indole	Nd	Nd	Nd	Nd	Nd	Nd	-
Nitratase	-	-	-	+	+	+	-
H_2S	Nd	Nd	Nd	Nd	Nd	Nd	-
Gelatinase	+	+	+		+	-	-
PHA	+	-	+	+	-	-	-
Protease	+	+	+	+	+	+	-
Tween 80	-	-	-	-	-	-	-
Amylase	+	+	+	+	+	+	-
Sorbitol	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Galacose	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	-
Acetate	+	+	-	+	-	-	-
Malate	+	+	-	+	-	-	-
Pyruvate	+	+	+	+	+	+	-
Succinate	+	+	+	+	+	-	-
Lactate	+	+	+	+	-	-	-
Formate	+	+	+	+	+	-	-
Arginine	-	+	+	-	-	+	-
Growth at: R.T.	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+
pH5	-	-	-	-	+	-	-
Tentative Identification	*Gluconobacter* sp.	*Gluconobacter* sp.	*Pseudomonas* sp.	*Halomonas* sp.	*Gluconobacter* sp.	*Acinetobacter* sp.	*Planococcus* sp.

Nd- not done

Table 19: Physiological and biochemical characteristics of eubacterial isolates

Bacteria	$GUFF_{121}$	$GUFF_{123}$	$GUFF_{124}$	$GUFF_{126}$	$GUFF_{128}$	$GUFF_{130}$	$GUFF_{132}$	$GUFF_{133}$
Gram character	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Catalase	+	+	+	+	+	+	+	+
Oxidase	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Indole	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Nitratase	-	-	+	-	-	-	+	+
H_2S	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Gelatinase	+	-	+	+	+	+	-	+
PHA	+	+	+	+	+	+	-	-
Protease	+	+	+	+	-	+	+	+
Tween 80	-	-	+	-	-	+	+	+
Amylase	+	+	+	+	+	+	+	-
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	-	-	-	-	-	-	-	-
Acetate	-	-	-	-	-	-	-	-
Malate	-	-	-	-	-	-	-	-
Pyruvate	-	-	-	-	-	-	-	-
Succinate	-	-	-	-	-	-	-	-
Lactate	-	-	-	-	-	-	-	-
Formate	-	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	+	+	+	+	+	+	-	-
Tentative Identification	*Acinetobacter* sp.	*Acinetobacter* sp.	*Acinetobacter* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Gluconobacter* sp.

Nd- not done

Table 19: Physiological and biochemical characteristics of eubacterial isolates

Bacteria	$GUFF_{138}$	$GUFF_{140}$	$GUFF_{141}$	$GUFF_{142}$	$GUFF_{144}$	$GUFF_{145}$	$GUFF_{146}$	$GUFF_{149}$
Gram character	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Catalase	+	+	+	+	+	+	+	+
Oxidase	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Indole	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Nitratase	+	-	+	+	+	-	-	+
H_2S	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Gelatinase	+	+	+	+	+	+	+	+
PHA	+	+	+	+	+	+	+	+
Protease	+	+	+	+	+	+	+	+
Tween 80	-	+	-	+	+	+	-	-
Amylase	-	-	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	-	-	-	-	-	-	-	-
Acetate	-	-	-	-	-	-	-	-
Malate	-	-	-	-	-	-	-	-
Pyruvate	-	-	-	-	-	-	-	-
Succinate	-	-	-	-	-	-	-	-
Lactate	-	-	-	-	-	-	-	-
Formate	-	-	-	-	-	-	-	-
Arginine	-	-	+	+	+	-	-	-
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	+	+	+	+	+	+	+	+
Tentative Identification	*Alteromonas aurantia*	*Pseudomonas* sp.	*Alteromonas aurantia*	*Alteromonas aurantia*	*Alcaligenes sp.*	*Alteromonas aurantia*	*Alteromonas rubra*	*Alcaligenes* sp.

Nd- not done

Table 19: Physiological and biochemical characteristics of eubacterial isolates

Bacteria	$GUFF_{150}$	$GUFF_{151}$	$GUFF_{152}$	$GUFF_{154}$	$GUFF_{163}$	$GUFF_{164}$
Gram character	+ve	-ve	-ve	+ve	-ve	-ve
Morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Catalase	+	+	+	+	+	+
Oxidase	Nd	Nd	Nd	-	Nd	Nd
Indole	Nd	Nd	Nd	-	Nd	Nd
Nitratase	+	+	+	-	+	+
H_2S	Nd	Nd	Nd	-	Nd	Nd
Gelatinase	+	+	+	+	+	+
PHA	-	+	+	-	+	+
Protease	+	+	+	+	+	+
Tween 80	-	-	-	-	-	-
Amylase	+	+	+	-	+	+
Sorbitol	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
Glucose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Ribose	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+
Xylose	+	+	+	+	+	+
Mannose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Citrate	-	-	-	-	-	-
Acetate	-	-	-	-	-	-
Malate	-	-	-	-	-	-
Pyruvate	-	-	-	-	-	-
Succinate	-	-	-	-	-	-
Lactate	-	-	-	-	-	-
Formate	-	-	-	-	-	-
Arginine	-	-	-	-	-	-
Growth at: R.T.	+	+	+	+	+	+
42°C	+	+	+	+	+	+
50°C	+	+	+	+	+	+
pH 5	+	+	+	-	-	-
Tentative Identification	*Planococcus* sp.	*Alcaligenes* sp.	*Alcaligenes* sp.	*Planococcus halophilus*	*Alcaligenes* sp.	*Acinetobacter* sp.

Nd- not done

Table 19: Physiological and biochemical characteristics of eubacterial isolates

Bacteria	$GUEFF_1$	$GUEFF_2$	$GUEFF_3$	$GUEFF_4$	$GUEFF_5$	$GUEFF_6$
Gram character, morphology	+ve Cocci	+ve Cocci	+ve Cocci	+ve Cocci	+ve Cocci	+ve Cocci
Pigmentation	Orange red	Cream	Yellow	Cream	Pale orange	White
Catalase	+	+	++	+	+	++
Oxidase	+	+	-	+	+	++
Motility	-	+	-	+	-	-
Pellicle	++	+	+	+	-	+
Sugar: Glucose	-	+	+	+	+	+
Sucrose	-	+	+	+	+	+
Fructose	-	+	+	+	+	+
Maltose	-	+	+	+	+	+
Starch	+	+	+	+	+	+
HL	FA	FA	FA	FA	FA	FA
Christensen's urea	-	+	-	+	-	-
Gelatin	+	++	-	-	-	-
H_2S	-	+	-	+	-	-
Growth at 37°C	+++	+	++++	+	+++	+++
Growth at 45°C	++	+	+++	+	++	++
NO_3	+	-	-	-	+	+
TSI : Colour change	-	Purplish blue	-	Purplish blue	-	-
growth	+	+	+	+	+	+
Indole	-	-	-	-	-	-
MR	-	-	-	-	-	-
VP	-	-	-	-	-	-
Citrate: Colour change	-	++	-	+	-	-
Growth	-	+	+	+	-	-
Tentative identification (3)	*Micrococcus roseus*	*Micrococcus varians*	*Micrococcus varians*	*Micrococcus varians*	*Micrococcus halobius*	*Micrococcus* sp.

+ growth, - no growth, FA facultative anaerobe

Table 19: Physiological and biochemical characteristics of eubacterial isolates

Isolates	$GUEFF_7$	$GUEFF_8$	$GUEFF_9$	$GUEFF_{10}$	$GUEFF_{11}$	$GUEFF_{12}$	$GUEFF_{13}$	$GUEFF_{14}$
Gram character	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Morphology	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Catalase	+	+	+	+	+	+	+	+
Oxidase	-	-	+	+	+	+	+	+
Sugar: Glucose	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
Sucrose	+	+	+	+	+	+	+	+
Fructose	-	+	+	+	+	+	+	+
Maltose	⊕	⊕	⊕	⊕	⊕	+	⊕	⊕
Starch	-	-	-	-	-	-	-	-
HL	FA	FA	FA	FA	FA	FA	FA	FA
Christensen's urea	+	+	+	+	+	-	+	+
Gelatin	-	-	-	-	-	-	-	-
NO_3	+	+	+	+	+	-	+	+
TSI : H_2S	+	+	+	+	+	+	+	+
Indole	+	+	+	+	+	+	+	+
MR	-	-	-	-	-	-	-	-
VP	+	+	-	-	-	-	-	-
Citrate	+	-	+	-	-	-	-	-
PPA	-	-	+	+	+	-	+	+
Tentative identification	*Klebseila* sp.	*Klebseila* sp.	*Proteus* sp.	*Proteus* sp.	*Proteus* sp.	*Salmonella* sp.	*Proteus* sp.	*Proteus* sp.

⊕ Acid and gas

Fig. 5: Retrieved extremely halotolerant eubacterial community of salt pans

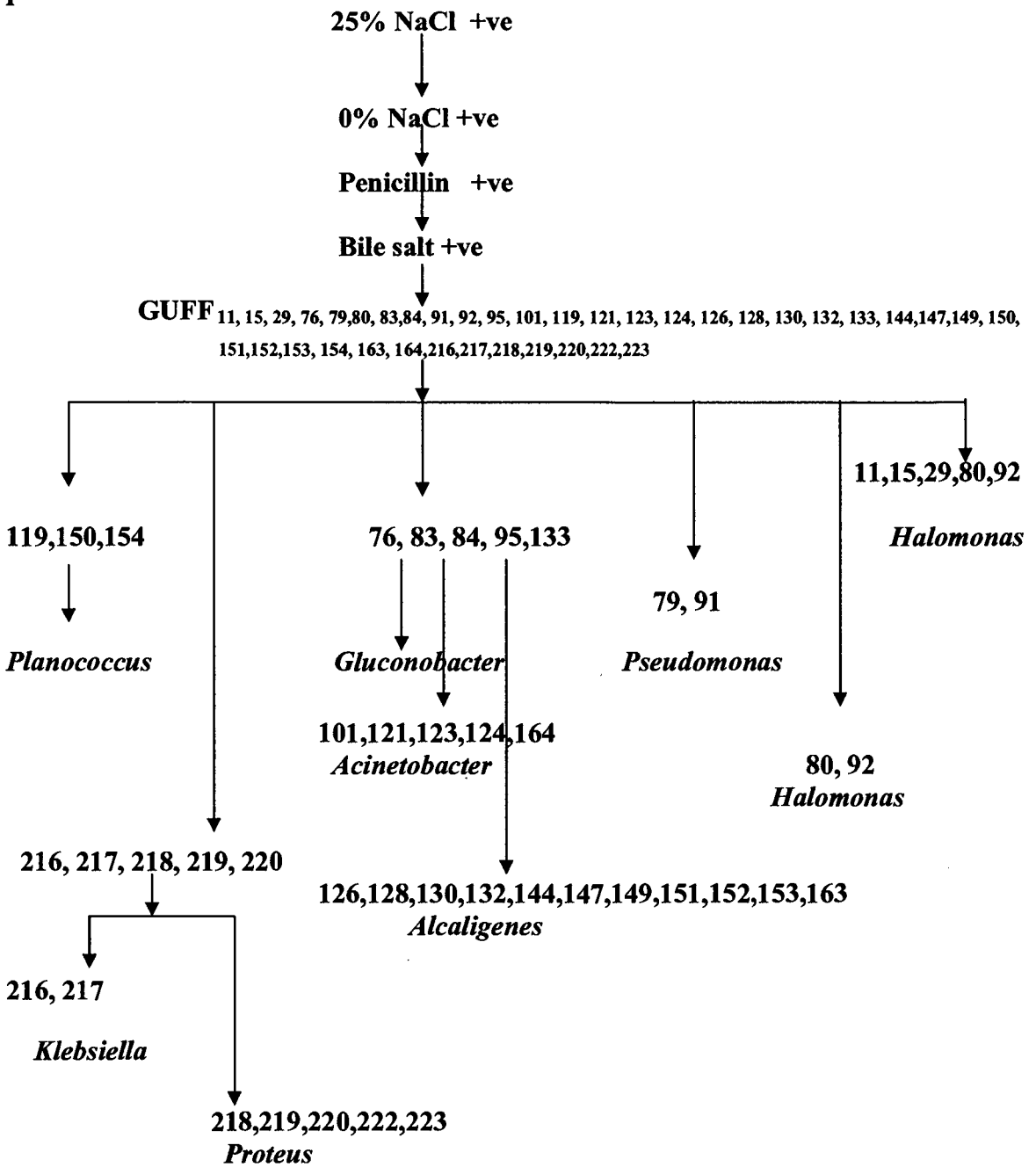


Fig. 5: Differentiation of euryhaline eubacteria

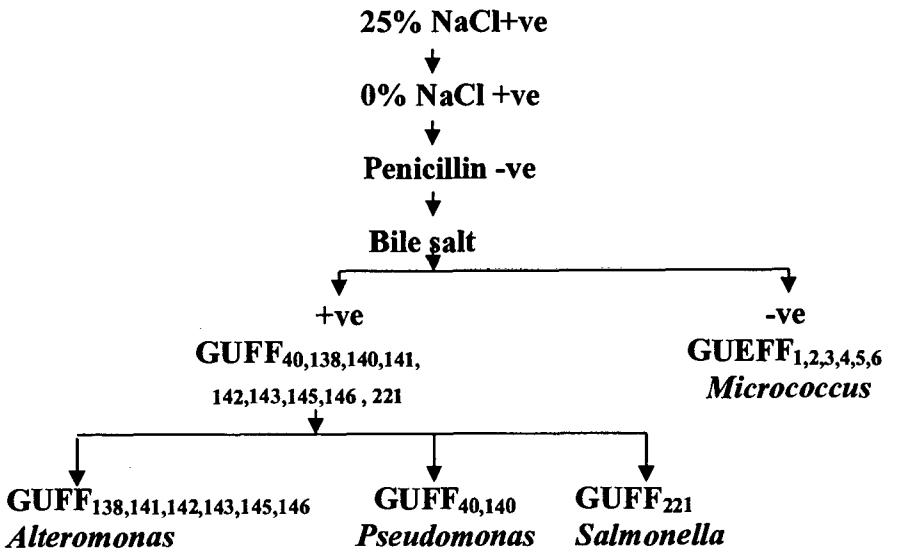


Table 20: Retrieved Eubacterial community of different salt pans during salt farming, monsoon and post monsoon

Salt pan	Samples from salt pan		
	Water	Brine	Sediment
A	-	*Pseudomonas* sp., *Alcaligenes* sp.	*Gluconobacter* sp.
Ag	-	*Alteromonas rubra*, *Alcaligenes* sp.	*Halomonas* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Alcaligenes* sp.
Ar	-	*Halomonas* sp.	*Acinetobacter* sp., *Alteromonas* sp.
N	-	*Pseudomonas* sp., *Alteromonas aurantia*, *Alteromonas* sp., *Gluconobacter* sp.	*Alcaligenes* sp., *Alteromonas aurantia*, *Alteromonas* sp.
R	*Acinetobacter* sp., *Klebsiella* sp.	*Planococcus halobius*, *Micrococcus roseus*, *Micrococcus varians*	*Alcaligenes* sp., *Micrococcus varians*, *Halomonas* sp., *Klebsiella* sp., *Proteus* sp., *Planococcus* sp.
S	*Acinetobacter* sp., *Proteus* sp., *Salmonella* sp.	*Alcaligenes* sp., *Gluconobacter* sp., *Micrococcus varians* , *Micrococcus halobius*.	*Alcaligenes* sp., *Micrococcus* sp., *Halomonas* sp., *Planococcus* sp., *Proteus* sp., *Gluconobacter* sp.

Differentiation of isolated Haloarchaeal cultures

Haloarchaea thus reported from various global laboratories are characterized on the basis of absolute requirement of NaCl for their growth at various specific pH, Mg^{2+} concentrations. One hundred and seventy-eight haloarchaea were firstly differentiated as in **Fig.6** on the basis of Penicillin, NaCl, bile salt. Bacterial cultures have been differentiated into 4 types : i) True haloarchaea- Penicillin +ve, 25% NaCl +ve, 0% NaCl –ve, bile salt –ve; ii) bile salt resistant haloarchaea; iii) haloarchaea producing penicillinase-Penicillin –ve, bile salt –ve, 25% NaCl+ve, 0% NaCl -ve; iv) euryhaline haloarchaea.

On the basis of pH, promoting their growth into : i) neutrophilic (pH 7); ii) weakly alkaline; iii) acidic; iv) normal pH range (pH 5 to pH 8.5); v) broad range (pH 5 to 10.5).

Differentiation of haloarchaeal isolates on basis of Mg^{2+} requirement for growth

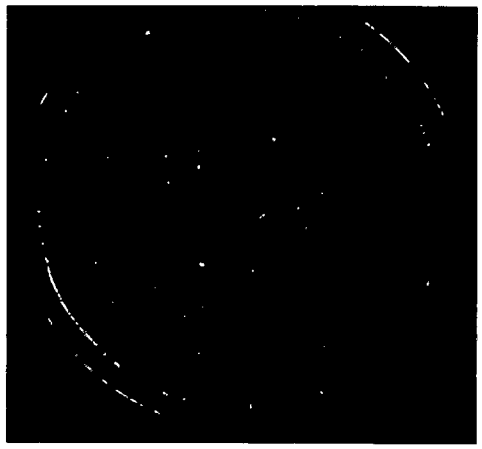
Secondly the haloarchaea from different pH groups were further differentiated on the basis of Mg^{2+} requirement for growth, yielded five groups **(Fig.6; Plate 7-9).** Haloarchaea could grow i) only at 10.0 mM ii) only at 790 mM iii) between 0.07-10 mM iv) between 0.07-790 mM v) between 10.0-790 mM.

Each of the group was further characterized on the basis of physiological & biochemical characteristics to a tentative genus of haloarchaea **(Plate 10; Table 21).**

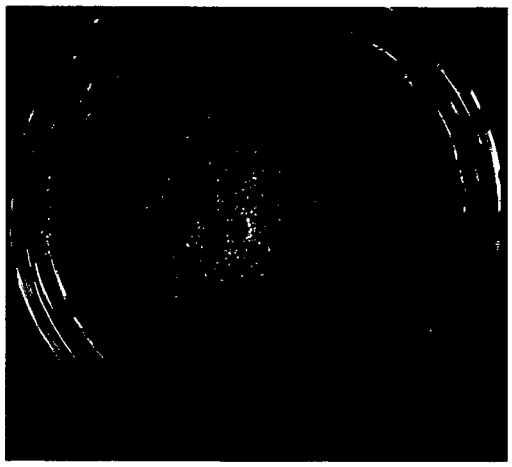
Biochemical characteristics of haloarchaeal isolates in various substrates

All the haloarchaeal isolates which were inoculated into various sugars - sorbitol, mannitol, glucose, sucrose, fructose, galactose, lactose, ribose, arabinose, xylose, mannose, maltose, produced acid, final pH was 4.0 to 6.0 after 5 days. However, $GUFF_{73}$, $GUFF_{76}$, $GUFF_{103}$, $GUFF_{104}$, $GUFF_{105}$, $GUFF_{108}$ and $GUFF_{112}$ did not grow on citrate. $GUFF_{13}$, $GUFF_{18}$,

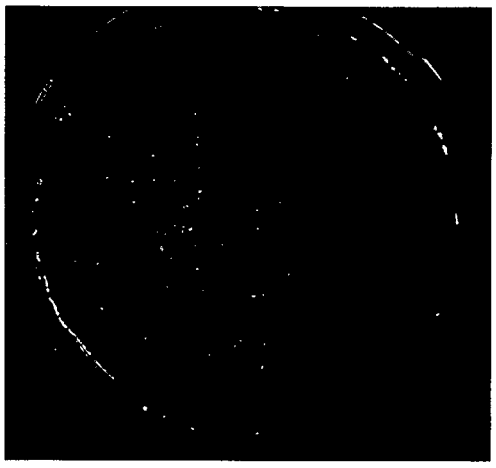
(a)



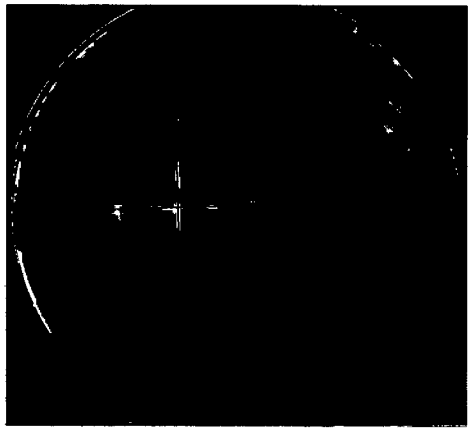
(b)



(c)



(d)



(e)

Plate 7: Growth of haloarchaea on (a) NTYE (b) PM (c) NGSM (d) HS (e) NTYE + Penicillin 700 U/m/Kanamycin 20 µg/ml.

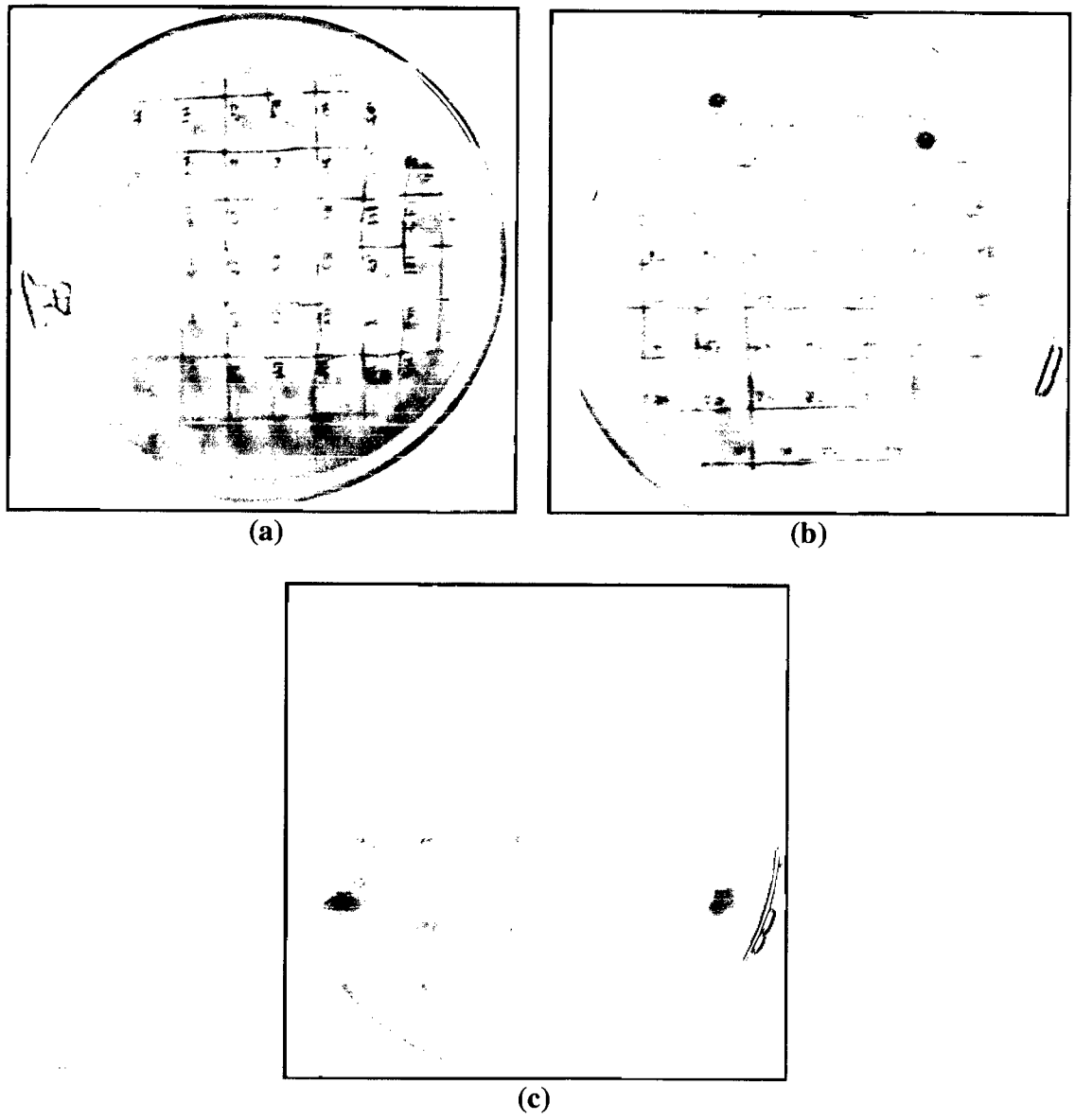


Plate 8: Growth of haloarchaea on NP, pH 8.5 (a) 10 mM Mg^{2+} (b) 0.7 mM Mg^{2+} (c) pH 10.5, 0.7 mM Mg^{2+}.

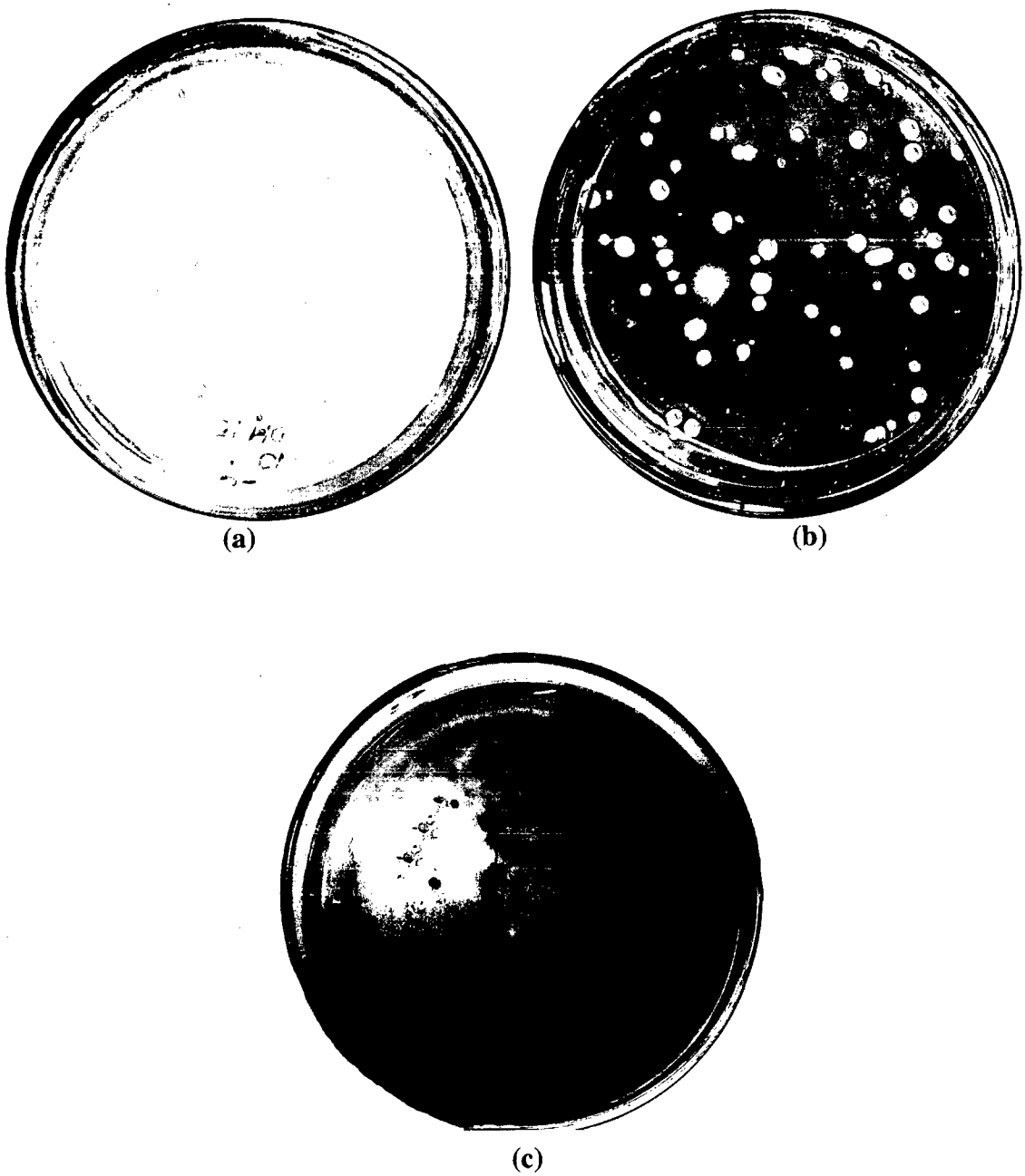


Plate 9: Haloarchaea growing on (a) NP, pH 10.5 some colonies showing antagonism ; NTYE, pH 5.0 - (b), (c) showing colonies with metallic sheen.

Fig. 6: Differentiation of haloarchaeal isolates on the basis of pH for growth

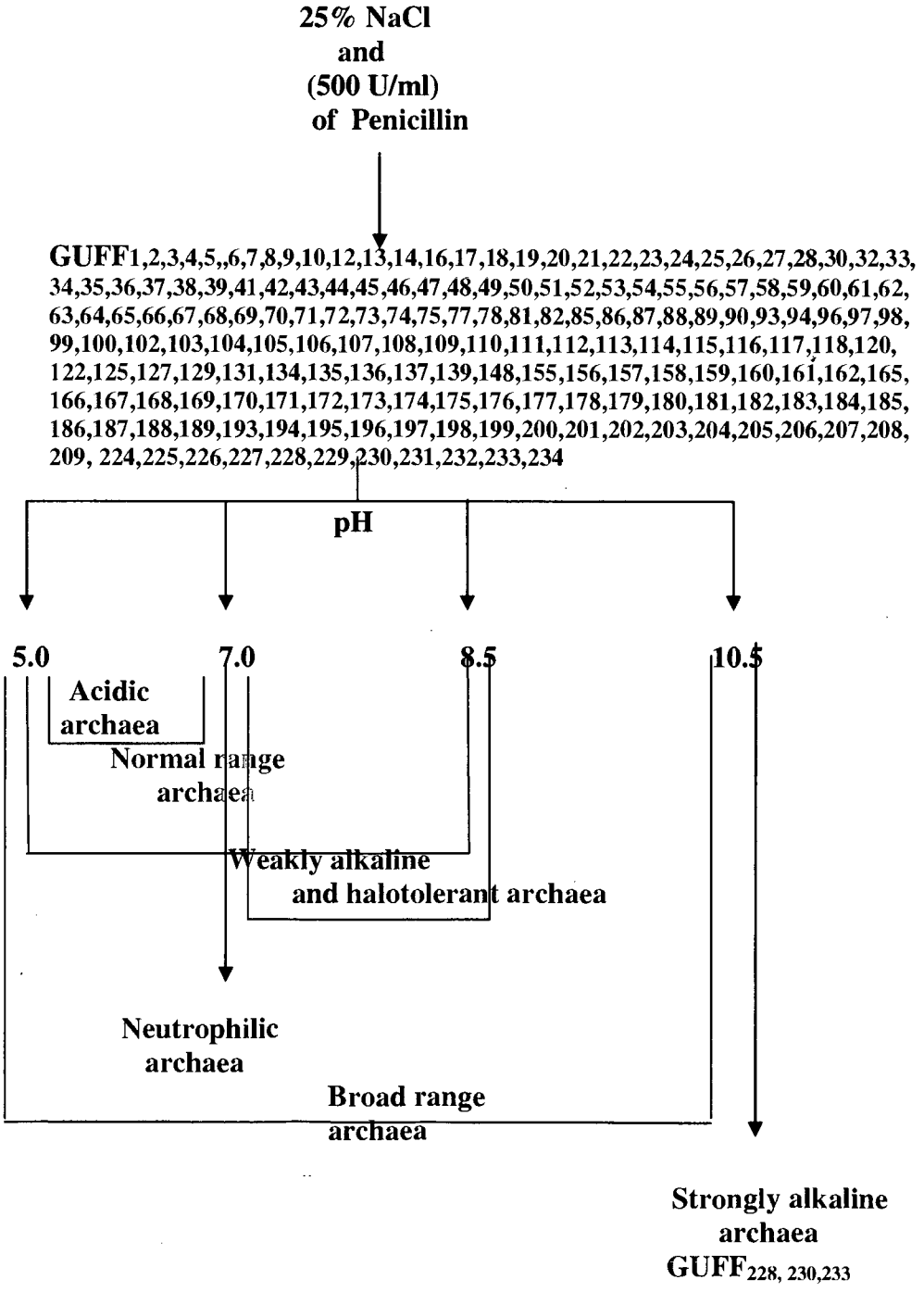


Fig. 6: Differentiation of acidophilic haloarchaeal isolates on the basis of Mg^{2+} requirement for growth

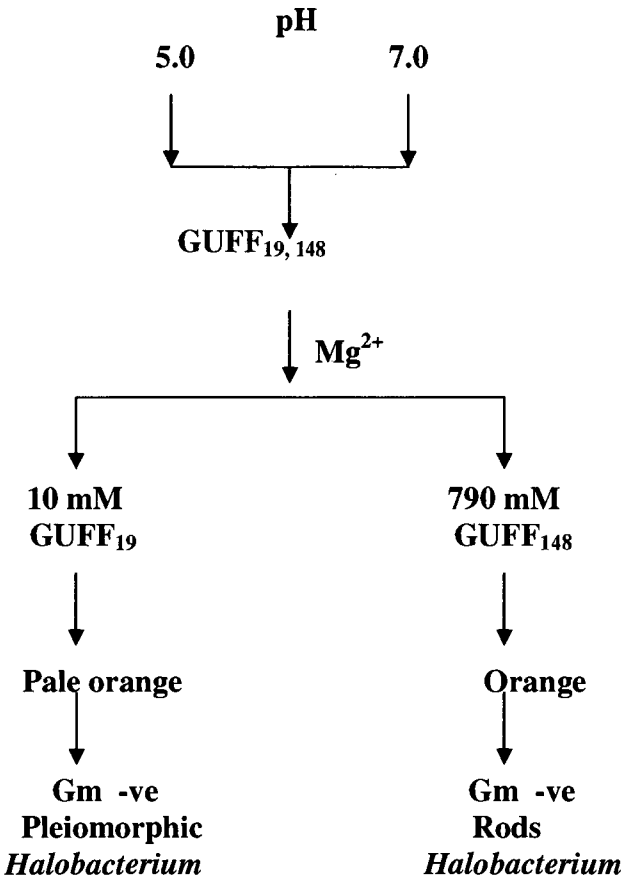


Fig. 6: Differentiation of halotolerant haloarchaea growing at

25% NaCl, 0% NaCl, Penicillin +ve

Bile salt -ve

GUFF1,5,8,13,17,21,24,30,31,36,74,77,78,81,85,86,89,90,94,97,98,100,103,105,106,107,108,109,110, 112,114,115,118,122,125,127,134,135,137,139,155,156,158,159,160,161,165,166,167,172,178,183,200, 203,207

Haloarchaea

Fig. 6: Differentiation of normal pH range haloarchaeal isolates on the basis of Mg^{2+} requirement for growth

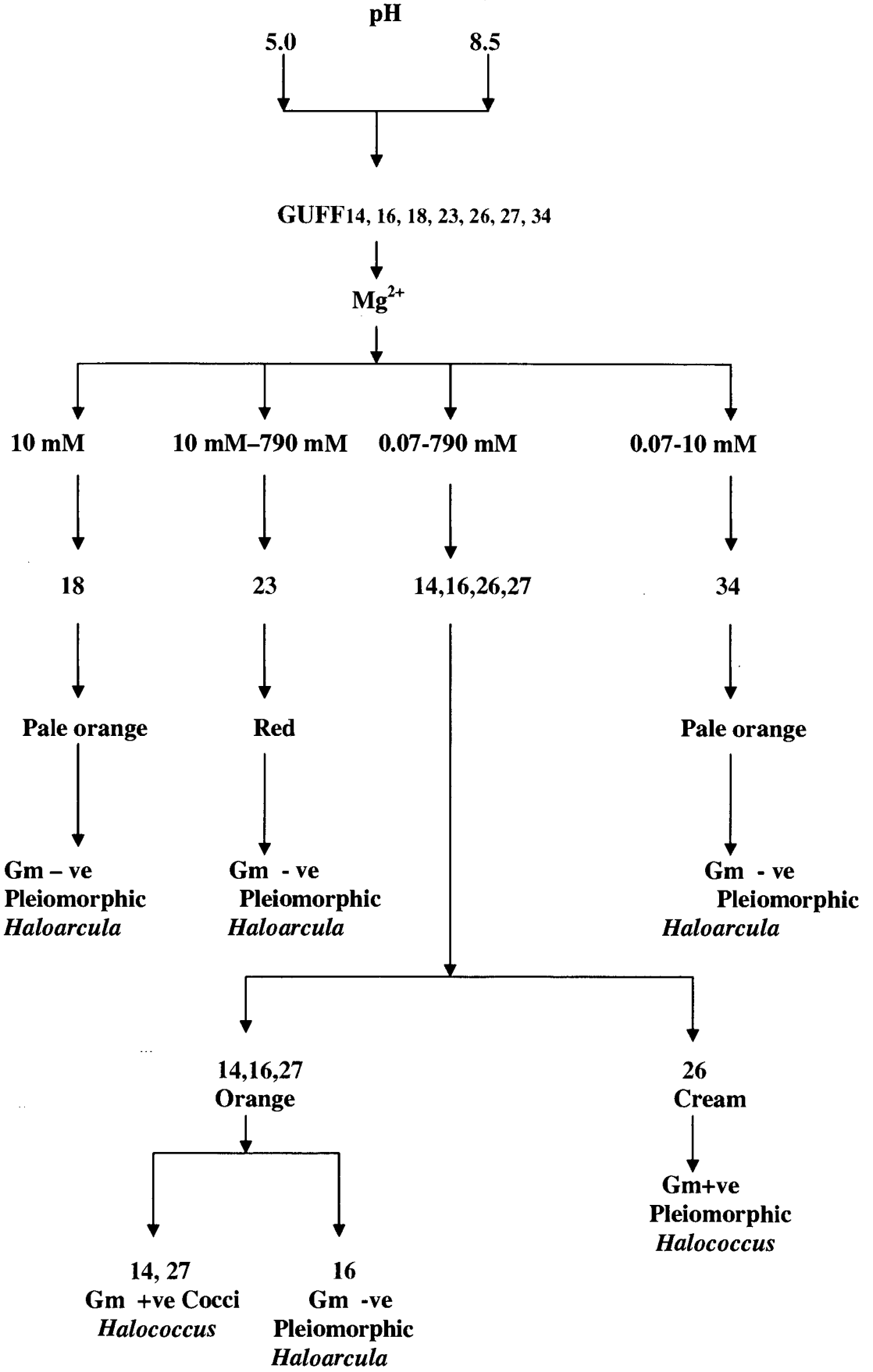


Fig. 6: Differentiation of neutrophilic haloarchaeal isolates on the basis of Mg^{2+} requirement for growth

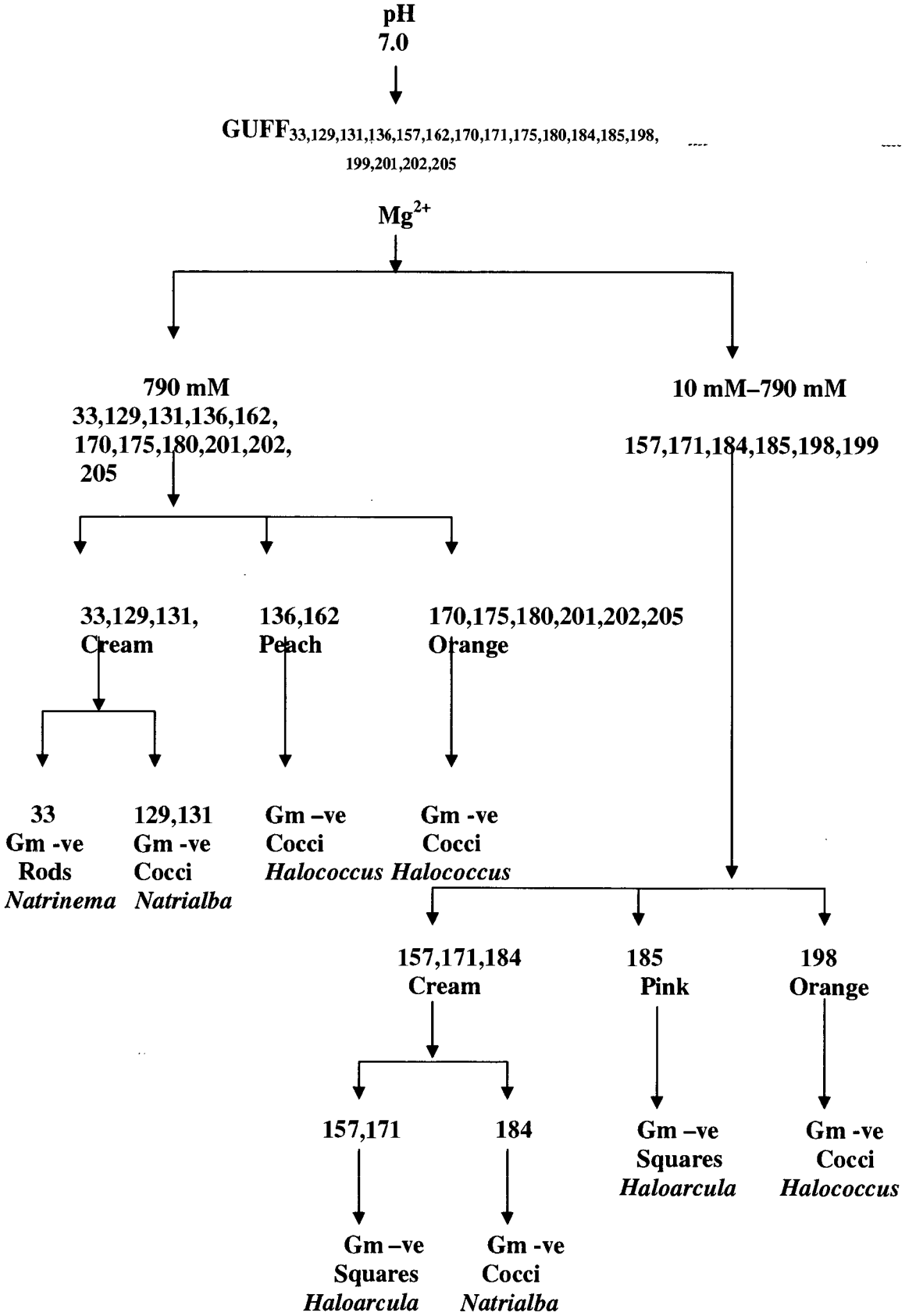


Fig. 6: Differentiation of weakly alkaline haloarchaeal isolates on the basis of Mg^{2+} requirement for growth

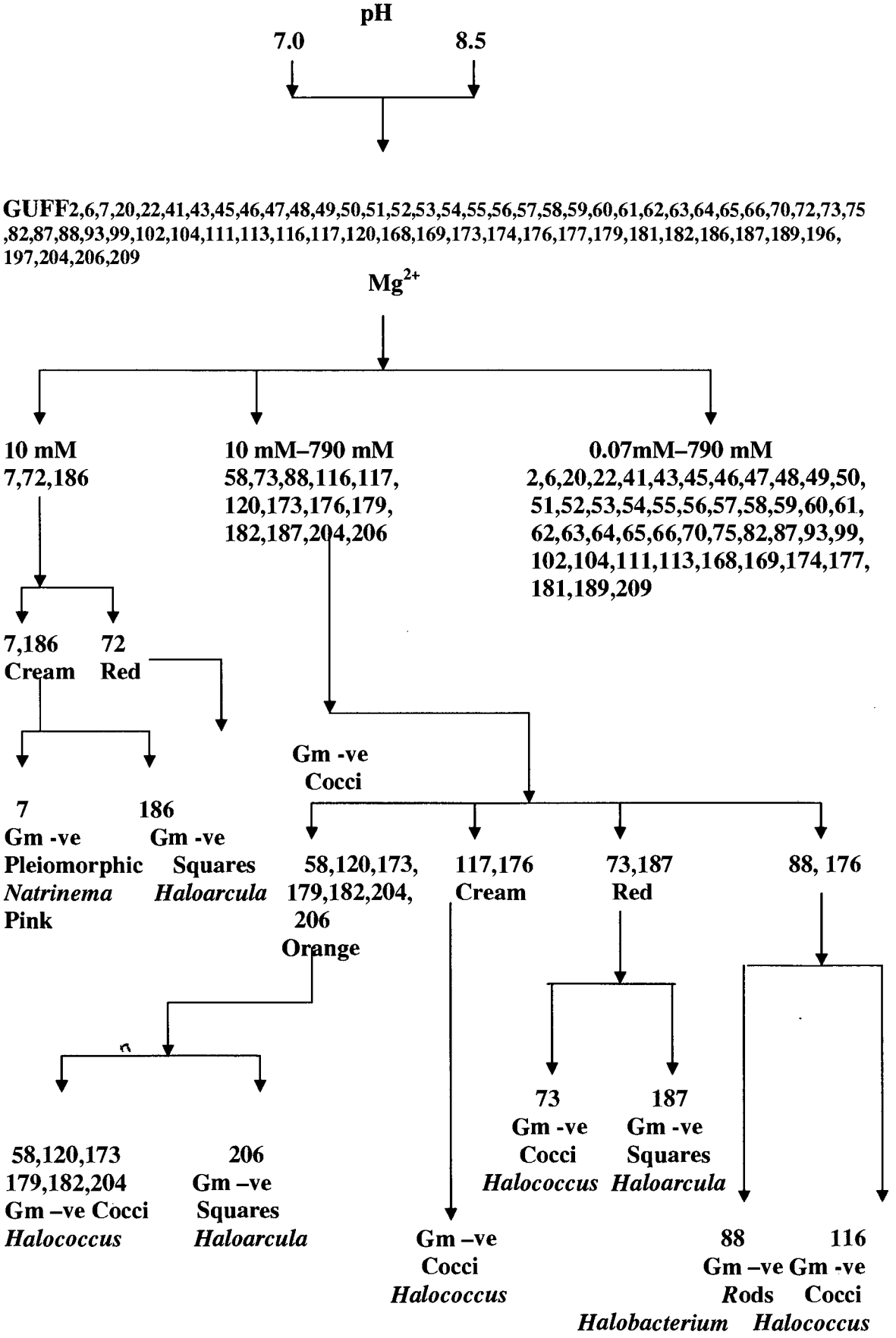


Fig. 6: Differentiation of neutrophilic haloarchaeal isolates on the basis of Mg^{2+} requirement for growth

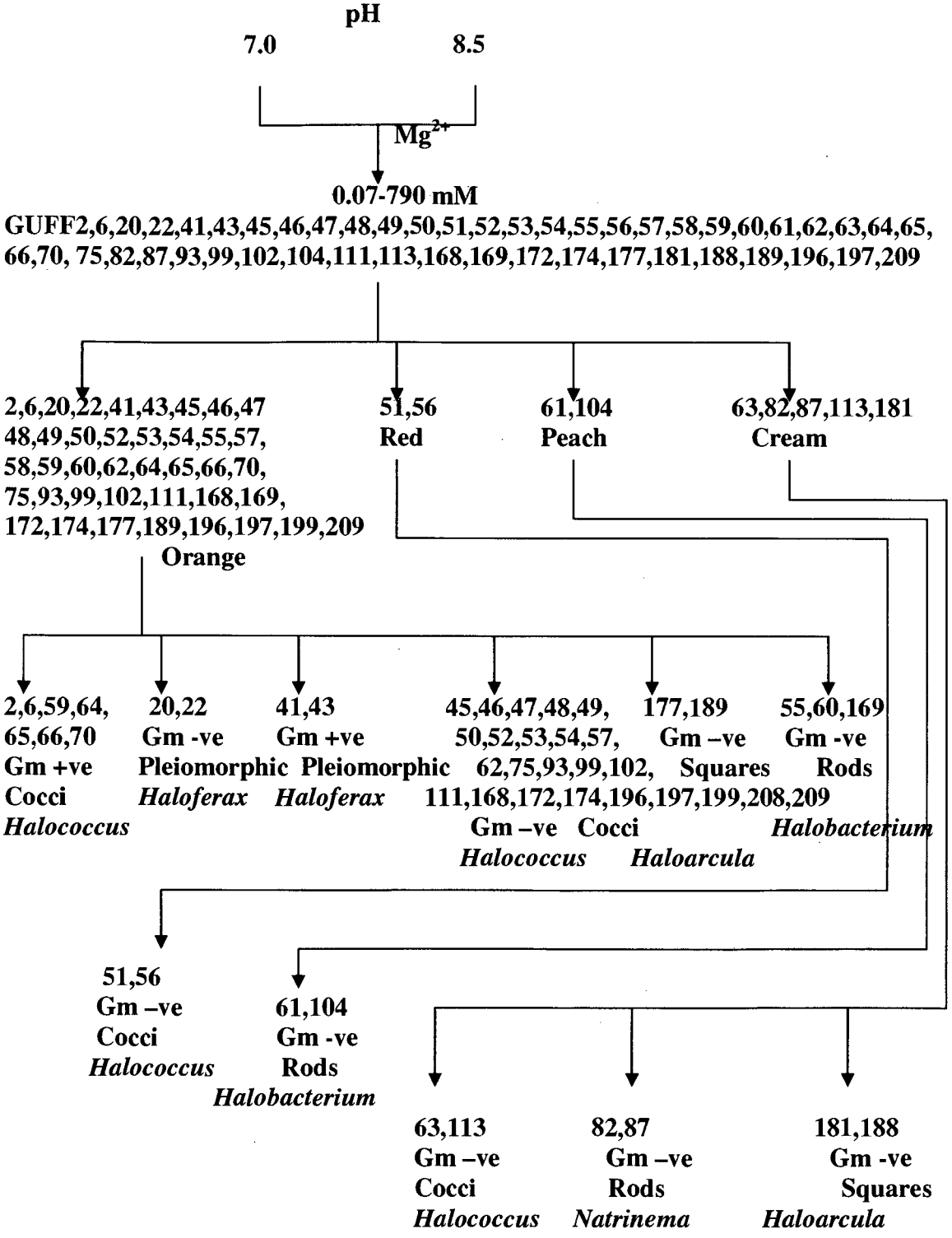
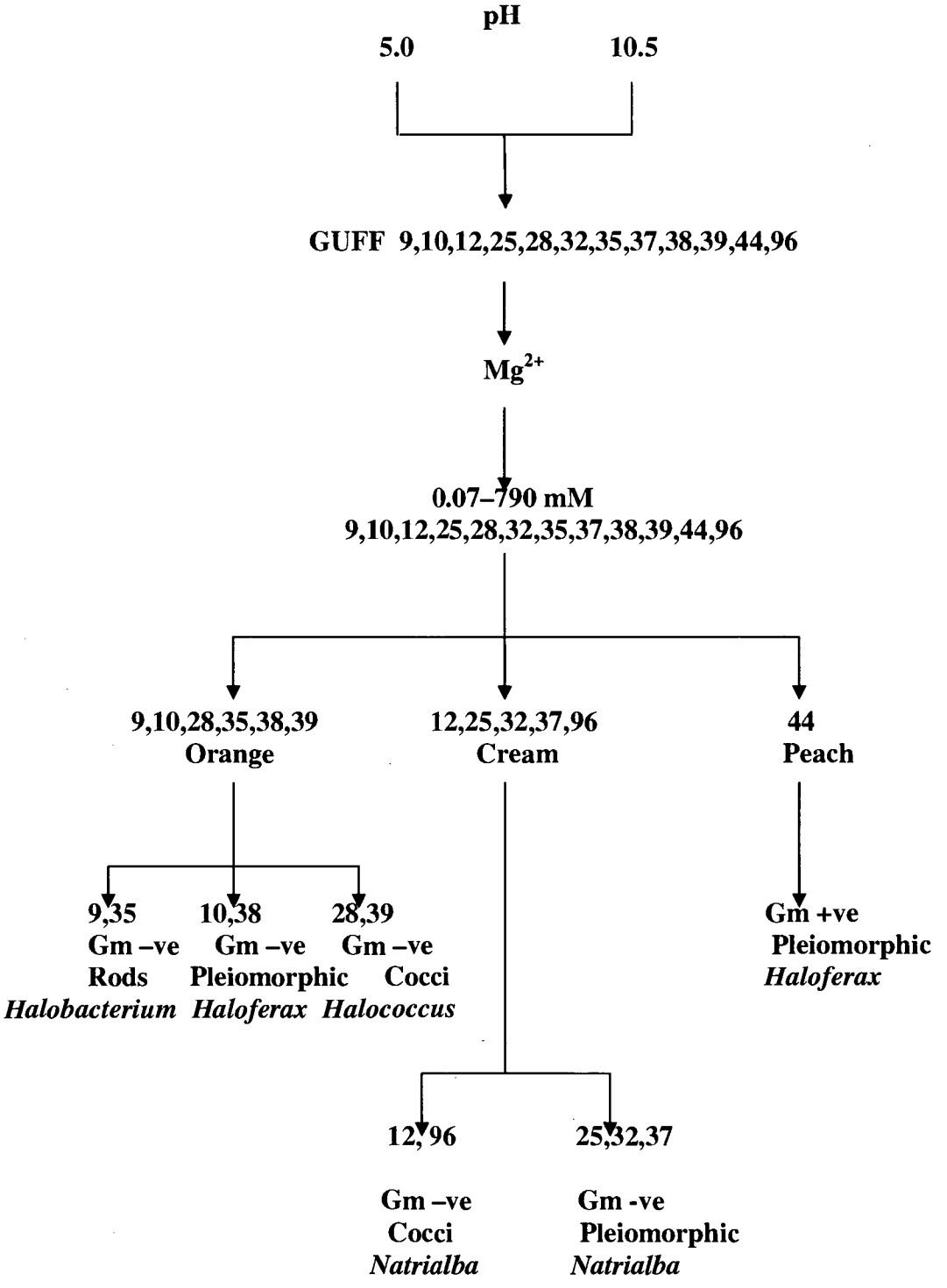


Fig. 6: Differentiation of haloarchaeal isolates with broad pH range, on the basis of Mg^{2+} requirement for growth



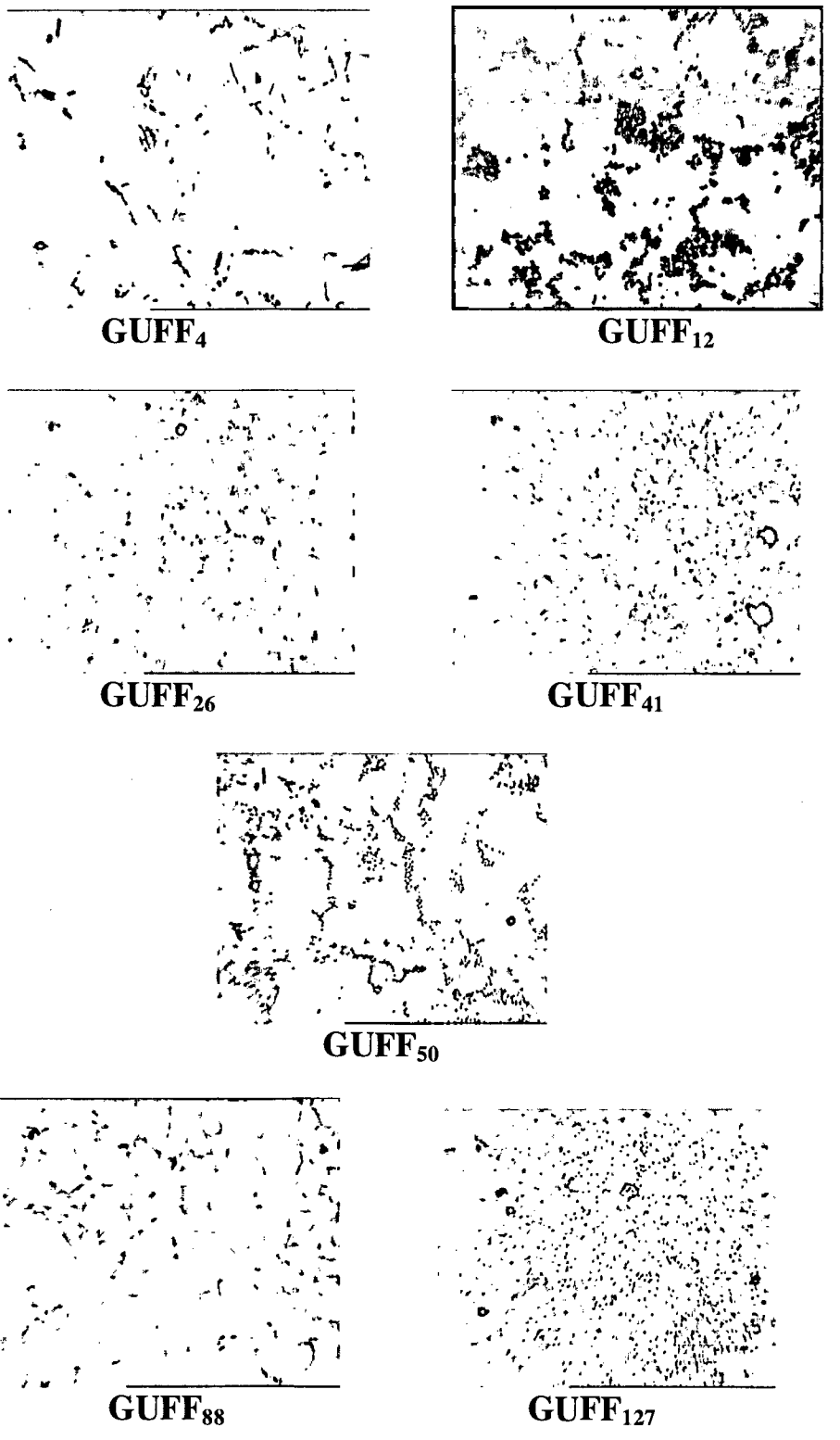


Plate 10: Gram stained photomicrographs of $GUFF_4$, $GUFF_{12}$, $GUFF_{26}$, $GUFF_{41}$, $GUFF_{50}$, $GUFF_{88}$, $GUFF_{127}$, on Olympus Magnus CH 20i Imaging Microscope (100x).

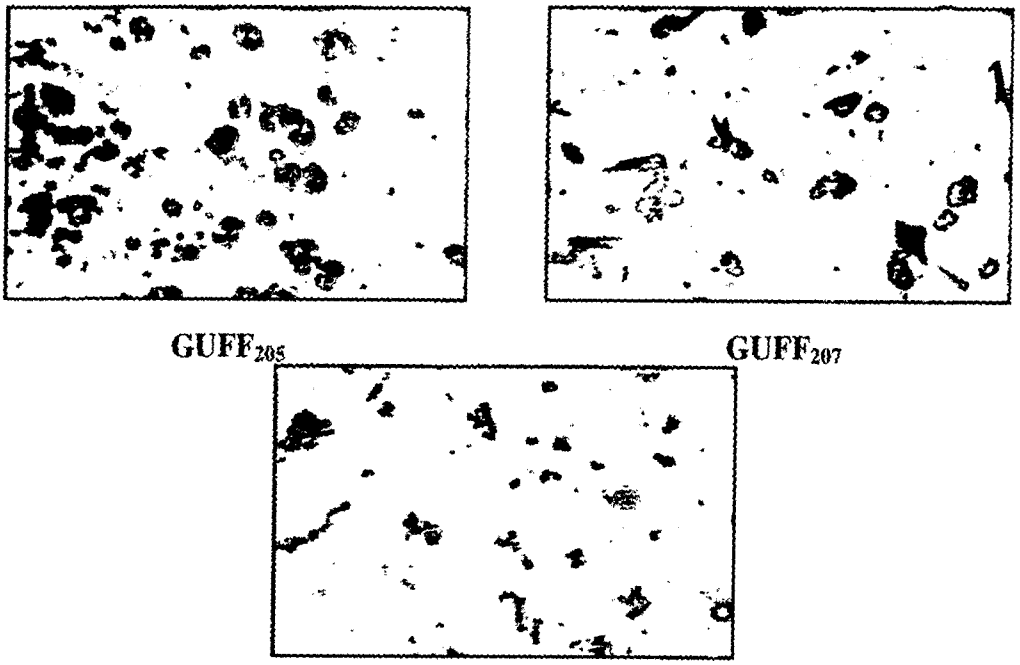


Plate 10: Gram's stained photomicrographs of $GUFF_{205}$, $GUFF_{207}$, $GUFF_{208}$ on Olympus Magnus CH 20i Imaging Microscope (100x)

$GUFF_{13}$, $GUFF_{19}$, $GUFF_{20}$, $GUFF_{25}$, $GUFF_{27}$, $GUFF_{28}$, $GUFF_{29}$, $GUFF_{31}$, $GUFF_{32}$, $GUFF_{33}$, $GUFF_{34}$, $GUFF_{35}$, $GUFF_{37}$, $GUFF_{40}$, $GUFF_{41}$, $GUFF_{43}$, $GUFF_{44}$, $GUFF_{45}$, $GUFF_{73}$, $GUFF_{76}$,

$GUFF_{22}$, $GUFF_{23}$, $GUFF_{24}$, $GUFF_{25}$, $GUFF_{28}$, $GUFF_{29}$, $GUFF_{30}$, $GUFF_{31}$, $GUFF_{32}$, $GUFF_{33}$, $GUFF_{34}$, $GUFF_{35}$, $GUFF_{36}$, $GUFF_{37}$, $GUFF_{38}$, $GUFF_{39}$, $GUFF_{40}$, $GUFF_{41}$, $GUFF_{43}$, $GUFF_{44}$, $GUFF_{76}$, $GUFF_{87}$, $GUFF_{91}$, $GUFF_{94}$, $GUFF_{95}$, $GUFF_{98}$, $GUFF_{101}$, $GUFF_{103}$, $GUFF_{104}$, $GUFF_{106}$, $GUFF_{109}$, $GUFF_{110}$, $GUFF_{111}$, $GUFF_{113}$, $GUFF_{157}$ did grow on acetate **(Table 21).**

$GUFF_{13}$, $GUFF_{17}$, $GUFF_{19}$, $GUFF_{21}$, $GUFF_{22}$, $GUFF_{23}$, $GUFF_{25}$, $GUFF_{29}$, $GUFF_{30}$, $GUFF_{32}$, $GUFF_{33}$, $GUFF_{34}$, $GUFF_{35}$, $GUFF_{36}$, $GUFF_{37}$, $GUFF_{38}$, $GUFF_{39}$, $GUFF_{40}$, $GUFF_{41}$, $GUFF_{44}$, $GUFF_{46}$, $GUFF_{73}$, $GUFF_{75}$, $GUFF_{76}$, $GUFF_{81}$, $GUFF_{85}$, $GUFF_{88}$, $GUFF_{91}$, $GUFF_{94}$, $GUFF_{95}$, $GUFF_{96}$, $GUFF_{97}$, $GUFF_{98}$, $GUFF_{101}$, $GUFF_{106}$, $GUFF_{107}$, $GUFF_{108}$, $GUFF_{109}$, $GUFF_{111}$, $GUFF_{112}$, $GUFF_{113}$ did not grow in malate.

$GUFF_{31}$, $GUFF_{34}$, $GUFF_{36}$, did not grow on pyruvate, while other isolates could grow. $GUFF_{13}$, $GUFF_{17}$, $GUFF_{18}$, $GUFF_{19}$, $GUFF_{22}$, $GUFF_{23}$, $GUFF_{24}$, $GUFF_{27}$, $GUFF_{28}$, $GUFF_{29}$, $GUFF_{30}$, $GUFF_{31}$, $GUFF_{32}$, $GUFF_{34}$, $GUFF_{35}$, $GUFF_{36}$, $GUFF_{37}$, $GUFF_{38}$, $GUFF_{39}$, $GUFF_{40}$, $GUFF_{41}$, $GUFF_{44}$, $GUFF_{65}$, $GUFF_{73}$, $GUFF_{76}$, $GUFF_{81}$, $GUFF_{85}$, $GUFF_{93}$, $GUFF_{101}$, $GUFF_{103}$, $GUFF_{104}$, $GUFF_{105}$, $GUFF_{106}$, $GUFF_{107}$, $GUFF_{109}$, $GUFF_{110}$, $GUFF_{112}$ and $GUFF_{113}$ did not grow on succinate.

$GUFF_{12}$, $GUFF_{13}$, $GUFF_{15}$, $GUFF_{19}$, $GUFF_{21}$, $GUFF_{26}$, $GUFF_{27}$, $GUFF_{28}$, $GUFF_{29}$, $GUFF_{30}$, $GUFF_{31}$, $GUFF_{32}$, $GUFF_{34}$, $GUFF_{35}$, $GUFF_{36}$, $GUFF_{37}$, $GUFF_{38}$, $GUFF_{39}$, $GUFF_{40}$, $GUFF_{41}$, $GUFF_{42}$, $GUFF_{43}$, $GUFF_{73}$, $GUFF_{76}$, $GUFF_{81}$, $GUFF_{85}$, $GUFF_{88}$, $GUFF_{94}$, $GUFF_{95}$, $GUFF_{96}$, $GUFF_{97}$, $GUFF_{98}$, $GUFF_{99}$, $GUFF_{101}$, $GUFF_{102}$, $GUFF_{105}$, $GUFF_{106}$, $GUFF_{107}$, $GUFF_{108}$, $GUFF_{109}$, $GUFF_{110}$, $GUFF_{112}$, $GUFF_{113}$, did not grow on lactate.

$GUFF_{81}$, $GUFF_{85}$, $GUFF_{93}$, $GUFF_{101}$, $GUFF_{103}$, $GUFF_{104}$, $GUFF_{105}$, $GUFF_{106}$, $GUFF_{109}$, $GUFF_{111}$, $GUFF_{112}$ did not grow on formate.

$GUFF_{16}$, $GUFF_{17}$, $GUFF_{22}$, $GUFF_{28}$, $GUFF_{29}$, $GUFF_{32}$, $GUFF_{34}$, $GUFF_{35}$, $GUFF_{83}$, $GUFF_{92}$, $GUFF_{95}$, $GUFF_{97}$, $GUFF_{99}$, did not grow on arginine

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_1$	$GUFF_2$	$GUFF_3$	$GUFF_4$	$GUFF_5$	$GUFF_6$	$GUFF_7$	$GUFF_8$
Gram character	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
Morphology	Pleio - morphic	Cocci	Rods	Rods	Pleio-morphic	Cocci	Pleio - morphic	Pleio-morphic
PHA	+	-	-	-	+	+	-	-
Gelatinase	+	+	+	-	+	+	-	+
Protease	+	-	-	-	+	-	-	-
Tween 80	-	-	-	-	+	-	-	-
Amylase	+	-	+	+	+	+	-	+
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Acetate	+	+	+	+	+	+	+	+
Malate	+	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+	+
Succinate	+	+	+	+	+	+	+	+
Lactate	+	+	+	+	+	+	+	+
Formate	+	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	+	-	-	-	+	-	-	-
0% NaCl	+	-	-	-	+	-	-	-
12.5% NaCl	+	+	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+	+	+
Tentative Identification	Halotolerant Haloarchaea	*Halococcus saccharolyticus*	*Haloarcula* sp.	*Halobacterium* sp.	Halotolerant Haloarchaea	*Halococcus salifodinae*	*Natrinema* sp.	Halotolerant Haloarchaea

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_9$	$GUFF_{10}$	$GUFF_{12}$	$GUFF_{13}$	$GUFF_{14}$	$GUFF_{16}$	$GUFF_{17}$	$GUFF_{18}$
Gram character	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
Morphology	Rods	Pleio-morphic	Cocci	Pleio-morphic	Cocci	Pleio - morphic	Pleio - morphic	Pleio - morphic
PHA	-	+	+	+	-	-	+	-
Gelatinase	+	+	+	+	+	+	+	+
Protease	+	+	+	-	+	+	+	-
Tween 80	-	-	+	-	+	+	-	+
Amylase	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Acetate	+	+	+	-	+	+	+	-
Malate	+	+	+	-	+	+	-	+
Pyruvate	+	+	+	+	+	+	+	+
Succinate	+	+	+	-	+	+	-	-
Lactate	+	+	-	-	+	+	+	+
Formate	+	+	+	-	+	+	+	+
Arginine	+	+	+	+	+	-	-	+
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	+	+	+	-	+	+	+	+
0% NaCl	+	+	+	-	+	+	+	+
12.5%NaCl	+	+	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+	+	+
Tentative Identification	*Halobacterium* sp.	*Haloferax* sp.	*Natrialba* sp.	Halotolerant Haloarchaea	*Halococcus* sp.	*Haloarcula* sp.	Halotolerant Haloarchaea	*Haloarcula* sp.

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_{19}$	$GUFF_{20}$	$GUFF_{21}$	$GUFF_{22}$	$GUFF_{23}$	$GUFF_{24}$	$GUFF_{25}$	$GUFF_{26}$
Gram character	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Morphology	Pleio-morphic	Pleio - morphic	Pleio-morphic	Pleio - morphic	Pleio - morphic	Pleio - morphic	Pleio - morphic	Pleio - morphic
PHA	-	-	-	-	-	-	-	+
Gelatinase	+	+	+	-	+	-	-	+
Protease	+	-	+	+	+	+	+	+
Tween 80	-	+	-	-	-	+	-	-
Amylase	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Acetate	+	+	+	-	-	-	-	+
Malate	-	+	-	-	-	+	-	+
Pyruvate	+	+	+	+	+	+	+	+
Succinate	-	+	+	-	-	-	+	+
Lactate	-	+	-	+	+	+	+	-
Formate	-	-	+	+	+	+	-	+
Arginine	+	+	+	-	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	+	-	+	-	+	+	+	+
0% NaCl	+	+	+	-	-	+	-	-
12.5%NaCl	+	+	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+	+	+
Tentative Identification	*Halobacterium* sp.	*Haloferax* sp.	Halotolerant Haloarchaea	*Haloferax sp.*	*Haloarcula* sp.	Halotolerant Haloarchaea	*Natrialba* sp.	*Halococcus* sp.

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_{27}$	$GUFF_{28}$	$GUFF_{30}$	$GUFF_{31}$	$GUFF_{32}$	$GUFF_{33}$	$GUFF_{34}$	$GUFF_{35}$
Gram character	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Morphology	Cocci	Cocci	Pleio-morphic	Pleio-morphic	Pleio - morphic	Rods	Pleio-morphic	Rods
PHA	+	-	-	+	-	-	-	+
Gelatinase	-	-	-	+	+	+	+	+
Protease	+	+	+	+	+	+	+	-
Tween 80	-	-	-	-	-	-	+	-
Amylase	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Acetate	+	-	-	-	-	-	-	-
Malate	+	+	-	+	-	-	-	-
Pyruvate	+	+	+	-	+	+	-	+
Succinate	-	-	-	-	-	+	-	-
Lactate	-	-	-	-	-	+	-	-
Formate	-	-	+	-	-	-	-	-
Arginine	+	-	+	+	-	+	-	-
Growth at R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	+	+	+	+	+	+	+	+
0% NaCl	-	-	+	+	-	-	-	-
12.5%NaCl	+	+	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+	+	+
Tentative Identification	*Halococcus* sp.	*Halococcus* sp.	Halotolerant Haloarchaea	Halotolerant Haloarchaea	*Natrialba* sp.	*Natrinema* sp.	*Haloarcula* sp.	*Halobacterium* sp.

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_{36}$	$GUFF_{37}$	$GUFF_{38}$	$GUFF_{39}$	$GUFF_{41}$	$GUFF_{43}$	$GUFF_{44}$	$GUFF_{45}$
Gram character	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve
Morphology	Pleio - morphic	Pleio - morphic	Pleio - morphic	Cocci	Pleio- morphic	Pleio - morphic	Pleio - morphic	Cocci
PHA	-	-	+	+	-	-	+	+
Gelatinase	+	+	-	+	+	+	+	+
Protease	+	+	+	+	+	+	+	+
Tween 80	-	+	+	-	+	-	-	+
Amylase	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Acetate	-	-	-	-	-	-	-	+
Malate	-	-	-	-	-	+	-	+
Pyruvate	-	+	+	+	+	+	+	+
Succinate	-	-	-	-	-	+	-	+
Lactate	-	-	-	-	-	-	+	+
Formate	+	-	+	+	-	-	-	-
Arginine	+	+	+	+	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	+	+	+	+	+	+	+	+
0% NaCl	+	-	-	-	-	-	-	-
12.5%NaCl	+	+	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+	+	+
Tentative Identification	Halotolerant Haloarchaea	*Natrialba* sp.	*Haloferax* sp.	*Halococcus* sp.	*Haloferax* sp.	*Haloferax* sp.	*Haloferax* sp.	*Halococcus* sp.

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_{46}$	$GUFF_{47}$	$GUFF_{48}$	$GUFF_{49}$	$GUFF_{50}$	$GUFF_{51}$	$GUFF_{52}$	$GUFF_{53}$
Gram character	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
PHA	-	-	-	-	+	-	-	-
Gelatinase	+	+	+	+	+	+	+	+
Protease	+	+	+	+	+	+	+	+
Tween 80	+	-	-	-	-	-	-	-
Amylase	+	+	+	+	+	+	-	-
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Acetate	+	+	+	+	+	+	+	+
Malate	-	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+	+
Succinate	+	+	+	+	+	+	+	+
Lactate	+	+	+	+	+	+	+	+
Formate	+	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	+	+	+	+	+	+	-	-
0% NaCl	-	-	-	-	-	-	-	-
12.5%NaCl	+	+	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+	+	+
Tentative Identification	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_{54}$	$GUFF_{55}$	$GUFF_{56}$	$GUFF_{57}$	$GUFF_{58}$	$GUFF_{59}$	$GUFF_{60}$	$GUFF_{61}$
Gram character	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Morphology	Cocci	Rods	Cocci	Cocci	Cocci	Cocci	Rods	Rods
PHA	-	+	+	+	+	+	+	-
Gelatinase	+	+	+	+	+	+	+	-
Protease	+	+	+	+	+	+	+	+
Tween 80	+	-	-	-	-	-	-	-
Amylase	+	+	+	+	+	+	+	-
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Acetate	+	+	+	+	+	+	+	+
Malate	+	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+	+
Succinate	+	+	+	+	+	+	+	+
Lactate	+	+	+	+	+	+	+	+
Formate	+	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	+	-	-	+	+	+	+	+
0% NaCl	-	-	-	-	-	-	-	-
12.5%NaCl	+	+	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+	+	+
Tentative Identification	*Halococcus* sp.	*Halobacterium* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halobacterium* sp.	*Halobacterium* sp.

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_{62}$	$GUFF_{63}$	$GUFF_{64}$	$GUFF_{65}$	$GUFF_{66}$	$GUFF_{69}$	$GUFF_{70}$	$GUFF_{72}$
Gram character	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve
Morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Squares	Cocci	Cocci
PHA	-	-	+	+	+	+	-	+
Gelatinase	-	-	+	+	+	+	-	+
Protease	+	+	+	+	+	+	+	-
Tween 80	+	-	+	-	-	+	-	+
Amylase	+	+	-	-	-	+	+	-
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Acetate	+	+	+	+	+	+	+	+
Malate	+	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+	+
Succinate	+	+	+	-	+	+	+	+
Lactate	+	+	+	+	+	+	+	+
Formate	+	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	-	+	-	+	+	+	-	-
0% NaCl	-	-	-	-	-	-	-	-
12.5% NaCl	+	+	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+	+	+
Tentative Identification	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halococcus* sp.	*Halorubrum* sp.

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_{73}$	$GUFF_{74}$	$GUFF_{75}$	$GUFF_{77}$	$GUFF_{78}$	$GUFF_{81}$	$GUFF_{82}$	$GUFF_{85}$
Gram character	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Rods	Rods	Rods
Gelatinase	+	+	+	+	-	-	-	-
PHA	-	-	-	-	-	-	-	-
Protease	+	+	+	+	+	-	+	+
Tween 80	+	-	-	+	+	-	-	-
Amylase	-	-	+	+	+	-	-	-
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+		+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	-	+	+	+	+	+	+	+
Acetate	+	+	+	+	+	+	+	+
Malate	-	+	-	+	+	-	+	-
Pyruvate	+	+	+	+	+	+	+	+
Succinate	-	+	+	+	+	-	+	-
Lactate	-	+	+	+	+	-	+	-
Formate	-	+	+	+	+	-	+	-
Arginine	-	+	+	+	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	-	-	-	-	-	-	-	-
0% NaCl	-	+	-	+	+	+	-	+
12.5% NaCl	+	+	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+	+	+
Tentative Identification	*Halococcus* sp.	Halotolerant Haloarchaea	*Halococcus* sp.	Halotolerant Haloarchaea	Halotolerant Haloarchaea	Halotolerant Haloarchaea	*Natrinema* sp.	Halotolerant Haloarchaea

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_{86}$	$GUFF_{87}$	$GUFF_{89}$	$GUFF_{90}$	$GUFF_{93}$	$GUFF_{94}$	$GUFF_{96}$	$GUFF_{98}$
Gram character	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Morphology	Rods	Rods	Rods	Cocci	Cocci	Cocci	Cocci	Cocci
PHA	-	+	+	+	-	-	+	-
Gelatinase	-	-	+	+	-	+	+	+
Protease	+	+	+	+	+	+	+	+
Tween 80	-	-	+	-	-	+	+	-
Amylase	-	-	-	+	-	-	-	+
Sorbitol	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Acetate	+	-	+	+	+	-	+	-
Malate	+	+	+	+	+	-	-	-
Pyruvate	+	+	+	+	+	+	+	+
Succinate	+	+	+	+	-	+	+	+
Lactate	+	+	+	+	+	-	-	-
Formate	+	+	+	+	-	+	+	+
Arginine	+	+	+	+	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+	+	+
42°C	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+
pH 5	-	-	-	-	-	-	+	-
0%NaCl	+	-	+	+	-	+	-	+
12.5%NaCl	+	+	+	+	+	+	+	+
25%NaCl	+	+	+	+	+	+	+	+
Tentative Identification	Halotolerant Haloarchaea	*Natrinema* sp.	Halotolerant Haloarchaea	Halotolerant Haloarchaea	*Halococcus* sp.	Halotolerant Haloarchaea	*Natrialba* sp.	Halotolerant Haloarchaea

Table 21: Physiological and biochemical characteristics of haloarchaea from salt pans

Haloarchaea	$GUFF_{99}$	$GUFF_{100}$	$GUFF_{102}$	$GUFF_{104}$	$GUFF_{105}$	$GUFF_{156}$
Gram character	-ve	-ve	-ve	-ve	+ve	+ve
Morphology	Cocci	Rods	Cocci	Rods	Cocci	Rods
Gelatinase	+	+	-	-	-	+
PHA	-	+	-	-	-	-
Protease	+	+	-	-	-	-
Tween 80	+	-	-	-	-	-
Amylase	+	-	+	+	-	+
Sorbitol	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
Glucose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Ribose	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+
Xylose	+	+	+	+	+	+
Mannose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Citrate	+	+	+	-	-	+
Acetate	+	+	+	-	+	+
Malate	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+
Succinate	+	+	+	-	-	+
Lactate	-	+	+	+	-	+
Formate	+	+	+	-	-	+
Arginine	-	+	+	+	+	+
Growth at: R.T.	+	+	+	+	+	+
42°C	+	+	+	+	+	+
50°C	+	+	+	+	+	+
pH 5	-	-	-	-	-	+
0% NaCl	-	+	-	-	+	+
12.5% NaCl	+	+	+	+	+	+
25% NaCl	+	+	+	+	+	+
Tentative Identification	*Halococcus* sp.	Halotolerant Haloarchaea	*Halococcus* sp.	*Halobacterium* sp.	Halotolerant Haloarchaea	Halotolerant Haloarchaea

.Characterization of Haloarchaea on basis of pigment characteristics

The cultures were all grown individually in NTYE containing 25% crude solar salt, showed various pigments (**Plate 11**). $GUFF_{32}$, $GUFF_{63}$ and $GUFF_{151}$ were cream pigmented colonies.

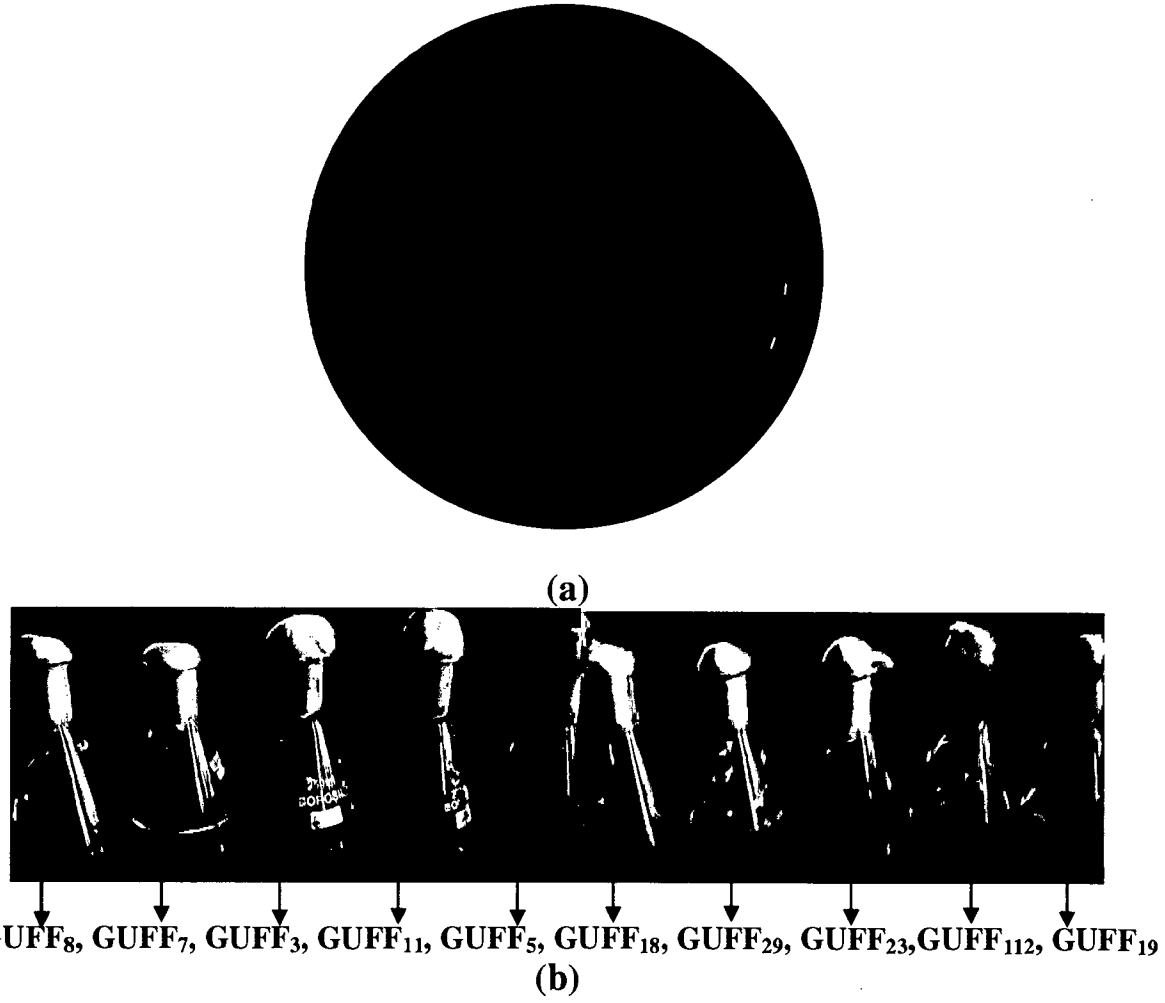


Plate 11: (a) Pigmented colonies of haloarchaea growing on NTYE agar; (b) Pigmented haloarchaeal cultures grown in liquid NTYE

As seen in **Table 22**, all showed a common peak at 416 λ. $GUFF_{63}$ and $GUFF_{151}$ showed common peaks at 334.5 λ, 282 λ and 252 λ. Hence, they can be placed together, even though they show differences/variations. $GUFF_{63}$ showed additional peaks at 292 λ, 274 λ and 218 λ; while $GUFF_{151}$ showed peaks at 304 λ (also shown by $GUFF_{32}$) and at 260 λ. $GUFF_{143}$ and $GUFF_{181}$ were cream coloured colonies. However, no peaks were seen.

$GUFF_2$, $GUFF_6$, $GUFF_{10}$, $GUFF_{47}$, $GUFF_{48}$, $GUFF_{49}$, $GUFF_{50}$ were orange pigmented colonies. All showed common peaks at 527 λ and 492 λ. $GUFF_6$, $GUFF_{10}$, $GUFF_{47}$, $GUFF_{49}$, $GUFF_{50}$, all showed a common peak at 599 λ.

But, most of the peaks at 469 λ, 282 λ, 271 λ and 240 λ were shared by $GUFF_2$ and $GUFF_6$, the difference being a peak at 305 λ of $GUFF_6$, while peaks of 416 λ and 296 λ of $GUFF_2$. $GUFF_{10}$ and $GUFF_{49}$, both shared peaks at 465 λ, difference being a peak at 321 λ of $GUFF_{10}$ **(Fig. 7)**.

$GUFF_{48}$ shared a peak of 465 λ common to $GUFF_2$, $GUFF_{10}$, $GUFF_{49}$, but was different from $GUFF_2$, $GUFF_{10}$, $GUFF_{49}$, in that it showed a peak at 329 λ. $GUFF_{47}$ and $GUFF_{50}$ of common group $GUFF_6$, $GUFF_{10}$, $GUFF_{49}$, differed in that $GUFF_{47}$ showed a peak at 416 λ while $GUFF_{50}$ at 313 λ. $GUFF_{70}$ had peaks at 599 λ, 305 λ and 282 λ common to $GUFF_6$, but was different from $GUFF_6$ at 416 λ and 335 λ. $GUFF_{53}$, $GUFF_{64}$, $GUFF_{69}$, all orange coloured showed common peaks at 495 λ. $GUFF_{53}$ differed from $GUFF_{69}$ as it had an

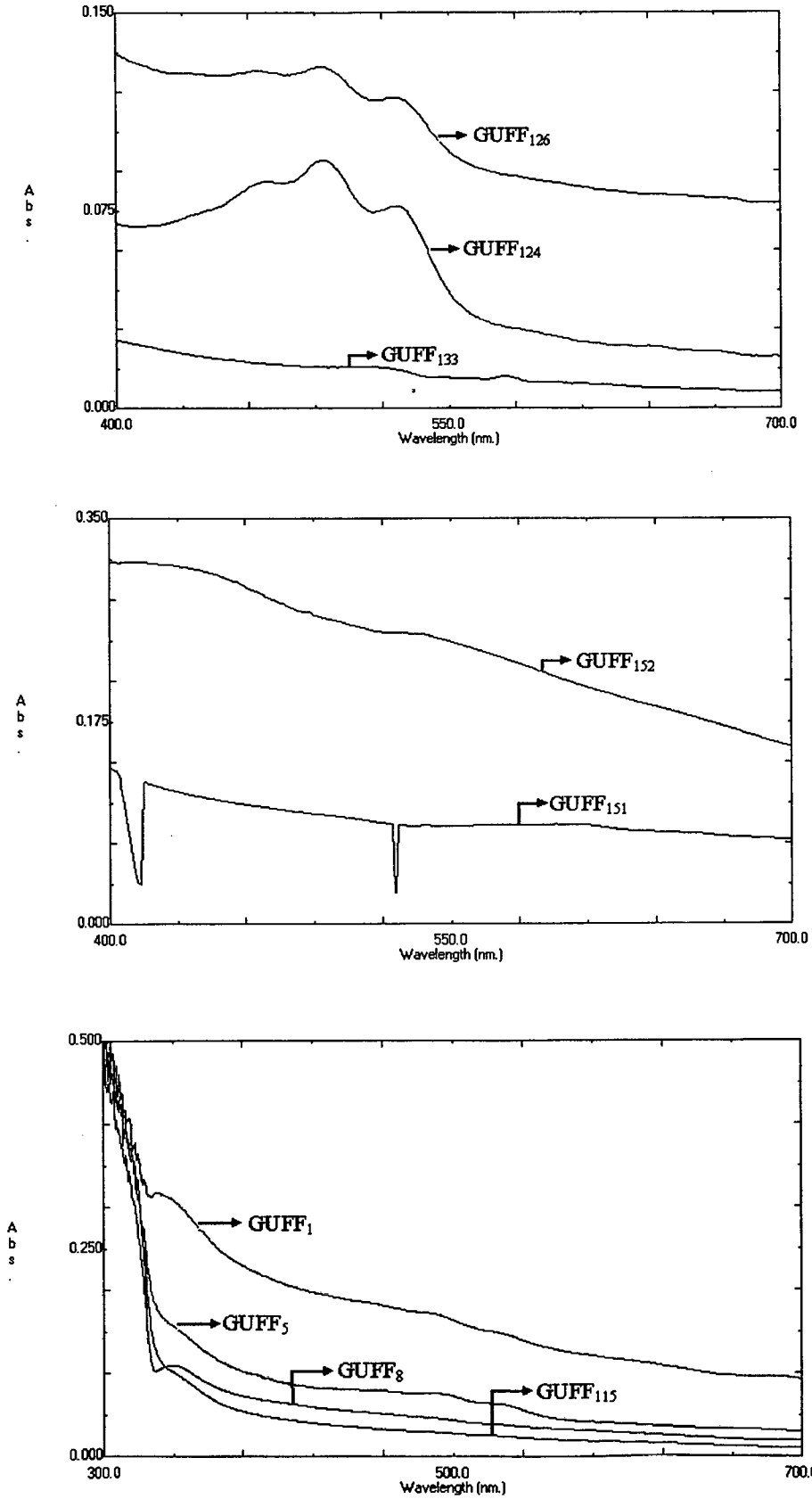


Fig. 7: Pigment scans of haloarchaeal isolates grown on NTYE, pH 7.0 in acetone.

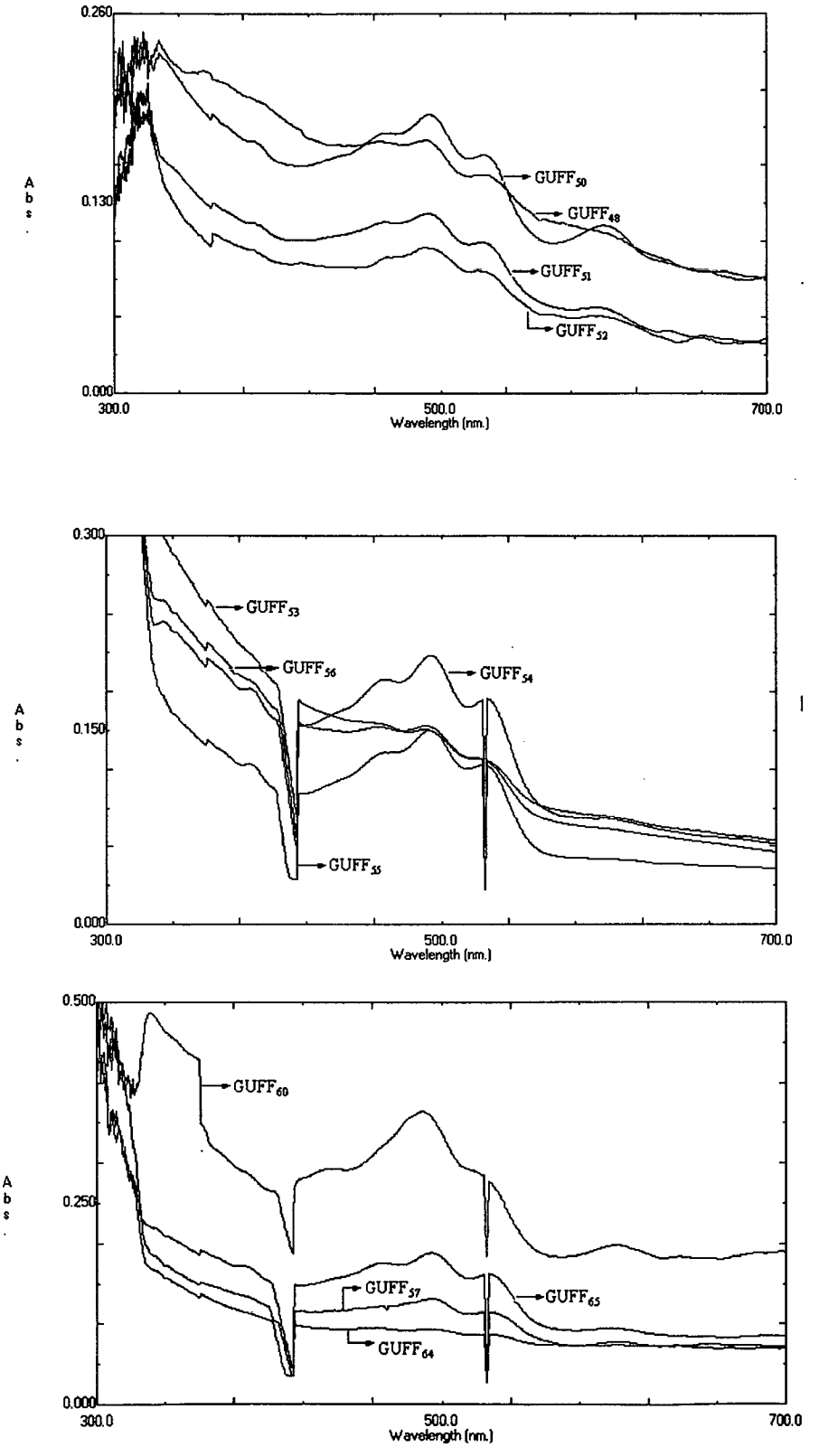


Fig. 7: Pigment scans of haloarchaeal isolates grown on NTYE, pH 7.0 in acetone.

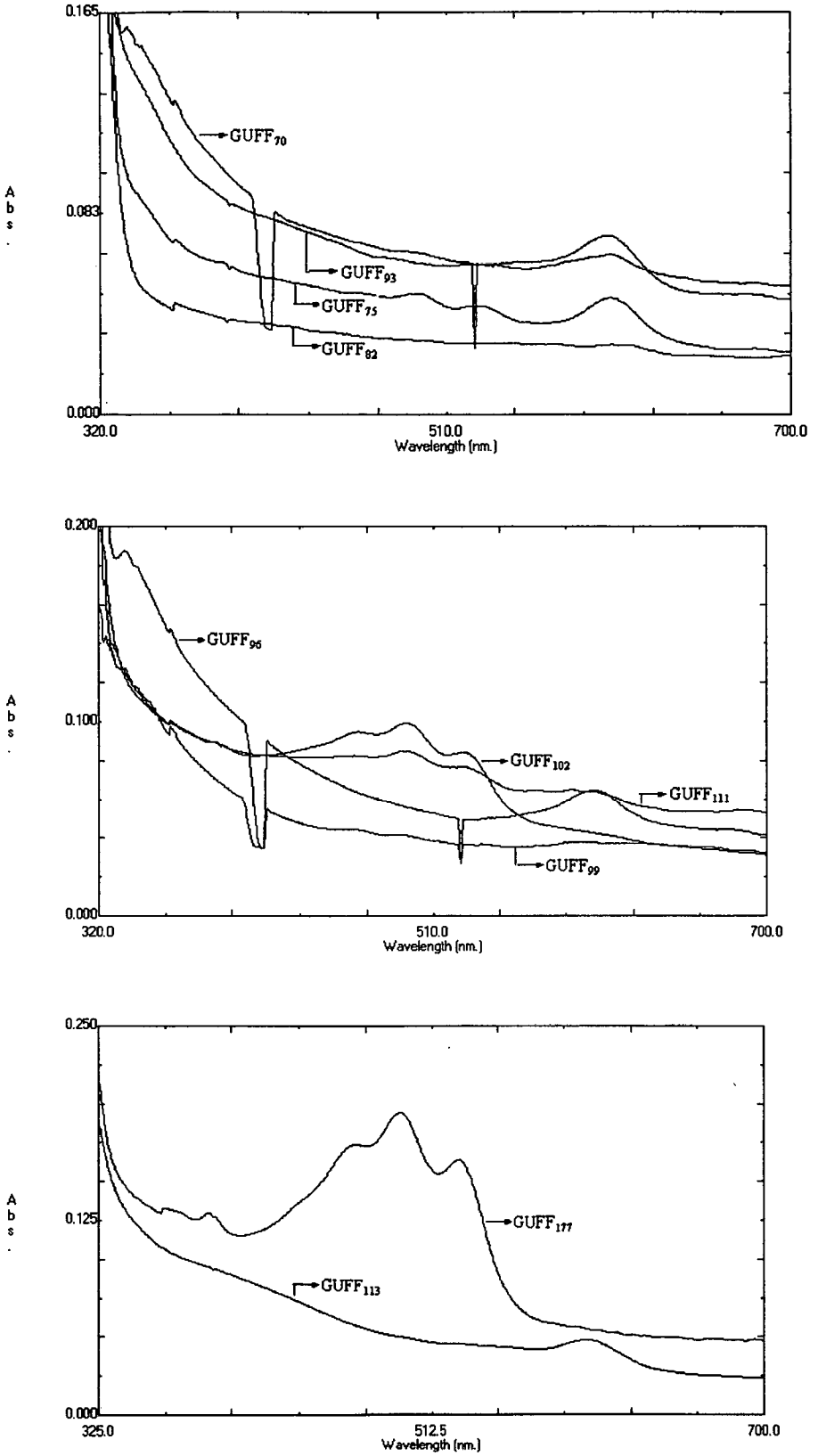


Fig. 7: Pigment scans of haloarchaeal isolates grown on NTYE, pH 7.0 in acetone.

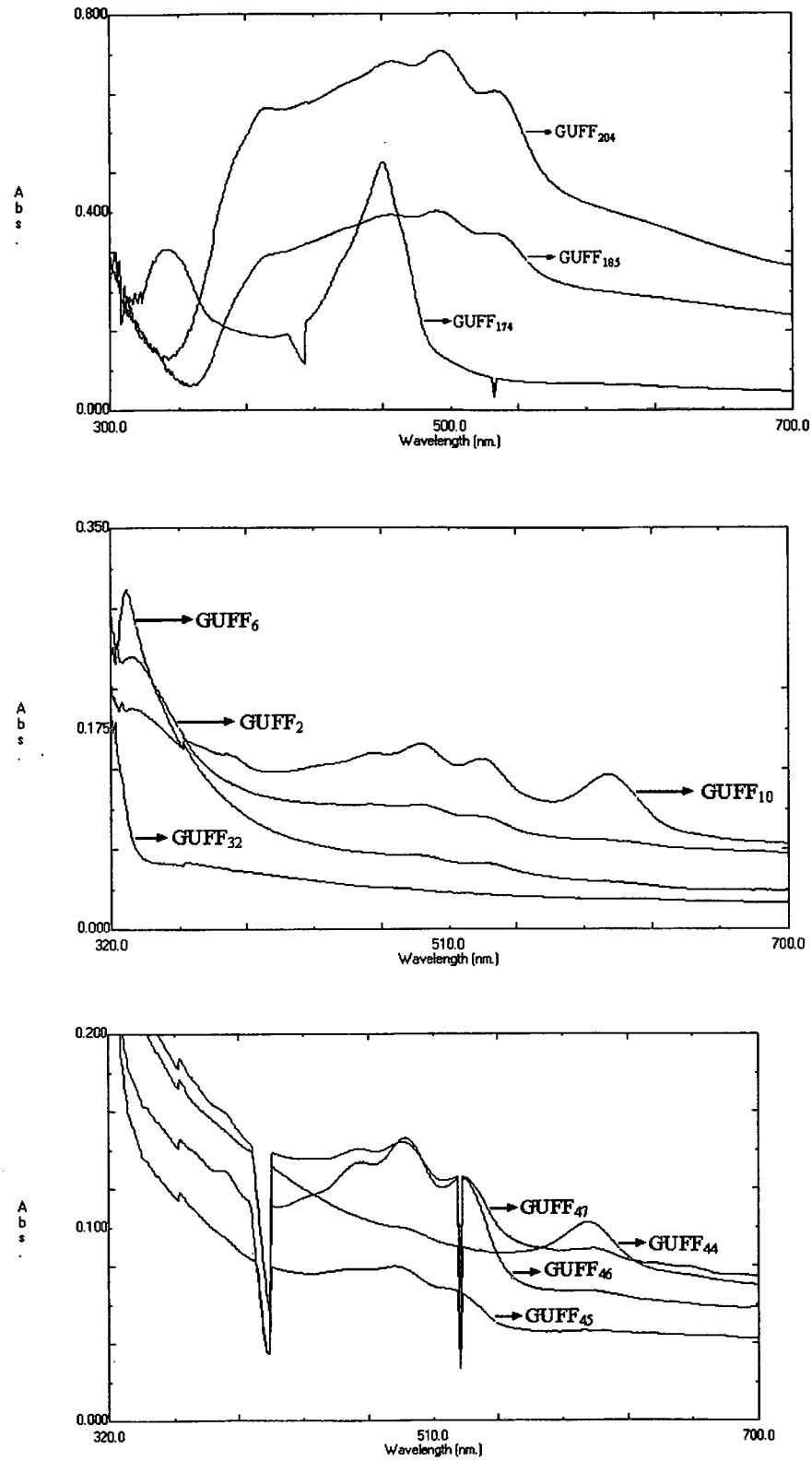


Fig. 7: Pigment scans of haloarchaeal isolates grown on NTYE, pH 7.0 in acetone.

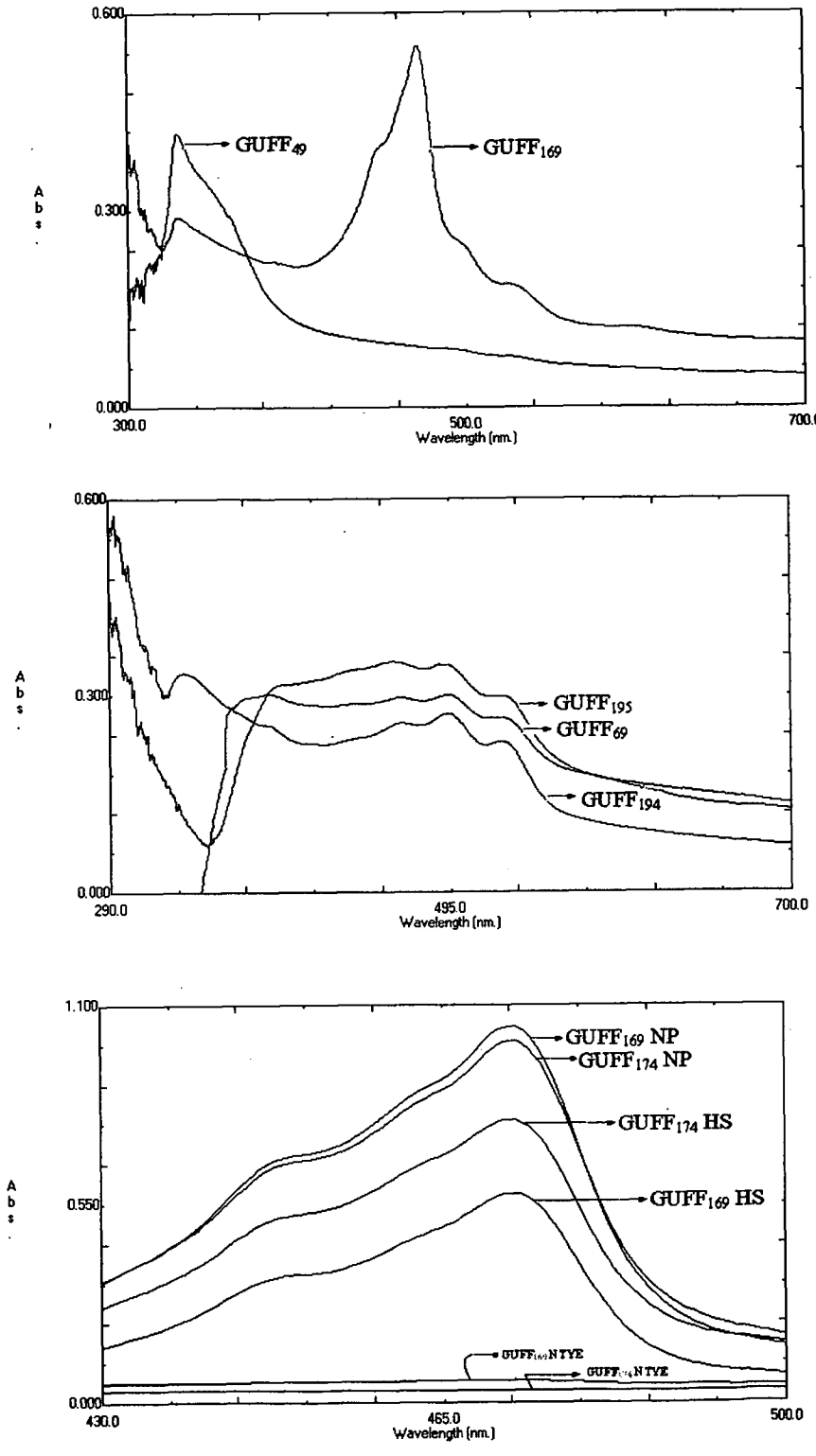


Fig. 7: Pigment scans of haloarchaeal isolates grown on NTYE, pH 7.0 in acetone.

additional peak at 416 λ, common to $GUFF_{64}$. $GUFF_{64}$ showed additional peaks at 309 λ and 301 λ. But peaks at 292 λ and 282 λ of $GUFF_{64}$ were common to $GUFF_{69}$ hence; the

difference between them was at peaks 470 λ, 335 λ,, 259 λ and 252 λ showed by $GUFF_{69}$. $GUFF_{46}$, $GUFF_{54}$, $GUFF_{55}$, $GUFF_{124}$, $GUFF_{177}$, $GUFF_{194}$ were orange pigmented colonies, yet all showed common peaks at 527 λ and 495 λ. Peak at 468 λ was common to $GUFF_{46}$, $GUFF_{54}$, $GUFF_{177}$ and $GUFF_{94}$. Amongst these, peak of 387 λ was common at $GUFF_{54}$ and $GUFF_{177}$. But $GUFF_{177}$ also showed an additional peak at 329 λ, not shown by $GUFF_{54}$.

$GUFF_{54}$, $GUFF_{194}$ and $GUFF_{124}$ showed common peaks at 338 λ, but $GUFF_{54}$, differed from $GUFF_{124}$ in showing a peak at 306 λ while $GUFF_{124}$ showed at 284 λ. $GUFF_{194}$ showed an additional peak at 275 λ.

$GUFF_{169}$ and $GUFF_{174}$ were orange until 6 days; they changed to yellow on the 7th day. Pigment scans of their yellow stage, showed common peaks at 334 λ and 308 λ. $GUFF_{169}$ differed from $GUFF_{174}$ in showing additional peak at 472 λ. $GUFF_{174}$ showed various peaks at 461 λ, 404 λ, 292 λ and 282 λ. The orange stage of $GUFF_{174}$ gave peaks at 526 λ, 494 λ and 269 λ.

$GUFF_9$ and $GUFF_{125}$ showed orange pigmented colonies. They showed a common peak at 295 λ. $GUFF_{125}$ gave a peak at 260 λ. Pigment scan of $GUFF_9$ showed additional peaks at 249 λ, 228 λ and 204 λ. $GUFF_{44}$, $GUFF_{115}$ and $GUFF_{126}$ were peach colonies. Yet, there were hardly any common peaks between them. $GUFF_{44}$ showed peaks at 600 λ, 416 λ, 313 λ and 301 λ, also shown by $GUFF_{115}$. $GUFF_{115}$ showed peaks at 229 λ, 288 λ, 283 λ, 243 λ and 212 λ. $GUFF_{126}$ gave peaks at 492 λ and 226 λ

$GUFF_1$, $GUFF_5$, $GUFF_8$, $GUFF_{62}$, $GUFF_{189}$, $GUFF_{209}$ were all pale orange colonies, yet each culture gave peaks at varied wavelengths. $GUFF_1$ at 331 λ, 278 λ, 243 λ, $GUFF_5$ gave peaks at 297 λ and 237 λ. $GUFF_8$ showed peaks at 339 λ, 296 λ and 248 λ. $GUFF_{62}$ at 275 λ and 261 λ, $GUFF_{189}$ and $GUFF_{209}$ gave single peak at 288 λ.

$GUFF_{57}$, $GUFF_{65}$, $GUFF_{75}$, $GUFF_{93}$, $GUFF_{99}$ and $GUFF_{111}$ were pale orange pigmented colonies. $GUFF_{57}$ was the only one showing a peak at 641 λ, besides peaks at 632 λ, 580 λ,

440 λ, 425 λ, 360 λ, 330 λ and 317 λ. $GUFF_{99}$ showed peaks at 416 λ, 303 λ, 282 λ, 270 λ and 264 λ, common to $GUFF_{93}$. $GUFF_{65}$, $GUFF_{75}$, $GUFF_{93}$, $GUFF_{111}$, all showed peaks at 600 λ. $GUFF_{111}$ shared peaks of 493 λ with $GUFF_{65}$, besides, $GUFF_{65}$ showed peaks at 527 λ, 466 λ, 309 λ and at 296 λ which is also common to $GUFF_{75}$. $GUFF_{75}$ showed peak at 274 λ.

Most of the isolates were rich in carotenoids. Extracts of cells in acetone showed absorption maxima which were similar to those reported for extracts of other halobacterial strains.

$GUFF_{45}$, $GUFF_{52}$, $GUFF_{60}$ were colonies with orange pigment. $GUFF_{45}$ and $GUFF_{60}$ showed common peaks at 697 λ. $GUFF_{45}$ showed peaks at 530 λ, 500 λ, 417 λ and 240 λ. $GUFF_{60}$ showed peaks at 368 λ, 601 λ, 489 λ, 437 λ and 331 λ. $GUFF_{52}$ showed peaks at 687 λ, 654 λ, 589 λ, 524 λ, 495 λ, 467 λ, 386 λ and 312 λ.

$GUFF_{12}$, $GUFF_{96}$, $GUFF_{113}$, $GUFF_{133}$, $GUFF_{156}$ were cream coloured colonies. $GUFF_{12}$, $GUFF_{96}$, $GUFF_{113}$, $GUFF_{156}$ showed common peaks at 600 λ. $GUFF_{12}$, $GUFF_{96}$, $GUFF_{156}$ showed common peaks at 416 λ. Therefore, they can be placed together. $GUFF_{96}$ and $GUFF_{156}$ also showed peaks common at 336 λ and 282 λ which were also seen for $GUFF_{133}$. But there are variations between $GUFF_{96}$ and $GUFF_{156}$ as $GUFF_{96}$ also showed peak at 319 λ, while $GUFF_{156}$ at 292 λ. $GUFF_{133}$ showed peaks at 574 λ, 299 λ and at 252 λ.

$GUFF_{153}$, $GUFF_{157}$, $GUFF_{160}$ were cream pigmented colonies. They can be grouped together, as they all show peaks at same- 282 λ, 247 λ, and 212 λ. $GUFF_{155}$, $GUFF_{158}$ and $GUFF_{193}$ were cream colonies. Common peak for all was seen at 284 λ. But $GUFF_{158}$ showed an additional peak at 264 λ. $GUFF_{128}$, $GUFF_{132}$, $GUFF_{135}$, $GUFF_{138}$, $GUFF_{140}$, $GUFF_{141}$, $GUFF_{149}$, $GUFF_{152}$, $GUFF_{154}$, all were cream coloured colonies.

$GUFF_{128}$, $GUFF_{132}$, $GUFF_{135}$, $GUFF_{140}$, $GUFF_{149}$, $GUFF_{152}$ and $GUFF_{154}$ showed common peaks at 299 λ. Further common peaks were seen at 246 λ for $GUFF_{128}$, $GUFF_{132}$, $GUFF_{135}$, $GUFF_{138}$, $GUFF_{149}$, $GUFF_{152}$ and $GUFF_{154}$. Variations between these cultures were that $GUFF_{128}$ showed an additional peak at 230 λ, $GUFF_{132}$ showed additional peaks at 329 λ and

at 205 λ. $GUFF_{135}$ at 219 λ also shared by $GUFF_{138}$. $GUFF_{138}$ showed a peak at 273 λ which is common for $GUFF_{141}$. $GUFF_{140}$ showed a peak at 256 λ, while $GUFF_{149}$ showed a peak at 212 λ and $GUFF_{154}$ at 227 λ.

$GUFF_{82}$, $GUFF_{87}$ and $GUFF_{117}$ were cream colonies showing common peaks at 266 λ. They differed from each other in that $GUFF_{87}$ showed additional peaks at 305 λ and 280 λ, while $GUFF_{117}$ showed at 255 λ.

Characterisation of lipids

Glycerol Diether Moiety

Thin layer chromatogram of lipids showed the presence of ether-linked lipids **(Plate 12)** similar to those of archaebacteria and distinctly different from the ester-linked lipids of *E. coli.*

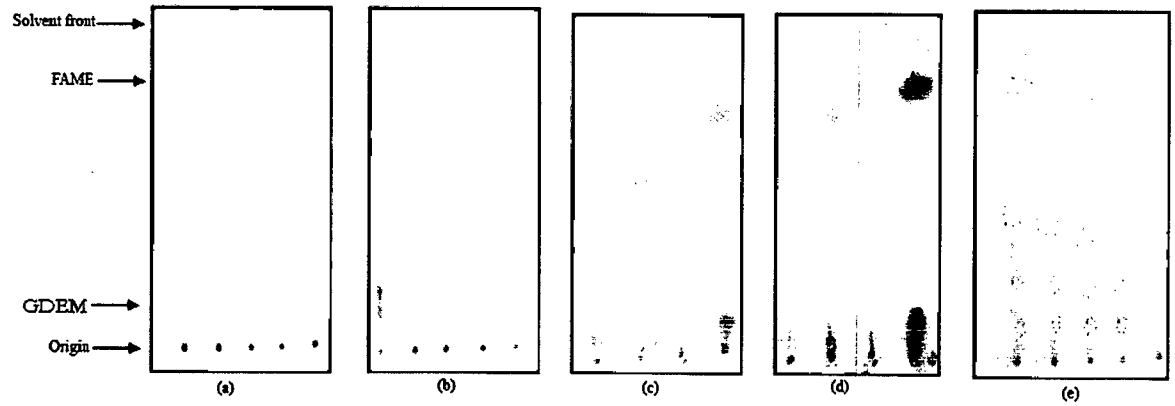


Plate 12 : Thin layer chromatographic analysis of whole organism methanolysates of haloarchaea. Plates were developed in solvent system petroleum ether (b.p. 60 to 80 ^{0}C): diethyl ether;85: 15, v/v, stained with (a-d) iodine; (e) 10 % dodecaphosphomolybdic acid in absolute ethanol. FAME, fatty acid methyl esters; GDEM, glycerol diether moieties

$GUFF_2$, $GUFF_6$, $GUFF_{53}$, $GUFF_{56}$ gave 2 spots R_f corresponding to 0.14 and 0.50, $GUFF_{57}$, $GUFF_{49}$, $GUFF_{12}$, $GUFF_{46}$, $GUFF_{52}$ gave 2 spots R_f 0.16, 0.54, $GUFF_{50}$ gave 2 spots, R_f 0.16, 0.61. $GUFF_{48}$ gave 4 spots R_f 0.18, 0.54, 0.70, 0.93, $GUFF_{32}$ gave 3 spots Rf 0.10, 0.50, 0.93, $GUFF_{54}$ gave 3 spots R_f 0.10, 0.50, 0.93, $GUFF_{44}$ gave 3 spots R_f 0.14, 0.53, 0.94,

$GUFF_{47}$ gave 5 spots R_f 0.12, 0.28, 0.41, 0.53, 0.96, $GUFF_{60}$ gave 1 spot R_f 0.06, $GUFF_{174}$ gave 1 spot R_f 0.06. $GUFF_1$, $GUFF_5$, $GUFF_{10}$, $GUFF_{19}$, $GUFF_{31}$ gave 5 spots R_f - 0.87, 0.76, 0.39, 0.21, 0.10. $GUFF_{40}$ gave 3 spots R_f 0.82, 0.18, 0.12, $GUFF_{41}$ gave 2 spots R_f 0.9, 0.47, $GUFF_{43}$ gave 3 spots R_f 0.87, 0.47, 0.3. $GUFF_{45}$ gave 4 spots R_f 0.9, 0.46, 0.37, 0.28. $GUFF_{51}$ gave 3 spots R_f 0.87, 0.45, 0.15. $GUFF_{55}$, $GUFF_{59}$ gave 2 spots R_f 0.88, 0.29. $GUFF_{63}$, $GUFF_{69}$ gave 7 spots R_f 0.88, 0.74, 0.57, 0.31, 0.22, 0.15, 0.07, $GUFF_{70}$ gave 6 spots R_f 0.91, 0.74, 0.59, 0.31, 0.23, 0.15. $GUFF_{137}$, $GUFF_{152}$, $GUFF_{169}$ gave 3 spots R_f 0.87, 0.74, 0.097. $GUFF_{127}$ gave 3 spots R_f 0.78, 0.67, 0.096. $GUFF_{185}$ gave 2 spots R_f 0.3, 0.09. $GUFF_{193}$ gave 4 spots R_f 0.65, 0.27, 0.15, 0.08. $GUFF_{195}$ gave 2 spots R_f 0.84, 0.29, $GUFF_{90}$ gave 2 spots R_f 0.84, 0.39. $GUFF_{79}$, $GUFF_{88}$ gave 4 spots R_f 0.87, 0.78, 0.46, 0.14. $GUFF_{13}$ gave 6 spots R_f 0.85, 0.75, 0.45, 0.36, 0.30, 0.16. $GUFF_{26}$ gave 5 spots R_f 0.86, 0.77, 0.44, 0.3, 0.16. $GUFF_3$ gave 3 spots R_f 0.85, 0.79, 0.48. $GUFF_{55}$ gave 3 spots, R_f 0.87, 0.54, 0.37 when sprayed for phospholipids, while $GUFF_{70}$ gave 4 spots, R_f 0.65, 0.55, 0.45 and 0.28.

Polar Lipids

Polar lipid compositions of isolates are as in **Plate 13.** $GUFF_1$ gave 5 spots, on spraying with ammonium molybdate R_f 0.9, 0.72, 0.66, 0.50, 0.4 while 2 spots with spray for glycolipids, R_f 0.41, 0.23. $GUFF_5$ gave 5 spots when sprayed for phospholipids, R_f 0.91, 0.70, 0.64, 0.51, 0.39 while 3 spots R_f 0.58, 0.44, 0.26 with glycolipid spray. $GUFF_{10}$ gave 4 spots, R_f 0.87, 0.69, 0.61, 0.50 when sprayed for phospholipids, while gave 3 spots with glycolipid spray, R_f 0.5, 0.38, 0.30. $GUFF_{19}$ gave 3 spots, R_f 0.68, 0.58, 0.40 when sprayed for phospholipids, while 1 spot, R_f 0.25 when sprayed for glycolipids.

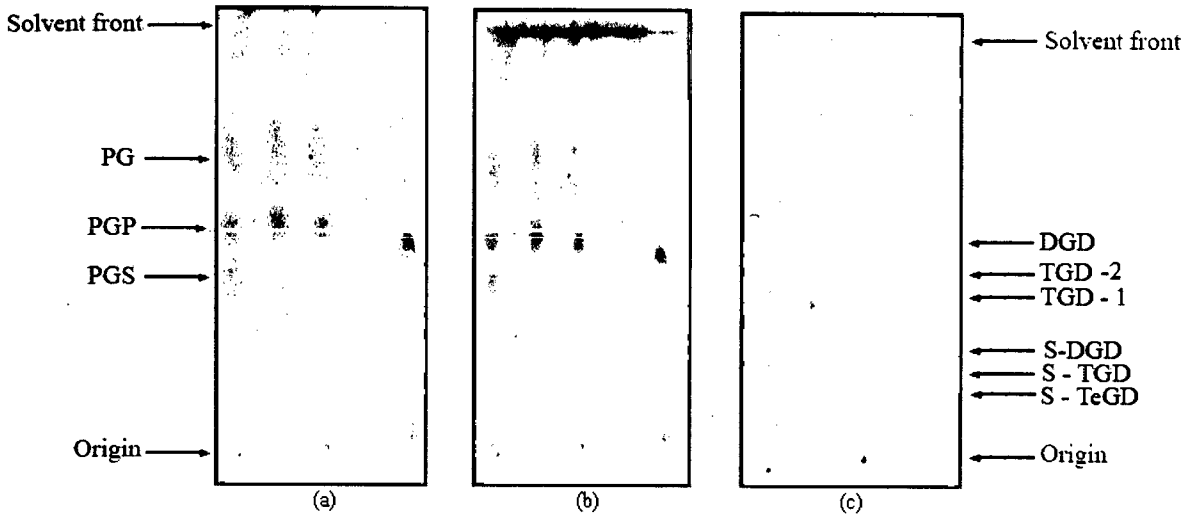
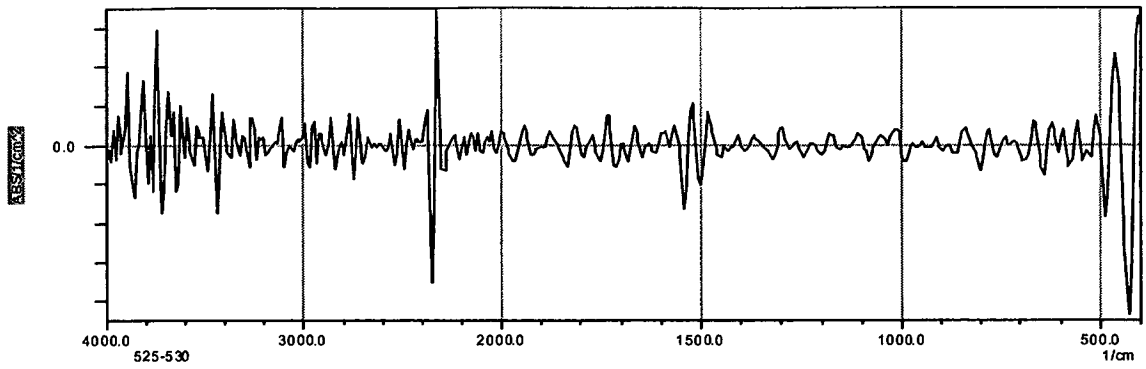


Plate 13: Thin layer chromatogram on silica gel G of polar lipids extracted from haloarcheal isolates. Plates were developed with solvent system chloroform: methanol: acetic acid: water, 85: 22.5: 10: 4 v/v. The tentative identification of the lipids spot, as based on literature data (Tindall, 1991; Torrenblanca et al, 1986; Kushwaha et al, 1982) is indicated. Phosphor lipids PG = phosphatidyl glycerol; PGP = phosphatidyl glycerol phosphate; PGS = phosphatidyl glycerol sulphate were stained by ammonium molybdate; glycolipids S-TGD, sulphated tetraglycosyldiether, triglycosyldiether TGD- 1, TGD- 2; DGD, diglycosyldiether were detected by spraying with 0.5% α-naphthol in 50% methanol/water followed by 5% H_2SO_4 in ethanol

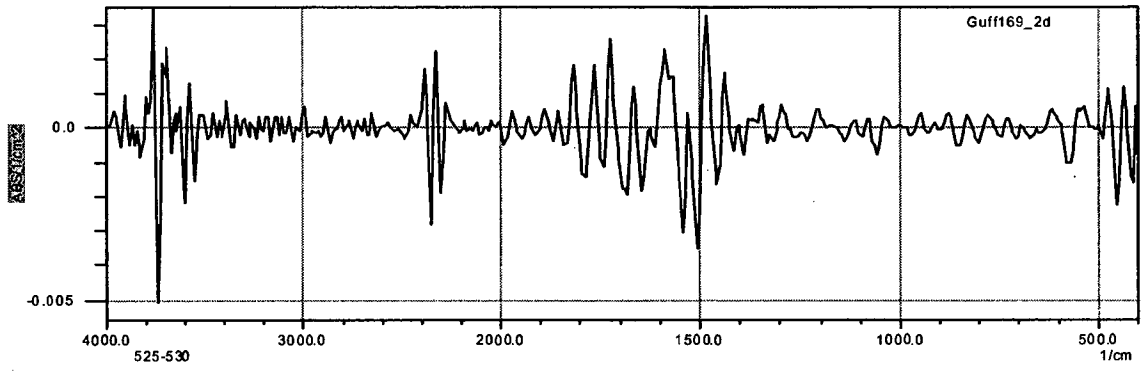
$GUFF_{31}$ gave 5 spots, R_f 0.92, 0.77, 0.70, 0.61, 0.44 when sprayed with ammonium molybdate while when sprayed for glycolipids gave 1 spot, R_f 0.21. $GUFF_{55}$ gave 3 spots, R_f 0.87, 0.54, 0.37 when sprayed for phospholipids, while $GUFF_{70}$ gave 4 spots, R_f 0.65, 0.55, 0.45 and 0.28.

Fluorescence Transmission Infra Red (FT-IR) spectroscopy of cultures

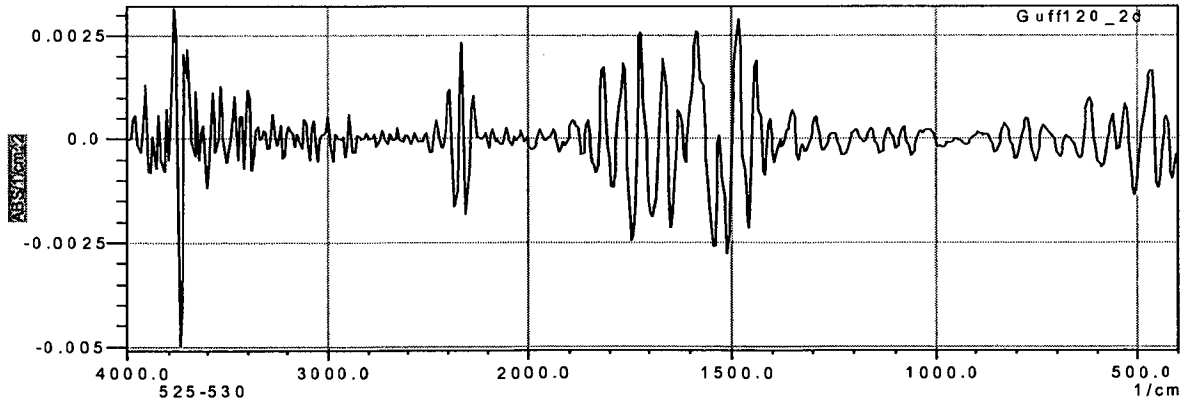
FT-IR of isolates were largely similar from 900-4000 nm, but showed distinct differences in the region between 600-900 nm, where characteristic features are noticed, in the "bacterial finger print region" (**Fig. 8**).



GUFF$_5$

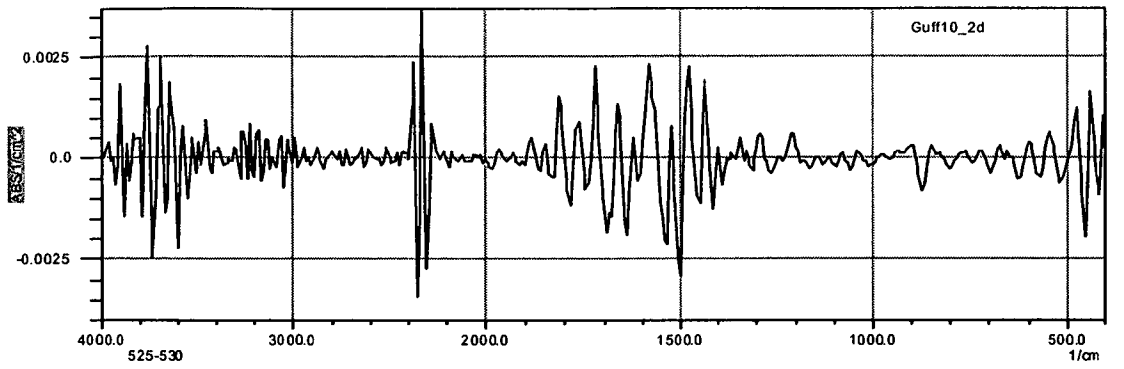


GUFF$_{169}$

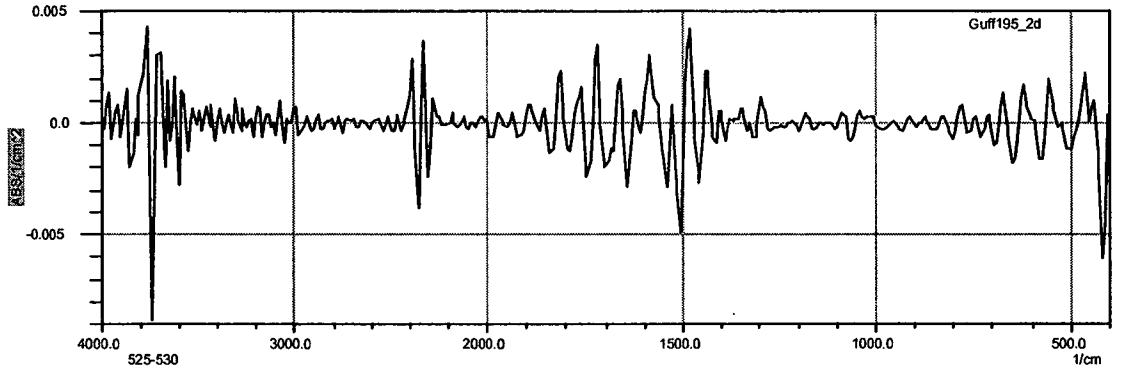


GUFF$_{120}$

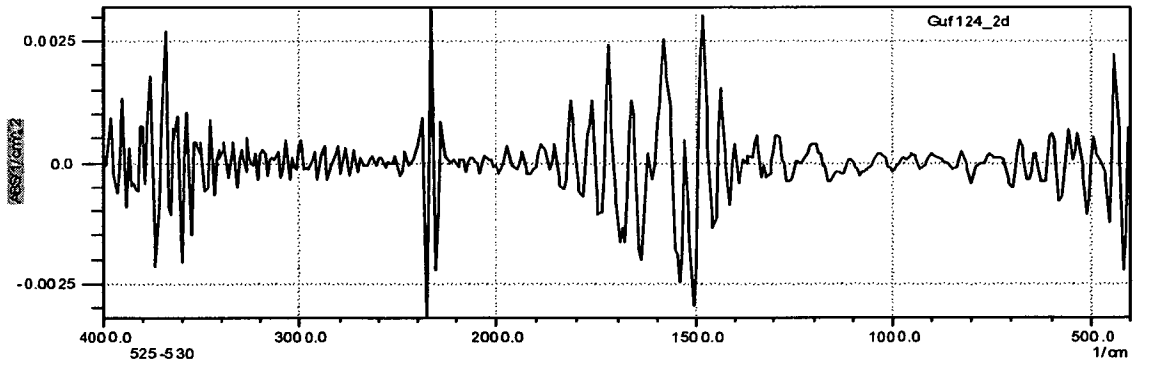
Fig. 8: FT-IR spectra of haloarchaeal isolates



GUFF$_{10}$



GUFF$_{195}$



GUFF$_{124}$

Fig 8: FT-IR spectra of haloarchaeal isolates

Scanning Electron Microscopy of selected isolates

Scanning electron photomicrographs of gold-coated cells grown in NTYE medium, demonstrated rods, cocci, spherical cup-shaped cells, C-shaped cells, triangular, rectangular cells, squared cells, pleiomorphic, cricket-ball shaped cells **(Plate 14).**

The haloarchaeal community of salt pans is as indicated in *Table 23.*

Table 23: Haloarchaeal community of salt pans

Salt pan	Samples from salt pan		
	Water	Brine	Sediment
A	-	*Halococcus* sp., *Haloferax* sp.,Halotolerant Haloarchaea	*Haloferax* sp., *Halobacterium* sp., *Natrialba* sp., *Haloarcula* sp., Halotolerant Haloarchaea
Ag	-	*Haloarcula* sp., *Halococcus* sp., *Natrinema* sp., N*atrialba* *sp.*,*Halobacterium* sp., Halotolerant Haloarchaea	*Halococcus* sp., *Haloferax* sp., *Haloarcula* sp., Halotolerant Haloarchaea
Ar	-	*Halococcus* sp., *Haloarcula* sp., *Haloferax* sp., *Halobacterium* sp., Halotolerant Haloarchaea	*Haloarcula* sp., *Halococcus* sp.,*Natrialba* sp., Halotolerant Haloarchaea
N	-	*Halococcus* sp., *Natrinema* sp., *Natrialba* sp., *Haloarcula* sp., *Halobacterium* sp., Halotolerant Haloarchaea	*Natrialba* sp., *Natrinema* sp., *Halococcus* sp., *Haobacterium* sp., *Haloarcula* sp., Halotolerant Haloarchaea
R	*Halococcus salifodinae*, *Halococcus* sp., Halotolerant Haloarchaea	*Halococcus saccharolyticus* sp, *Halococcus* sp., *Halorubrum* sp., *Halobacterium* sp., Halotolerant Haloarchaea,unidentified $GUFF_{193,194,195}$	*Halococcus* sp., *Halobacterium* sp., *Haloarcula* sp., Halotolerant Haloarchaea
S	*Halococcus* sp., Halotolerant Haloarchaea	*Halobacterium* sp., *Halococcus* sp., *Natrialba* sp., *Natrinema* sp., *Haloarcula* sp., *Haloferax* sp., Halotolerant Haloarchaea	*Natrialba* sp.,*Halococcus* sp.,*Haloarcula* sp., *Halobaculum* sp., *Haloferax* sp., *Natronomonas* sp., *Natronococcus* sp., Halotolerant Haloarchaea

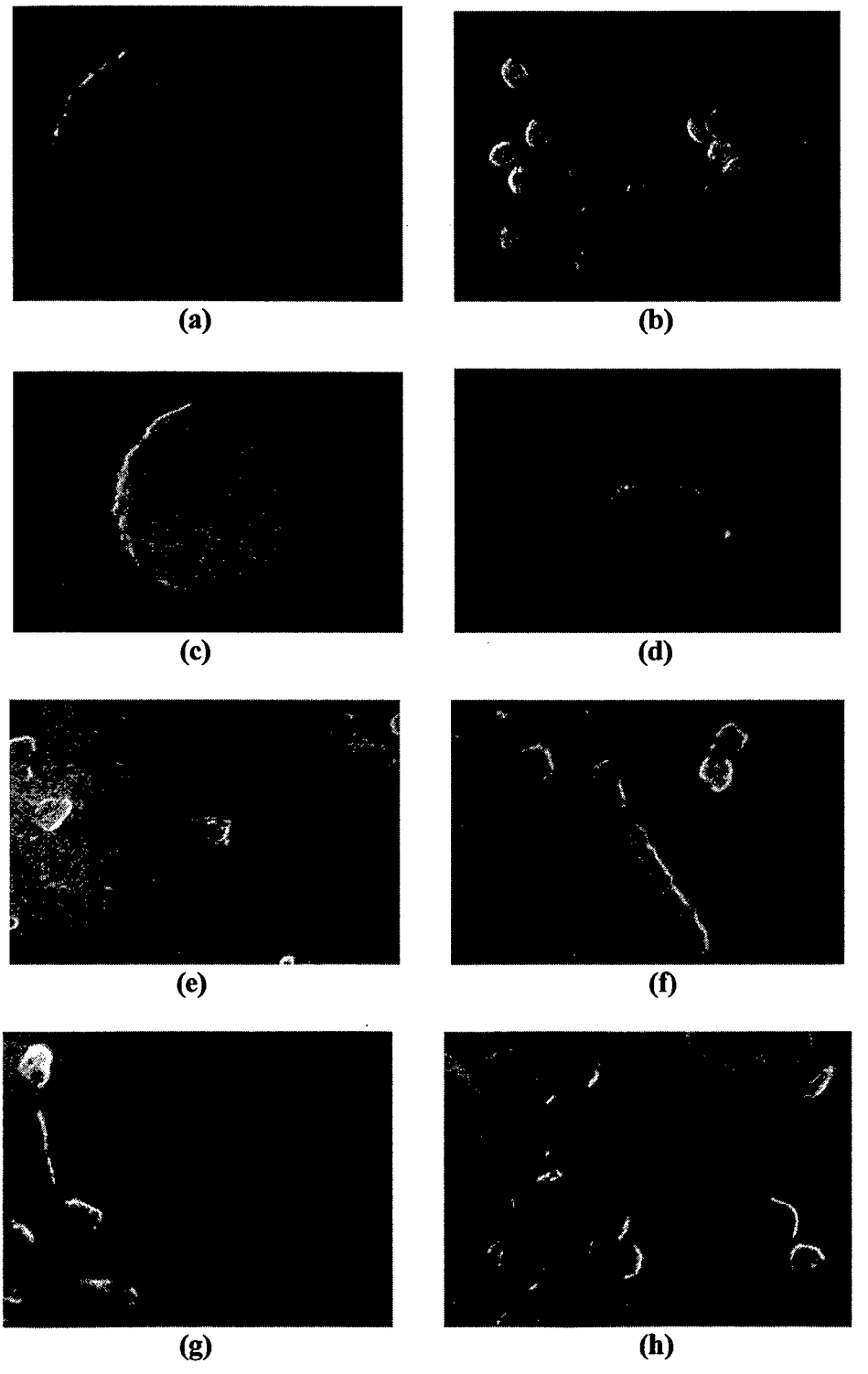


Plate 14: Scanning electron micrographs of gold-plated cells grown in liquid culture NTYE medium, pH 7.0 (a) 13,000x; (b) 1,800x (c) 13,000x (d) 20,000x (e) 13,000x of $GUFF_{63}$; (f), (g), (h) 13,000x of $GUFF_{70}$

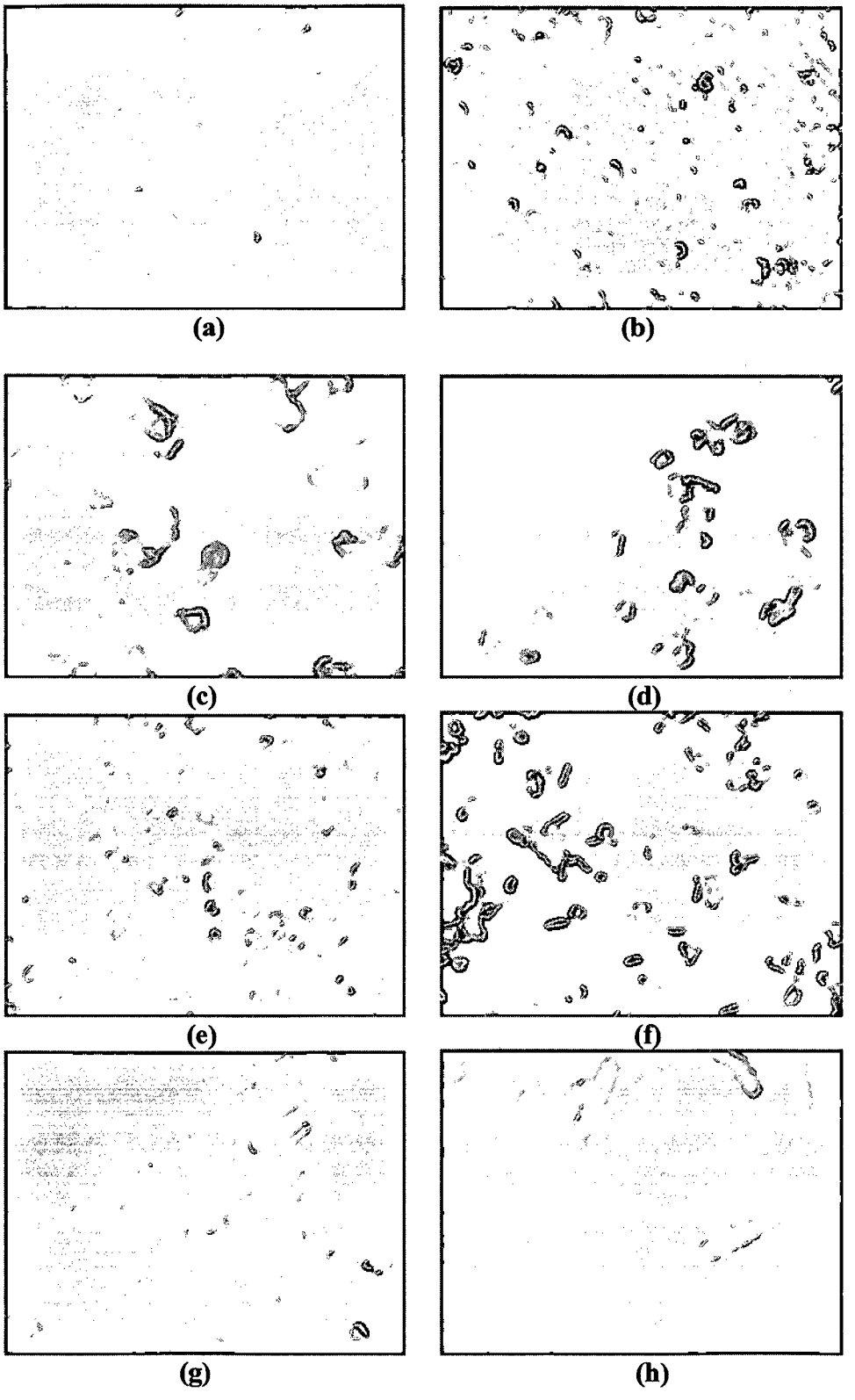


Plate 13: Scanning electron micrographs of gold-plated cells grown in liquid culture NTYE medium, pH 7.0 (a) $GUFF_{40}$ 13,000x; (b) $GUFF_{41}$ 5,000x; $GUFF_{69}$-(c) 13,000x (d) 7,000x (e) 5,000x (f) 5,000x of $GUFF_{63}$; (g) $GUFF_{79}$ 7,000x (h) $GUFF_{88}$ 13,000x

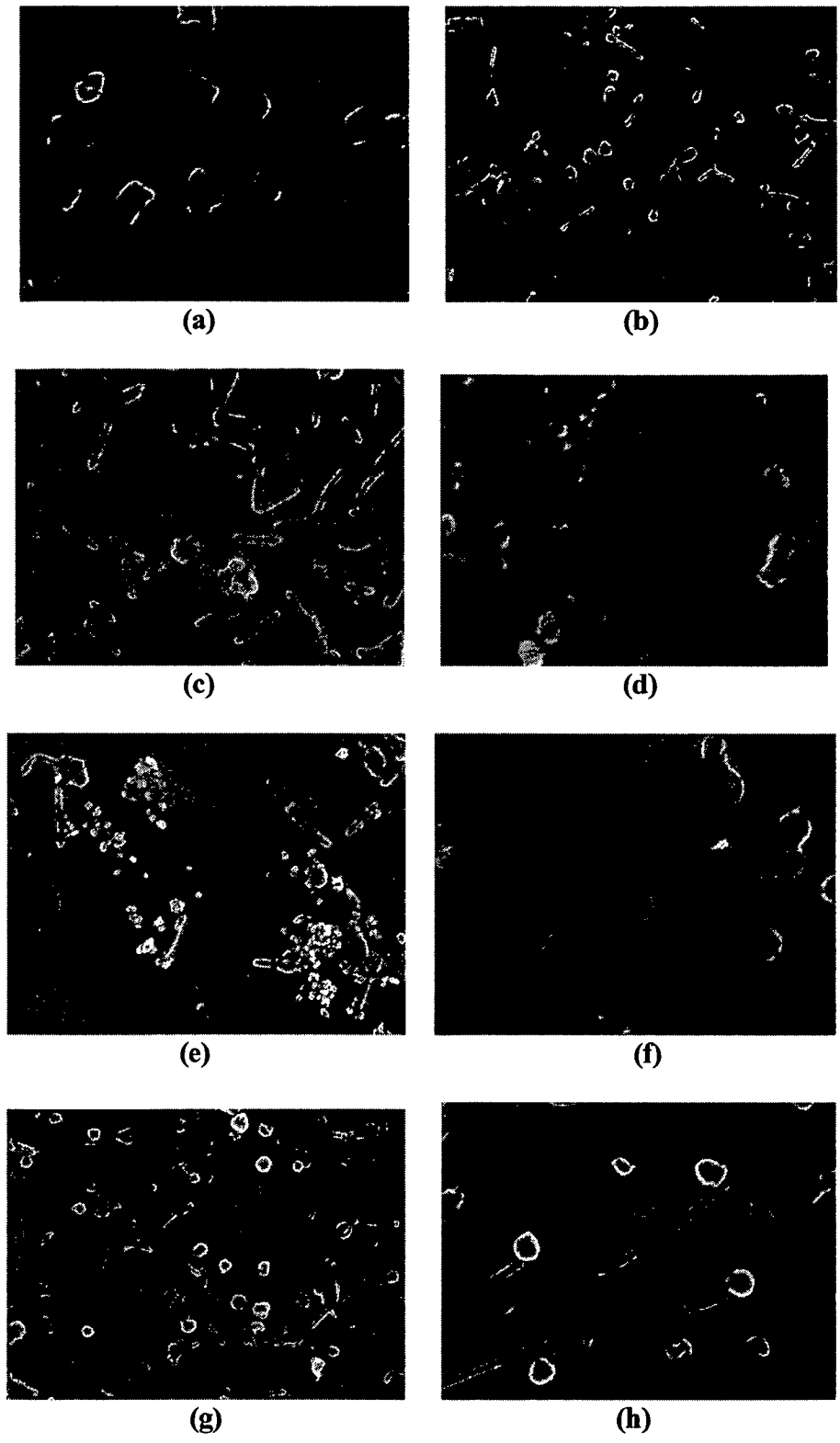


Plate 13: Scanning electron micrographs of gold-plated cells grown in liquid culture NTYE medium, pH 7.0 (a) $GUFF_3$ 13,000x; (b) $GUFF_5$ 5,000x; $GUFF_{13}$-(c) 7,000x (d) 22,000x; (e) 7,000x (f) $GUFF_{19}$ 13,000x; $GUFF_{26}$- (g) 5,000x (h) 10,000x

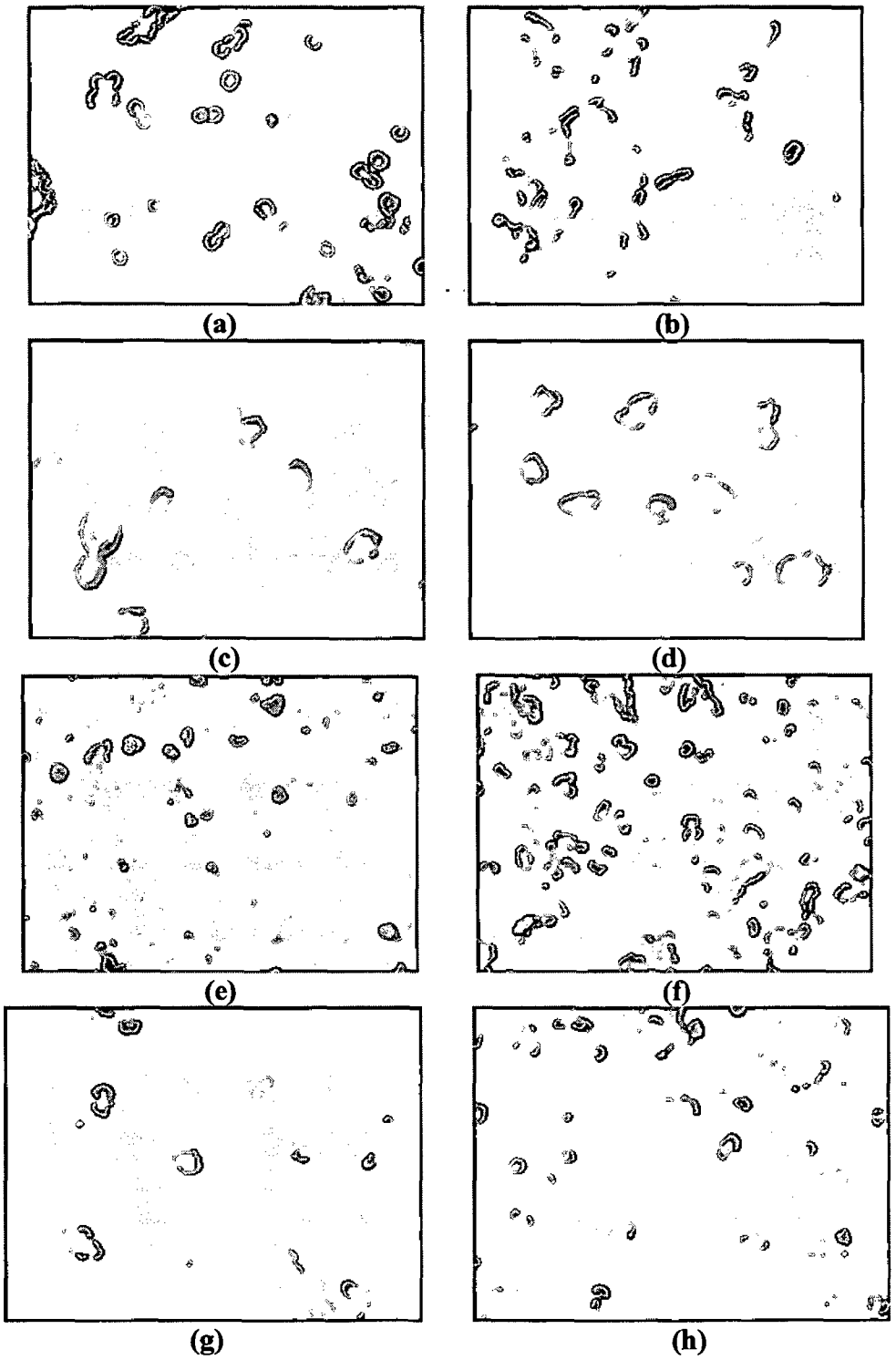


Plate 13: Scanning electron micrographs of gold-plated cells grown in liquid culture NTYE medium, pH 7.0 (a) $GUFF_{127}$ 5,000x; (b) $GUFF_{137}$ 7,000x;-(c) $GUFF_{90}$ 13,000x (d) $GUFF_{169}$ 13,000x; and pH 5.0 (e) $GUFF_{224}$ 5,000x (f) $GUFF_{227}$ 5,000x, $GUFF_{177}$- (g) 5,000x; (h) $GUFF_{185}$ 4,000x

The retrievable microbial types obtained from various salt pans studied are presented in **Fig.9.**

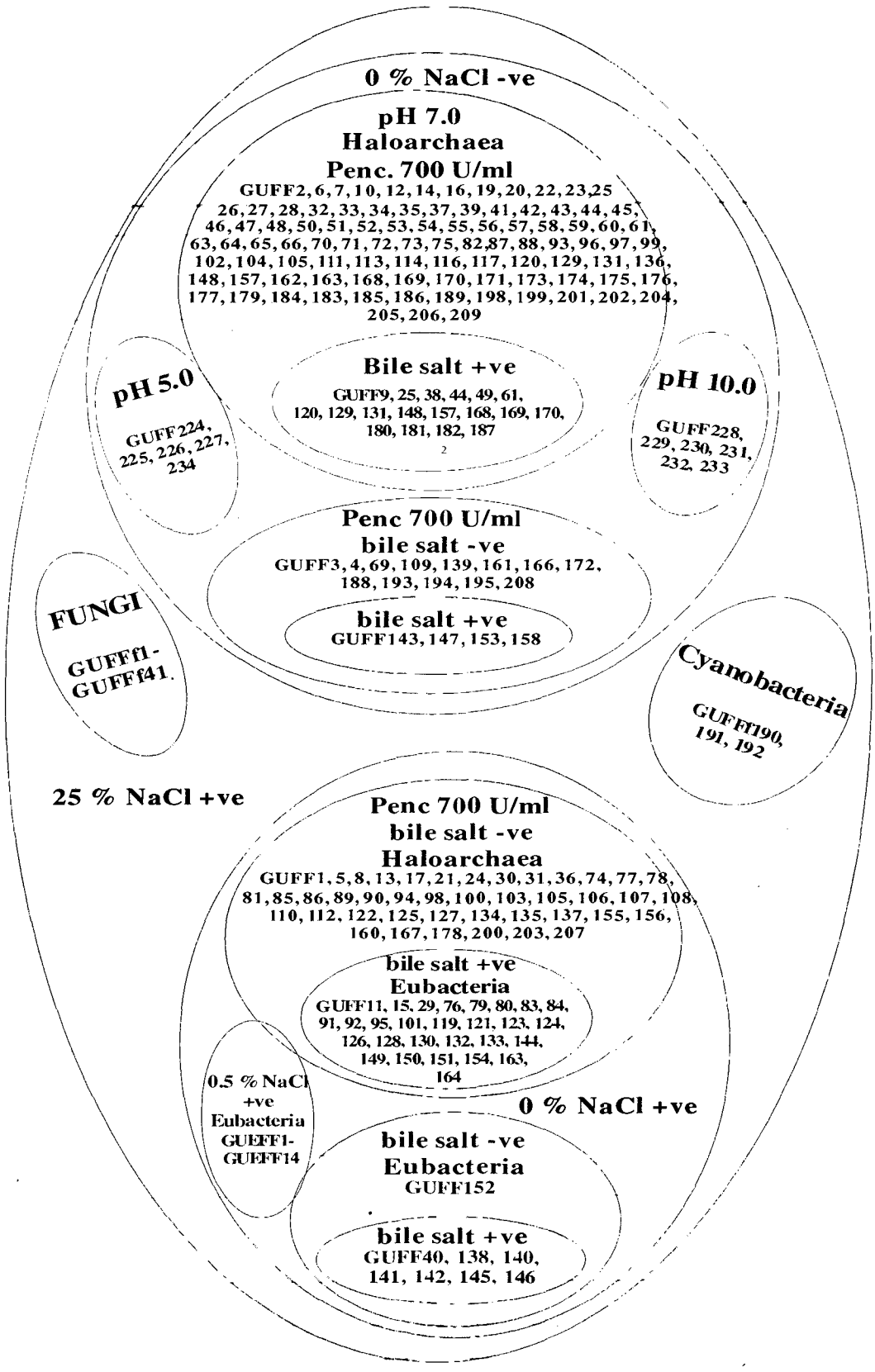


Fig. 9: Total retrievable microbial community and types from salt pans of Goa

SECTION B

DIVERSITY OF HALOARCHAEA AND ASSOCIATE MICROBIOTA IN SALT PANS OF GOA

Influence of annual climatic changes on physico-chemical status and microbiota of salt pan econiche

Land used for evaporation and crystallization of salt from riverine waters gather water with the onset of monsoon and finally is submerged to 1.0–1.5 metres with progress of monsoon (i.e. from June to September) annually (submerged phase). It is, therefore, the endeavour of this study to check the impact of such changes pH, soil conditions and the microbiota of the sediment of these lands during the monsoon **(Plate 15a)** as well as post-monsoon, a period when the land is submerged nearly by a water cover of 15-20 cms., during September to December (shallow water phase) **(Plate 15b).**

(a) **(b)**

Plate 15: (a) Submerged (monsoon), (b) Shallow water (post monsoon)

Effect of Monsoon

On an average, Goa received a total rainfall of 120 cm during 2001, 2003, 2005. Salt pans at Ribandar and Siridao had a water column of 1.5 and 1.0 metre above sediment respectively. Both, at post-monsoon stage, had water column nearly 20 cm above sediment. Colour and

texture of these sediments were brownish black and clay loam during both seasons. Moisture content in monsoons and post monsoons was 1.3 g and 0.74 g; 1.4 g and 0.8 g for sediments of R and S salt pans respectively. Temperature during the two seasons was around 25^0 C. The pH was 7.0, 7.5 & 7.4 for R & S salt pan water samples and sediments respectively.

Most probable counts of bacteria

During the monsoons of 2003, water samples from salt farming land at R & S gave counts of $1.1x10^5$ cfu/ml while sediment samples gave counts of $1.1x10^6$ cfu/g of **non-halophiles** as in **Table 24.** HS medium for **moderate halophiles** had only column & sediment growers. Total MPN counts of moderate halophiles in waters of R & S were $7.2x10^4$ cfu/ml while that of sediments were $7.2x10^5$ cfu/g. MPN counts of **extreme halophiles** in water of R were found to be $7.2x10^4$ cfu/ml while that of S were $1.1x10^5$ cfu/ml. Extreme halophilic pellicle growers were not observed in waters of R. Total MPN counts of extreme halophiles in sediments of R and S were found to be $1.1x10^6$ cfu/g. As in **Table 24,** during post monsoons, counts of $4.4x10^2$ to $2.8x10^3$ cfu/ml, $3.6x10^5$ to $7.2x10^5$ cfu/g, $2.8x10^3$ to $7.2x10^4$ cfu/ml and $8.5x10^4$ to $7.2x10^5$ cfu/g were recorded for R_w, R_s, S_w, S_s respectively.

Most probable counts of haloarchaea

Sediment & water samples from R & S salt pans gave a total of $1.1x10^6$ cfu/g in NTYE and PM medium, the same in HS gave $7.2x10^4$ to $7.2x10^5$ cfu/ml, while a count of $3.6x10^4$ to $3.9x10^5$ cfu/g during monsoons and $1.6x10^2$ to $1.1x10^5$ cfu/ml during post monsoons in NGSM **(Table 25).** Haloarchaea capable of growing on synthetic medium with single carbon source, i.e. glucose, casein, starch, agar were enumerated. Counts for sediments of R & S salt pans were 10 fold higher than for water samples as seen in **Table 26** and ranged between $2.0x10^1$ to $3.9x10^5$ cfu/g.

Table 24 : Most probable number of bacteria in water & sediments of salt pans during monsoon and post-monsoon

Salt pans	Counts of bacteria						
	Bacteria	Non-halophiles		Moderate halophiles		Extreme halophiles	
	Culture Media	NB NB		HS HS		NTYE NTYE	
	Year	2003	TC	2003	TC	2003	TC
R_w	P	$3.6x10^4$		0		0	
		$9.6x10^0$	$1.1x10^5$	0	$7.2x10^4$	$4.8x10^0$	$7.2x10^4$
	C	$3.6x10^4$		$3.6x10^4$		$3.6x10^4$	
		$1.4x10^3$	$2.8x10^3$	$4.4x10^2$	$4.4x10^2$	$1.4x10^3$	$2.8x10^3$
	B	$3.6x10^4$		$3.6x10^4$		$3.6x10^4$	
		$1.4x10^3$		0		$1.4x10^3$	
R_s	P	$3.6x10^5$		0		$3.6x10^5$	
		$1.0x10^3$	$1.1x10^6$	0	$7.2x10^5$	$1.2x10^3$	$1.1x10^6$
	C	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
		$3.6x10^5$	$7.2x10^5$	$3.6x10^5$	$3.6x10^5$	$3.6x10^5$	$7.2x10^5$
	B	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
		$3.6x10^5$		0		$3.6x10^5$	
S_w	P	$3.6x10^4$		0		$3.6x10^4$	
		$2.0x10^2$	$1.1x10^5$	0	$7.2x10^4$	$1.2x10^2$	$1.1x10^5$
	C	$3.6x10^4$		$3.6x10^4$		$3.6x10^4$	
		$3.6x10^4$	$7.2x10^4$	$2.8x10^3$	$2.8x10^3$	$3.6x10^4$	$7.2x10^4$
	B	$3.6x10^4$		$3.6x10^4$		$3.6x10^4$	
		$3.6x10^4$		0		$3.6x10^4$	
S_s	P	$3.6x10^5$		0		$3.6x10^5$	
		$1.2x10^3$	$1.1x10^6$	0	$7.2x10^5$	$3.0x10^3$	$1.1x10^6$
	C	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
		$3.6x10^5$	$7.2x10^5$	$8.5x10^4$	$8.5x10^4$	$3.6x10^5$	$7.2x10^5$
	B	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
		$3.6x10^5$		0		$3.6x10^5$	

TC-Total Count, w-water, s-sediment, counts in water-cfu/ml, counts in sediment-cfu/g, counts in blue-Postmonsoon (shallow) , counts in black-monsoon (submerged)

Table 25: Most probable number of haloarchaea in water and sediments of salt pans on various media during monsoon and post- monsoon

Salt pan	Count of bacteria						
	Media						
	Culture Media	NTYE / PM NTYE		HS HS		NGSM NGSM	
	Year	2003	TC	2003	TC	2003	TC
R_w	P	0		0		$8.0x10^1$	
		$4.8x10^0$	$7.2x10^4$	0	$7.2x10^4$	0	$3.6x10^4$
	C	$3.6x10^4$		$3.6x10^4$		$3.6x10^4$	
		$1.4x10^3$	$2.8x10^3$	$4.4x10^2$	$4.4x10^2$	$1.6x10^2$	$1.6x10^2$
	B	$3.6x10^4$		$3.6x10^4$		$2.0x10^2$	
		$1.4x10^3$		0		0	
R_s	P	$3.6x10^5$		0		$8.0x10^2$	
		$1.2x10^3$	$1.1x10^6$	0	$7.2x10^5$	0	$3.9x10^5$
	C	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
		$3.6x10^5$	$7.2x10^5$	$3.6x10^5$	$3.6x10^5$	$3.5x10^4$	$3.5x10^4$
	B	$3.6x10^5$		$3.6x10^5$		$2.0x10^3$	
		$3.6x10^5$		0		0	
S_w	P	$3.5x10^4$		0		0	
		$1.2x10^2$	$1.1x10^5$	0	$7.2x10^4$	0	$3.6x10^4$
	C	$3.6x10^4$		$3.6x10^4$		$3.6x10^4$	
		$3.6x10^4$	$7.2x10^4$	$2.8x10^3$	$2.8x10^3$	$1.0x10^3$	$1.0x10^3$
	B	$3.6x10^4$		$3.6x10^4$		0	
		$3.6x10^4$		0		0	
S_s	P	$3.6x10^5$		0		0	
		$3.0x10^3$	$1.1x10^6$	0	$7.2x10^5$	0	$3.6x10^5$
	C	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$	
		$3.6x10^5$	$7.2x10^5$	$8.5x10^4$	$8.5x10^4$	$1.1x10^5$	$1.1x10^5$
	B	$3.6x10^5$		$3.6x10^5$		0	
		$3.6x10^5$		0		0	

TC-Total Count, w-water, s-sediment, counts in water-cfu/ml, counts in sediment-cfu/g, counts in blue-Postmonsoon (shallow) , counts in black-monsoon (submerged), NP medium had no growth

Table 26: Most probable number of haloarchaea in water & sediment of salt pans growing on synthetic medium with single carbon source during monsoon and post monsoon

Counts of bacteria									
Salt pan	Culture media	NGSM		NCSM		NSSM		NSAM	
	Year	2003	TC	2003	TC	2003	TC	2003	TC
R_w	P	$8.0x10^1$		$3.0x10^2$		$3.0x10^2$		0	
		0	$3.6x10^4$	0	$3.6x10^4$	0	$3.6x10^4$	0	$1.8x10^2$
	C	$3.6x10^4$		$3.6x10^4$		$3.6x10^4$		$1.8x10^2$	
		$1.6x10^2$	$1.6x10^2$	$1.6x10^2$	$1.6x10^2$	$1.8x10^2$	$1.8x10^2$	$2.4x10^1$	$2.4x10^1$
	B	$2.0x10^2$		0		0		0	
		0		0		0		0	
R_s	P	$8.0x10^2$		$3.0x10^3$		$3.0x10^3$		0	
		0	$3.9x10^5$	0	$3.6x10^5$	0	$3.6x10^5$	0	$1.8x10^3$
	C	$3.6x10^5$		$3.6x10^5$		$3.6x10^5$		$1.8x10^3$	
		$3.5x10^4$	$3.5x10^4$	$7.0x10^3$	$7.0x10^3$	$3.6x10^5$	$3.6x10^5$	$5.0x10^3$	$5.0x10^3$
	B	$2.0x10^3$		0		0		0	
		0		0		0		0	
R_w	P	0		$3.4x10^2$		$3.4x10^2$		0	
		0	$3.6x10^4$	0	$1.7x10^3$	0	$1.4x10^3$	0	$2.0x10^1$
	C	$3.6x10^4$		$1.4x10^3$		$1.4x10^3$		$2.0x10^1$	
		$1.0x10^3$	$1.0x10^3$	$2.2x10^2$	$2.2x10^2$	$3.6x10^3$	$3.6x10^3$	$5.0x10^2$	$5.0x10^2$
	B	0		0		0		0	
		0		0		0		0	
R_s	P	0		$3.4x10^3$		$3.4x10^3$		0	
		0	$3.6x10^5$	0	$1.7x10^4$	0	$1.7x10^4$	0	$2.0x10^2$
	C	$3.6x10^5$		$1.4x10^4$		$1.4x10^4$		$2.0x10^2$	
		$1.1x10^5$	$1.1x10^5$	$4.0x10^3$	$4.0x10^3$	$3.2x10^5$	$3.2x10^5$	$5.0x10^3$	$5.0x10^3$
	B	0		0		0		0	
		0		0		0		0	

TC-Total Count, w-water, s-sediment, counts in water-cfu/ml, counts in sediment-cfu/g, counts in blue-Postmonsoon (shallow) , counts in black-monsoon (submerged)

Post monsoon phase runs into the preparative stage for harvesting of crystalline salt during the annual summer season i.e. (January to May).

Microbial status during progression of salt crystal precipitation

The crystallisation of salt involves preparative stage, water evaporation and finally in the formation of crystals.

One salt pan at Ribandar in Tiswadi Taluka was studied for the entire process of salt farming for microflora as detailed below.

a) Preparative stage: The water of R salt pan which was prepared for carrying out salt farming, when inoculated into medium containing 0% NaCl gave counts of $2.8x10^3$ cfu/ml, while medium containing 25% crude solar salt gave counts which were same in number as that for medium containing 0% NaCl. Total MPN counts of both the types were $5.6x10^3$ cfu/ml.

b) Microflora of inlet waters: Water evaporation stage consisted of taking in waters (inlet waters) into the salt pan; total counts of bacteria in media growing at 0% NaCl and 25% crude solar salt were $4.1x10^4$ cfu/ml.

c) Microflora of stagnant evaporating waters: These waters which were taken into the salt pan were allowed to stagnate & then allowed to evaporate. When stagnant evaporating waters were inoculated into media containing 0% NaCl and 25% crude solar salt, gave total MPN counts of $8.3x10^4$ cfu/ml.

d) Microflora of salt crystallization stage/brine: Studies carried out during this phase are detailed in the earlier **Section A.**

e) Microflora associated with salt crystals: Crystals of salt often had orangish red colouration. On an average, the counts on NTYE were $1.6x10^4$ cfu/ml. In some of the salt pans the crystals had a tendency to adhere and give rise to large flakes as in **Plate 16.** These showed distinct green cyanobacteria growing as mats. Effect of seasonal changes on diversity of microbial community is as seen in **Fig.10**.

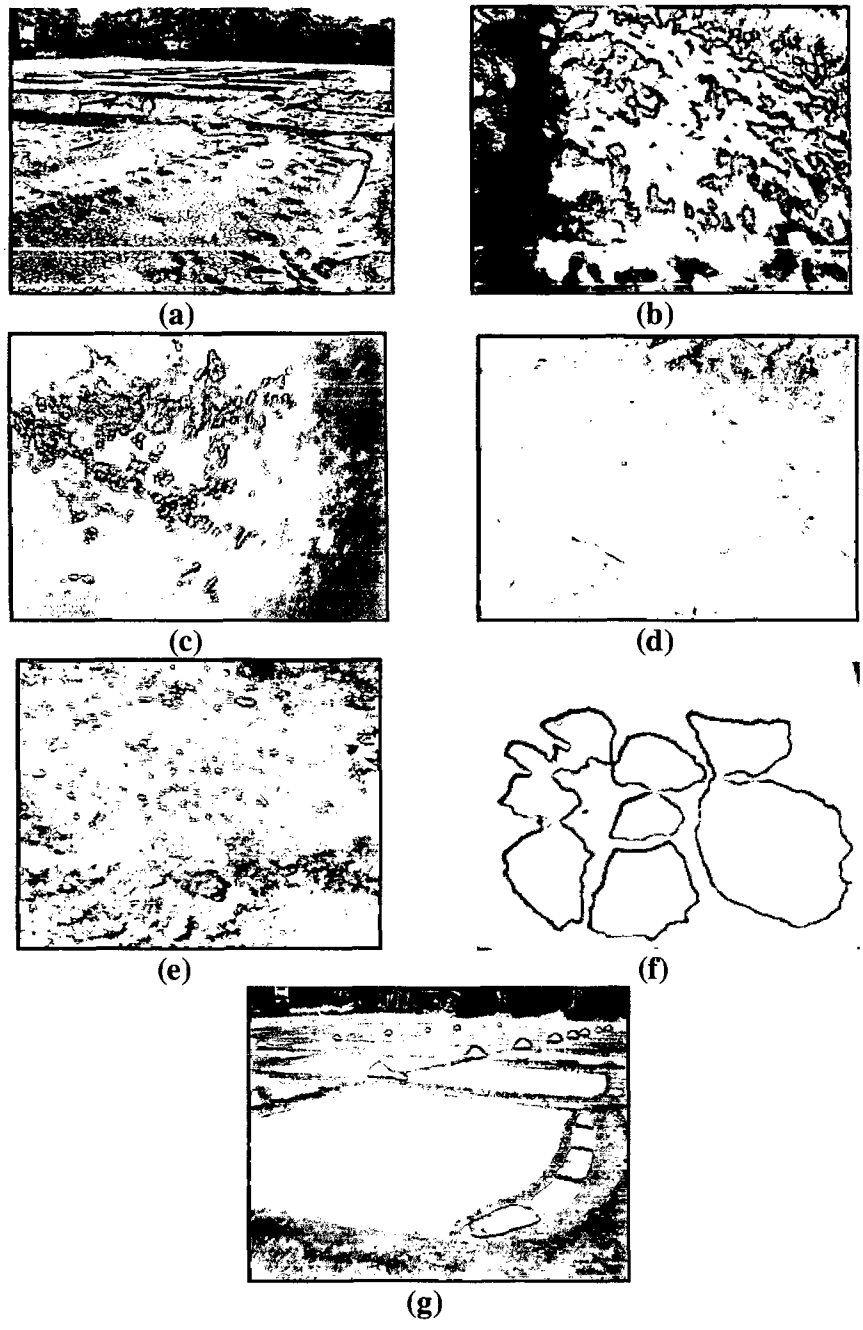
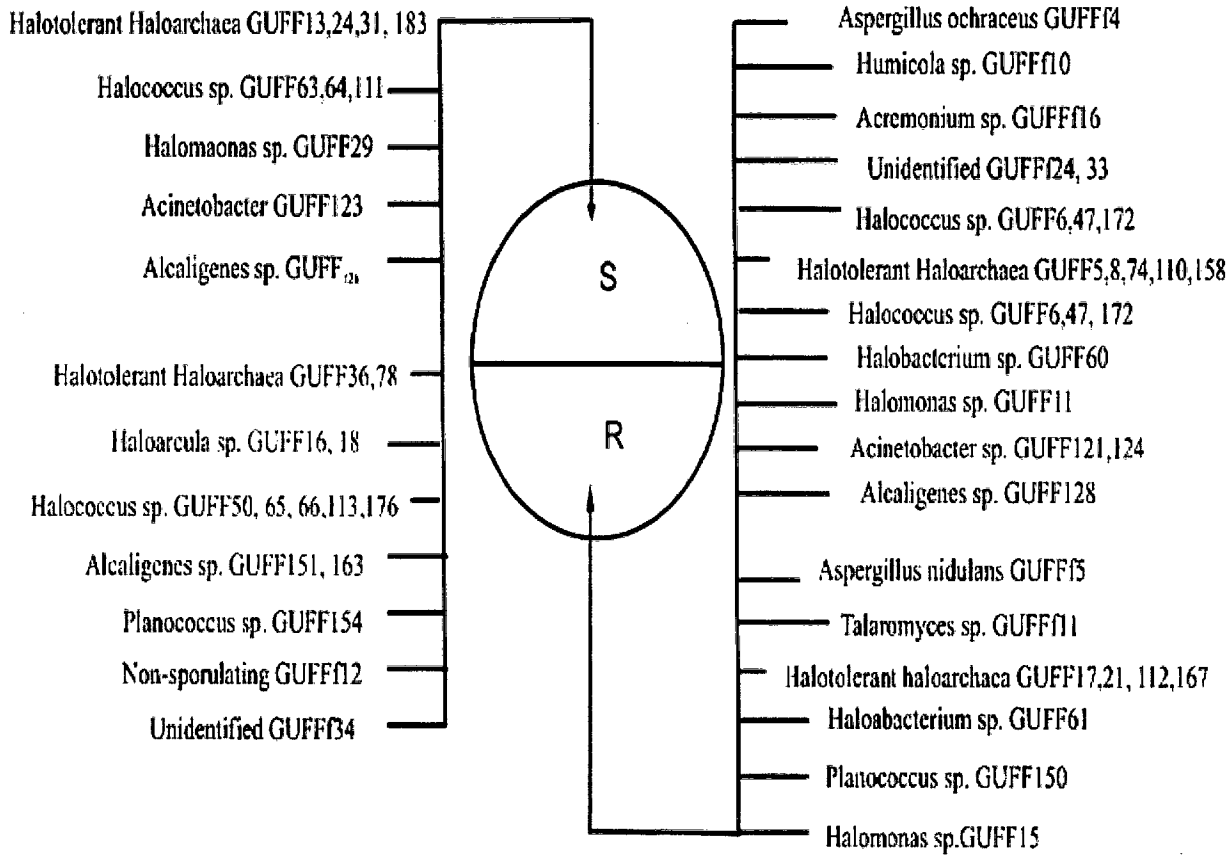


Plate 16: Salt farming stage – (a) preparation of land, (b) extensive salt precipitation (c-e) halobacterial bloom, (f) salt flakes from salt pan, (g) harvest of crude salt as heaps.

Fig. 10: Microbial community in monsoon and post-monsoon



Printed in black: monsoon, printed in pink: post-monsoon

SECTION C

SIGNIFICANCE OF HALOARCHAEA & METAL AND OTHER POLLUTANTS IN SALT PANS

1. Role of Haloarchaea in C and N cycles of econiche

Microflora in all soils/sediments are known to engage themselves in geochemical recycling of elements, either through degradative processes (heterotrophy) or through processes involving direct fixation of atmospheric elements. The land used for salt processing, although has no vegetation, is fringed by mangroves and by saline rice paddies. This study tried to evaluate the role of haloarchaea (the dominant inhabitants of salt pans) in geochemical re-cycling of Carbon & Nitrogen through very simple experiments of enumerating haloarchaeal degraders and fixers in brine & sediment samples of salt pan.

a) Most probable number of Carbon degraders in brine and sediments of salt pans

Brine samples from six salt pans were inoculated separately, into NSM medium with 0.2% glucose/0.5% starch/0.5% agar/0.5% casein as sole source of carbon, growth without addition of growth factors/supplements, as in **Table 27.**

Glucose supported total MPN counts of haloarchaea of $5.2x10^3$ cfu/ml, higher for S salt pan than R salt pan in 2001. Counts obtained were highest of $6.4x10^3$ cfu/ml for R salt pan and lowest of $2.4x10^3$ cfu/ml for Ar salt pan in 2003, while counts were the same for R & S salt pans in 2005.

Starch : Total MPN counts of $4.1x10^4$ cfu/ml were obtained for S salt pan in 2001; while counts were highest for S salt pan of $9.2x10^4$ cfu/ml and lowest of $4.7x10^3$ cfu/ml for Ag salt pan in 2003. R salt pan showed higher counts of $4.1x10^4$ cfu/ml than S salt pan in 2005.

Table 27: MPN of haloarchaea in brine of salt pans growing on synthetic medium with single carbon source

Counts of haloarchaea (cfu/ml) on synthetic media																			
Salt Pan		NGSM / NG(Fe)SM / NG(Mn)SM						NSSM / NCSM						NSAM					
	Year	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC
A	P	-	-	-	-	-	-	-		$5.5x10^2$		-		-		0		-	
		-	-	0	0	-	-			$3.6x10^4$									
		-	-	0	0	-	-												
	C	-	-	-	-	-	-	-	-	$4.6x10^3$	$8.8x10^3$	-	-	-	-	$2.1x10^1$	$2.1x10^1$	-	-
		-	-	$4.0x10^3$	$4.0x10^3$	-	-			$3.6x10^4$	$1.1x10^5$								
		-	-	$1.3x10^3$	$1.3x10^3$	-	-												
	B	-	-	-	-	-	-	-		$3.6x10^3$		-		-		0		-	
		-	-	0	0	-	-			$3.6x10^4$									
		-	-	0	0	-	-												
AgD	P	-	-	-	-	-	-	-		$5.2x10^2$		-		-		0		-	
		-	-	0	0	-	-			$3.6x10^4$									
		-	-	0	0	-	-												
	C	-	-	-	-	-	-	-	-		$4.7x10^3$	-	-	-	-	$2.0x10^1$	$2.0x10^1$	-	-
		-	-	$3.0x10^3$	$3.0x10^3$	-	-			$6.0x10^2$	$1.1x10^5$								
		-	-	$1.2x10^3$	$1.2x10^3$	-	-			$3.6x10^4$									
	B	-	-	-	-	-	-	-				-		-		0		-	
		-	-	0	0	-	-			$3.6x10^3$									
		-	-	0	0	-	-			$3.6x10^4$									
Ar_1T	P	-	-	-	-	-	-	-		$7.0x10^3$		-		-		0		-	
		-	-	0	0	-	-			$3.6x10^4$									
		-	-	0	0	-	-												
	C	-	-	-	-	-	-	-	-		$7.5x10^4$	-	-	-	-	$6.0x10^1$	$6.0x10^1$	-	-
		-	-	$3.6x10^4$	$3.6x10^4$	-	-			$3.2x10^4$	$1.1x10^5$								
		-	-	$5.0x10^3$	$5.0x10^3$	-	-			$3.6x10^4$									
	B	-	-	-	-	-	-	-				-		-		0		-	
		-	-	0	0	-	-			$3.6x10^4$									
		-	-	0	0	-	-			$3.6x10^4$									

TC Total Count; w-water; s-sediment; counts in blue-NG(Fe)SM; counts in green–NG(Mn)SM; counts in black–NGSM; counts in red–NSSM; counts in orange–NSAM; counts in purple–NCSM

Table 27: MPN of haloarchaea in brine of salt pans growing on synthetic medium with single carbon source

		Counts of haloarchaea (cfu/ml) on synthetic media																	
lt Pan		NGSM / NG(Fe)SM / NG(Mn)SM						NSSM / NCSM						NSAM					
	Year	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC	2001	TC	2003	TC	2005	TC
	P	-	-	-	-	-	-	-		$1.6x10^2$		-		-		0		-	
		-	-	0	0	-	-			$3.6x10^4$									
		-	-	0	0	-	-												
N	C	-	-	-	-	-	-	-	-		$6.8x10^4$	-	-	-	-	$1.0x10^2$	$1.0x10^2$	-	-
		-	-	$9.0x10^3$	$9.0x10^3$	-	-			$3.2x10^4$	$1.1x10^5$								
		-	-	$3.6x10^4$	$3.6x10^4$	-	-			$3.6x10^4$									
	B	-	-	-	-	-	-	-				-		-		0		-	
		-	-	0	0	-	-			$3.6x10^4$									
		-	-	0	0	-	-			$3.6x10^4$									
	P	$2.0x10^2$		$2.4x10^2$		$4.0x10^2$		0		$8.2x10^3$		$2.5x10^3$		0		0		0	
		0		0		0		$3.6x10^4$		$3.6x10^4$		$3.6x10^4$							
		0		0		0													
R	C	$4.0x10^2$	$4.2x10^3$	$5.1x10^2$	$6.4x10^3$	$3.6x10^3$	$7.6x10^3$	$5.0x10^1$	$5.0x10^1$		$4.9x10^4$	$2.8x10^4$	$4.1x10^4$	$5.0x10^1$	$5.0x10^1$	$1.0x10^1$	$1.0x10^1$	$1.5x10^1$	$1.5x10^1$
		$2.3x10^3$	$2.3x10^3$	$4.0x10^3$	$4.0x10^3$	$3.6x10^3$		$3.6x10^4$	$1.1x10^5$	$4.5x10^6$	$1.1x10^5$	$3.6x10^4$	$1.1x10^5$						
		$3.6x10^3$	$3.6x10^3$	$3.6x10^3$	$3.6x10^3$	$3.0x10^3$	$3.6x10^3$			$3.6x10^4$									
	B	$3.6x10^3$		$5.6x10^3$		$3.6x10^3$	$3.0x10^3$	0				$3.6x10^4$		0		0		0	
		0		0		0		$3.6x10^4$		$3.6x10^4$		$3.6x10^4$							
		0		0		0				$3.6x10^4$									
	P	$4.0x10^2$		$4.8x10^2$		$3.6x10^2$		0		$4.0x10^3$		$1.1x10^3$		0		0		0	
		0		0		0		$3.6x10^4$		$3.6x10^4$		$3.6x10^4$							
		0		0		0													
S	C	$1.2x10^3$	$5.2x10^3$	$5.7x10^2$	$6.0x10^3$	$3.6x10^3$	$7.6x10^3$		$2.0x10^1$		$9.2x10^4$	$2.8x10^3$	$3.4x10^4$	$2.0x10^1$	$2.0x10^1$	$2.5x10^1$	$2.5x10^1$	$2.1x10^1$	$2.1x10^1$
		$3.6x10^2$	$3.6x10^2$	$3.6x10^2$		$3.0x10^2$	$3.0x10^2$	$2.0x10^1$	$1.1x10^5$	$5.2x10^4$	$1.1x10^5$	$3.6x10^4$	$1.1x10^5$						
		$1.4x10^3$	$1.4x10^3$	$2.6x10^3$	$3.6x10^2$	$3.1x10^3$	$3.1x10^3$	$3.6x10^4$		$3.6x10^4$									
	B	$3.6x10^3$		$4.9x10^3$	$2.6x10^3$	$3.6x10^3$						$3.0x10^4$		0		0		0	
		0		0		0		0		$3.6x10^4$		$3.6x10^4$							
		0		0		0		$3.6x10^4$		$3.6x10^4$									

TC Total Count; w-water; s-sediment; counts in blue-NG(Fe)SM; counts in green-NG(Mn)SM; counts in black–NGSM; counts in red–NSSM; counts in orange–NSAM; counts in purple–NCSM

Agar supported growth of agar degraders which constituted only of column growers. Total MPN counts of $5.0x10^1$ cfu/ml for R salt pan were higher than S salt pan in 2001. Agar digesters grew throughout the column, counts of $1.0x10^2$ cfu/ml were obtained for N salt pan & lowest for R salt pan of $1.0x10^1$ cfu/ml in 2003. S salt pan gave higher count of $2.5x10^1$ cfu/ml than R salt pan in 2005. One of the isolate $GUFF_{177}$, when inoculated separately into NSM with 0.1%/0.2%/0.3%/0.4%/0.5% of agar, digested agar in tubes containing 0.3%, 0.4%, 0.5%, into flakes/blobs of agar. Phenol sulphuric acid method of estimation gave 0.9 % total carbohydrate in terms of glucose while DNSA method yielded 0.08% of reducing sugar in the liquefied medium. 10 ml of liquefied agar culture medium now could be pipetted and had a flow of 4 seconds/ml.

Casein Total MPN counts of haloarchaea were $1.1x10^5$cfu/ml in the years of 2001, 2003, 2005 in all the salt pans.

Most probable number of haloarchaea obtained in brine & sediment of salt pans during post monsoon on synthetic medium with a single carbon source are recorded in **Table 27.**

Total viable counts for fungi in brine/sediment ranged from $1.0x10^4$ cfu/g to $2.4x10^4$ cfu/ml $2.0x10^3$ cfu/g to $1.5x10^4$ cfu/ml, and $1.1x10^3$ cfu/ml to $8.0x10^3$ cfu/g in glucose, starch and casein synthetic media respectively. Of the 42 fungal cultures isolated during study of Section A, 18 had absolute requirement of NaCl for their growth and grew to 20% crude solar salt concentration in SAB medium. These interestingly, degraded glucose, cellulose, cellobiose, pectin and even hydrocarbon as a sole source of carbon during growth in synthetic medium as seen in **Table 28; Fig. 11 & Plate no.17**. $GUFFf_{31}$ and $GUFFf_{39}$ showed mobilisation of calcium, **Plate no.18.**

Table 28: Total viable counts of fungi in sediments/brine on synthetic media with single source of carbon

Taluka	Salt pan		Counts of fungi in brine and sediments		
			NGSM	NSSM	NCSM
Pernem	A	b	$1.7x10^4$	$1.5x10^4$	$3.0x10^3$
	AgD	s	$1.0x10^4$	$2.0x10^3$	$2.0x10^3$
Bardez	Ar_1T	b	$1.7x10^4$	$2.1x10^4$	$3.5x10^3$
	N	b	$2.4x10^4$	$1.3x10^4$	$1.1x10^3$
Tiswadi	R	s	$1.1x10^4$	$4.8x10^3$	$8.0x10^3$
	S	b	$1.6x10^4$	$1.1x10^4$	$6.0x10^3$

b-brine; s-sediment; counts in brine-cfu/ml, counts in sediment-cfu/g

Table 28: Degradation potential of fungi on various sources of carbon

Salt pan	Fungal isolates	Zone of growth(mm) in NSM with 20% crude solar salt																	
		Carbohydrates															Hydrocarbon		
		Glucose			Starch			Cellulose			Cellobiose			Pectin			Grease		
		Days of incubation and Temp. (28+ 2^0C)																	
		7	10	23	7	10	23	7	10	23	7	10	23	7	10	23	7	10	23
R_S	$GUFFf_2$	3	10	11	4	7	13	2	3	8	2	5	7	3	5	12	-	4	9
S_s	$GUFFf_3$	5	9	10	6	12	18	2	3	9	5	7	12	3	5	16	-	5	18
R_s	$GUFFf_4$	4	10	15	5	13	25	2	4	13	5	8	17	3	6	25	-	4	12
S_s	$GUFFf_5$	5	7	17	7	12	24	2	5	23	3	12	16	3	5	23	-	5	20
Ag_s	$GUFFf_6$	7	7	17	7	13	18	2	5	19	7	8	16	3	5	18	-	4	17
A_s	$GUFFf_7$	10	10	16	7	15	21	2	5	21	8	10	18	3	5	20	-	4	15
R_s	$GUFFf_{10}$	6	12	22	9	15	18	2	5	20	3	5	19	3	3	20	-	5	20
R_s	$GUFFf_{11}$	9	10	17	10	16	23	2	5	23	7	8	20	3	5	23	-	5	22
N_s	$GUFFf_{19}$	5	9	13	7	12	22	2	5	23	5	7	15	3	5	18	-	4	16
Ar_b	$GUFFf_{20}$	6	8	9	7	11	20	2	6	24	5	11	14	3	3	17	-	4	18
Ar_b	$GUFFf_{f21}$	8	10	17	6	14	22	3	6	26	3	12	17	3	8	25	-	5	21
Sw	$GUFFf_{24}$	7	10	14	7	17	26	2	5	23	8	12	20	3	6	18	-	4	19
Ar_s	$GUFFf_{25}$	8	8	18	8	15	24	2	5	25	7	14	20	3	9	24	-	5	21
Ar_s	$GUFFf_{26}$	8	9	15	9	18	25	3	8	26	7	13	18	3	8	20	-	5	25
Ag_s	$GUFFf_{35}$	9	10	12	7	15	23	2	4	26	10	16	22	3	6	17	-	4	20
N_s	$GUFFf_{37}$	8	9	11	10	19	25	4	7	20	4	5	17	3	6	13	-	5	20
A_b	$GUFFf_{39}$	7	9	18	8	16	23	2	5	23	6	13	20	3	9	14	-	4	20
R_s	$GUFFf_{40}$	8	10	17	6	16	21	4	5	22	5	10	18	3	6	14	-	3	18

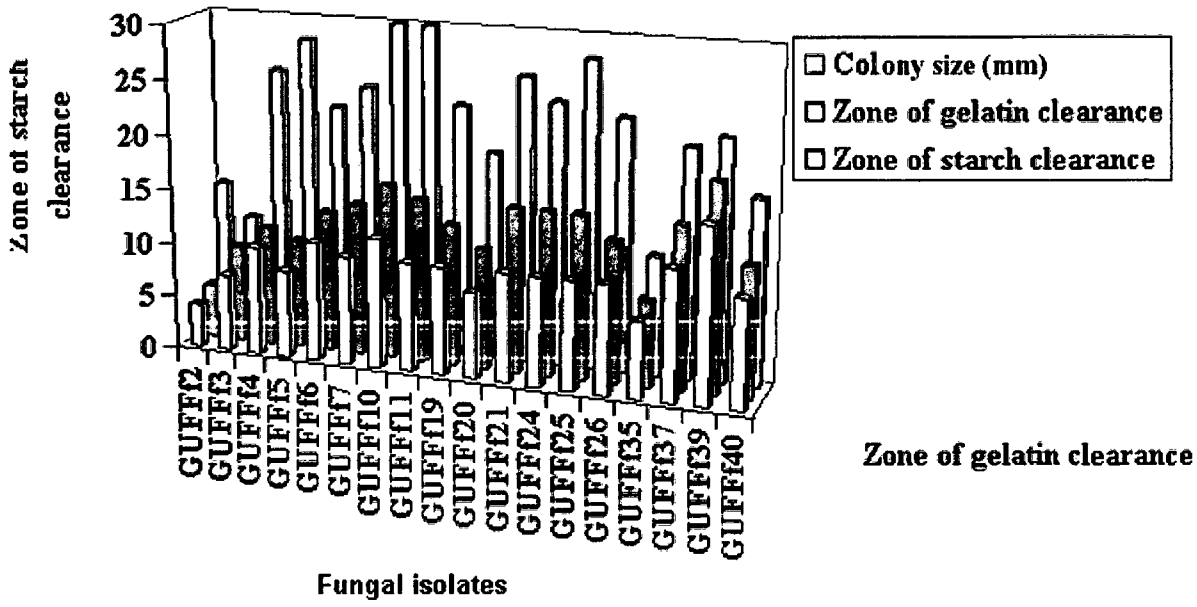


Fig. 11: Degradation of starch and gelatin by fungal isolates

(a) (b) (c)

Plate 17: Growth of fungi on synthetic medium NSM (a) Glucose (b) Cellobiose (c) Starch

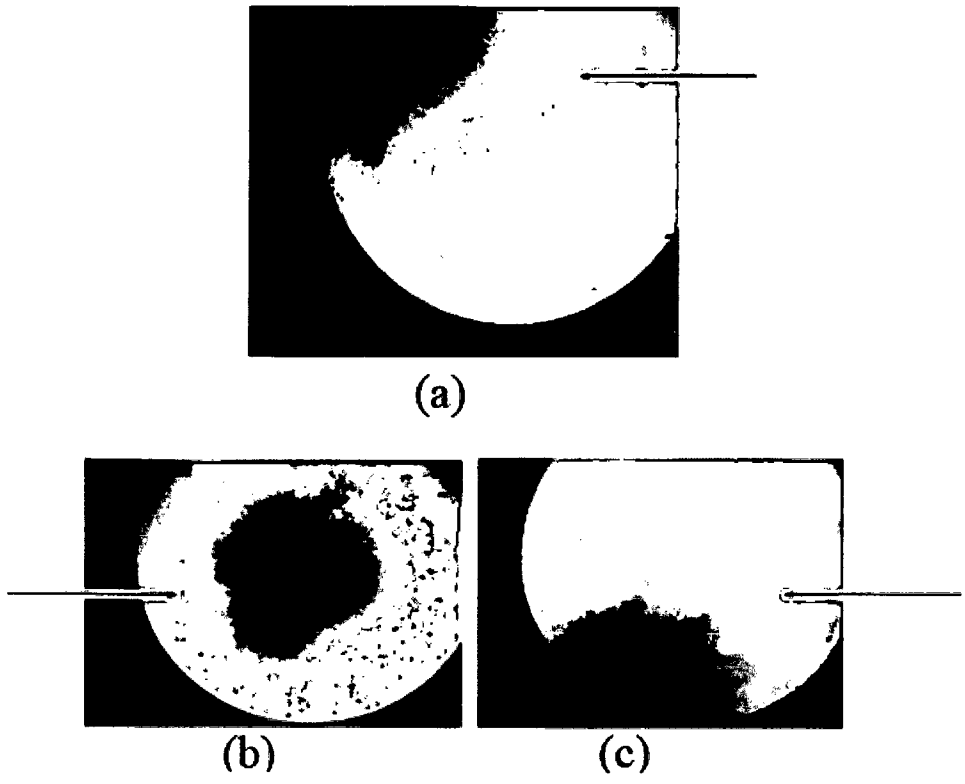


Plate 18: Calcium oxalate crystals (indicated by an arrow) produced by $GUFFf_{31}$ (a, b) and (c) $GUFFf_{39}$ on synthetic medium containing glucose

Colony forming units were greater by 10 fold in sediment than in water in both salt pans. The number of degraders in R salt pan were of the same order for glucose, starch & casein. In agar, however, the numbers were 2 fold less. The growth of degraders in each of the substrates could be distinctly differentiated into those growing as pellicle on surface and reaching a cell count range from $3.0x10^2$ cfu/ml to $3.4x10^3$ cfu/ml for starch during monsoons. Interestingly, pellicle degraders were absent for glucose in water as well as sediment samples of R & S salt pans during post monsoons; column growers occupying the entire column of growth medium with numbers of $3.5x10^4$ cfu/ml, these were two fold higher in numbers than pellicle formers. Further, they were a thousand to ten thousand times more in sediments than in waters of both salt pans. The numbers degrading single carbon source i.e. glucose were nearly the same as those degrading complex carbon sources, such as casein and starch.

The number of degraders over the period of 2001, 2003 & 2005 were nearly consistent with $3.4x10^4$ cfu/ml to $3.4x10^5$ cfu/g for glucose, $3.4x10^5$ cfu/g for starch, $3.6x10^5$ cfu/g for casein and $2.0x10^1$ cfu/ml to $1.8x10^3$ cfu/ml for agar degraders for water and sediment of both salt pans; and degraders utilizing glucose, starch, casein and agar as sole source of carbon, were also enumerated in brine samples. Interestingly, brine from salt pans gave identical counts of $1.1x10^5$cfu/g for casein.

b) Nitrogen degraders in brine and sediments of salt pans

Halophilic fungi showed growth in terms of zone sizes on synthetic media containing gelatin, chitin, chitosan **(Table 29).**

Table 29: Degradation potential of fungi on proteins

Fungal isolates	Zone of growth (mm) in NSM with 20% crude solar salt											
	Casein			Chitin			Chitosan			Gelatin		
	Days of incubation and Temp. (28± 2^{0}C)											
	7	10	23	7	10	23	7	10	23	7	10	23
GUFFf$_2$	5	11	21	2	14	22	5	9	20	2	4	13
GUFFf$_3$	3	7	12	2	6	8	2	3	6	2	4	12
GUFFf$_4$	2	5	12	2	5	15	2	5	10	2	4	12
GUFFf$_5$	5	9	20	3	6	20	2	6	19	2	5	22
GUFFf$_6$	10	16	23	3	5	28	3	7	22	2	5	23
GUFFf$_7$	8	13	19	3	7	19	3	7	22	2	4	19
GUFFf$_{10}$	4	8	11	2	5	12	2	5	11	2	3	11
GUFFf$_{11}$	7	10	23	7	10	23	7	10	23	7	10	23
GUFFf$_{19}$	6	9	14	3	6	28	2	4	21	2	3	13
GUFFf$_{20}$	7	13	23	3	5	20	2	5	23	2	4	21
GUFF$_{f21}$	7	10	23	7	10	23	7	10	23	7	10	23
GUFF$_{f24}$	5	10	22	5	10	23	4	11	23	2	4	21
GUFF$_{f25}$	5	7	23	3	7	23	4	13	23	2	5	21
GUFF$_{f26}$	6	12	21	4	8	24	3	10	25	2	5	22
GUFFf$_{35}$	6	12	17	2	5	18	3	7	23	2	4	17
GUFFf$_{37}$	6	10	22	2	4	25	3	6	20	2	4	24
GUFFf$_{39}$	5	10	25	3	6	24	7	15	25	2	4	23
GUFFf$_{40}$	6	9	22	3	5	24	6	12	24	2	5	24

c) Most probable counts of Carbon fixers

MPN counts of $8.0x10^2$ cfu/ml and $4.0x10^2$ cfu/ml as column growers were obtained for S and R salt pans in 2001. Surprisingly, no counts of carbon fixing haloarchaea were obtained for all salt pans in 2003 and for R & S salt pans in 2005. Neither pellicle nor bottom growers were observed in MPN tubes. A total of three isolates designated as $GUFF_{190, 191, 192}$ were obtained, identified as *Synechococcus* sp., *Nodularia* sp., and *Spirilina* sp. respectively, detailed in Section A.

d) Nitrogen fixers in salt pans

Haloarchaeal cultures capable of growing in synthetic media in absence of Nitrogen were recovered from Ribandar brine only, at cfu of $6.0x10^1$ per ml. These were purified and designated as namely, $GUFF_{193}$, $GUFF_{194}$ and $GUFF_{195}$, however, could not be assigned to any known genera **(Plates 19, 20, 21).**

2. Occurrence of Faecal microorganisms in salt pans

Most probable counts of coliforms

No counts were obtained in brine and sediments of any of the salt pans during salt farming/ summer season while counts of $1.2x10^1$ cfu/ml and $2.0x10^1$ cfu/ml were obtained in water and $5.0x10^2$ cfu/g and $6.0x10^2$ cfu/g in sediment of R salt pan in 2003 and 2005; $2.0x10^1$ cfu/ml in water and $5.0x10^2$ cfu/g and $6.0x10^2$ cfu/g, were obtained in sediment of S salt pan in 2003 and 2005 respectively **(Table 30)** during the seasons of monsoon and post monsoon.

Characterization of isolates

Three bacterial cultures were isolated and purified from Mac Conkey's tubes designated as $GUEFF_{7.8.9}$, and five from Mac Conkey's tubes containing 20% crude solar salt, designated as $GUEFF_{10,11,12,13,14.}$ Characterization of the isolates obtained during monsoon and post-monsoon is detailed in Section A.

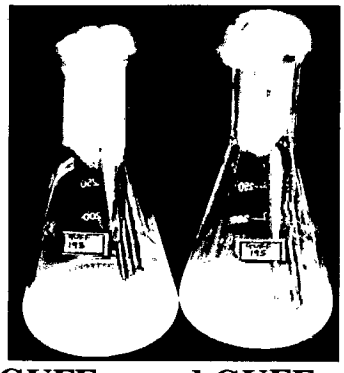


Plate 19 : Growth of $GUFF_{193}$ and $GUFF_{195}$ in NGSMN medium

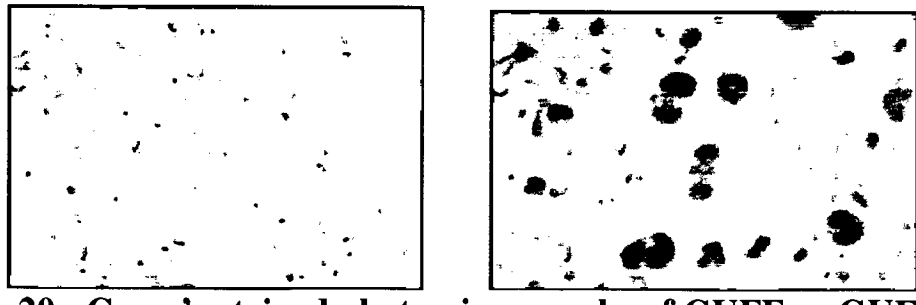


Plate 20 : Gram's stained photomicrographs of $GUFF_{193}$, $GUFF_{195}$ on Olympus Magnus CH 20i Imaging microscope

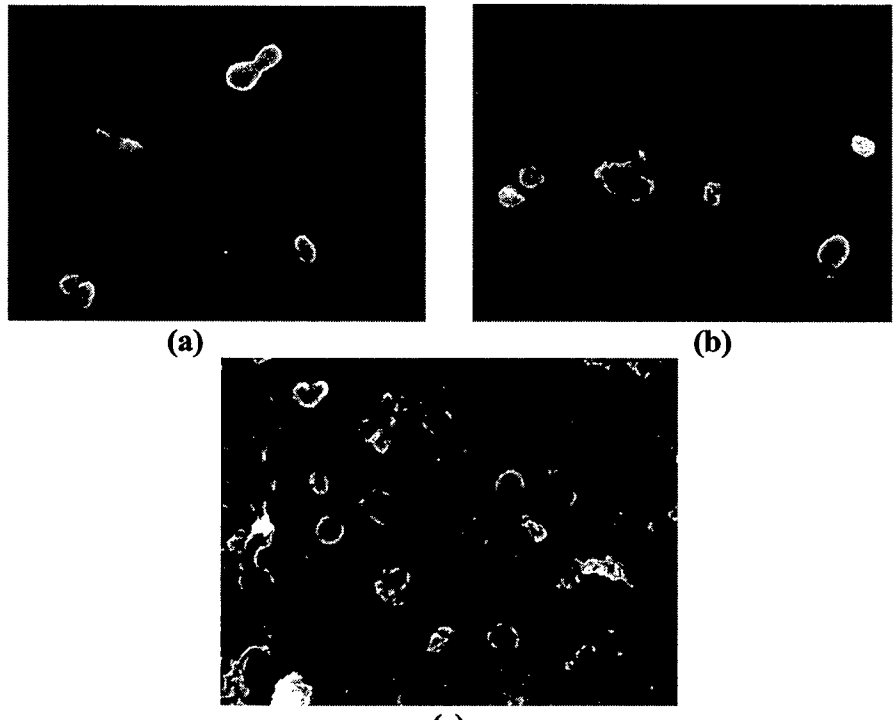


Plate 21: Scanning electron micrographs of gold-plated cells grown in liquid culture NGSM medium without nitrogen, pH 7.0, a) $GUFF_{193}$ 4,500x (b) $GUFF_{194}$ 4,000x ; (c) $GUFF_{195}$ 7,500x

Table 30: Most probable counts of coliforms growing in Mac Conkey's broth, in water/brine/sediment of salt pans during different seasons

Salt pans		Counts of coliforms (cfu)		
		Culture Media	Mac Conkey's broth	
Sample		Year	2003 TC	2005 TC
Ar	b	P	-	-
		C	0	0
		B	-	-
	s	P	-	-
		C	0	0
		B	-	-
N	b	P	-	-
		C	0	0
		B	-	-
	s	P	-	-
		C	0	0
		B	-	-
R	b	P	$2.0x10^1$	$1.8x10^1$
		C		$*1.3x10^2$
		B	0	0
			$1.2x10^1$	$2.0x10^1$
	s	P	$3.6x10^2$	$2.0x10^2$
		C		$*1.3x10^2$
		B	0	0
			$5.0x10^2$	$6.0x10^2$
S	b	P	$1.5x10^1$	$1.8x10^1$
		C		$*1.8x10^1$
		B	0	0
			$2.0x10^1$	$2.0x10^1$
	s	P	$3.6x10^2$	$4.0x10^2$
		C		$*1.8x10^2$
		B	0	0
			$5.0x10^2$	$6.0x10^2$

b-brine; s-sediment, TC-Total Count, *MacConkey's broth with 20% crude solar salt Red: Summer; Salt farming stage, Blue: Monsoon; Submerged stage, Black: Post monsoon; Shallow water stage

Metal pollution indicators in salt pans

Occurrence of heavy metal pollutants

Analysis of heavy metals by Atomic Absorption Spectrophotometer revealed 183 ppm of Cd^{2+}, 308 ppm of Hg^{2+}, 208 ppm of Cu^{2+}, 414 ppm of Pb^{2+} in sediment of Batim salt pan. Brine of Siridao gave 142 ppm, 694 ppm, 0 ppm, 0 ppm of Cd^{2+}, Hg^{2+}, Cu^{2+}, and Pb^{2+} respectively. These heavy metals were found to be within the range of regulatory limits.

Haloarchaeal population in sediments/brine of salt pans growing in presence of Fe^{2+}/ Mn^{2+}

As seen in **Table 27,** a total MPN count was observed in **NG(Mn)SM** for brine samples as column growers, $3.6x10^3$ cfu/ml for R salt pan in 2001; counts of $3.6x10^4$ cfu/ml were highest for N salt pan & lowest for AgD salt pan of $1.2x10^3$ cfu/ml in 2003. Counts were about the same for R & S salt pans in 2005. Similarly, in **NG(Fe)SM**, a total MPN count of $3.6x10^2$ cfu/ml were higher for S salt pan than observed for R salt pan in 2001; N salt pan showed highest counts of $9.0x10^3$ cfu/ml while AgD salt pan showed lowest counts of $3.6x10^3$ cfu/ml in 2003. R salt pan showed higher counts of $3.6x10^3$ cfu/ml than S salt pan in 2005. Pellicle and bottom growers were absent in the MPN tubes.

MPN of haloarchaea in sediment

In **NG(Mn)SM,** a total MPN count of haloarchaea were obtained only as column growers. Pellicle and bottom growers were absent. Sediments of R and S salt pans showed counts of $3.6x10^4$ cfu/g. **NG(Fe)SM** medium gave similar total MPN counts of haloarchaea as obtained for NG(Mn)SM.

In all 14 isolates were obtained, eight from NG(Fe)SM and six from NG(Mn)SM media, these were designated as $GUFF_{196,197,198,199,200,201,202,203,204,205,206,207,208,209}$ and characterized as

Halococcus sp., *Haloarcula* sp. and Halotolerant haloarchaea. This record is available in **Table 25, Fig.6** of Section A.

Cultures $GUFF_{200}$, $GUFF_{206}$ and our previous isolate, namely GUSF MTCC 3265 grown in NG(Fe)SM and the latter two in NG(Mn)SM (**Plate 22**) grew as pink/orange culture. Cells grown with & without Fe/Mn showed clumping in SEM profiles **Plate 23.**

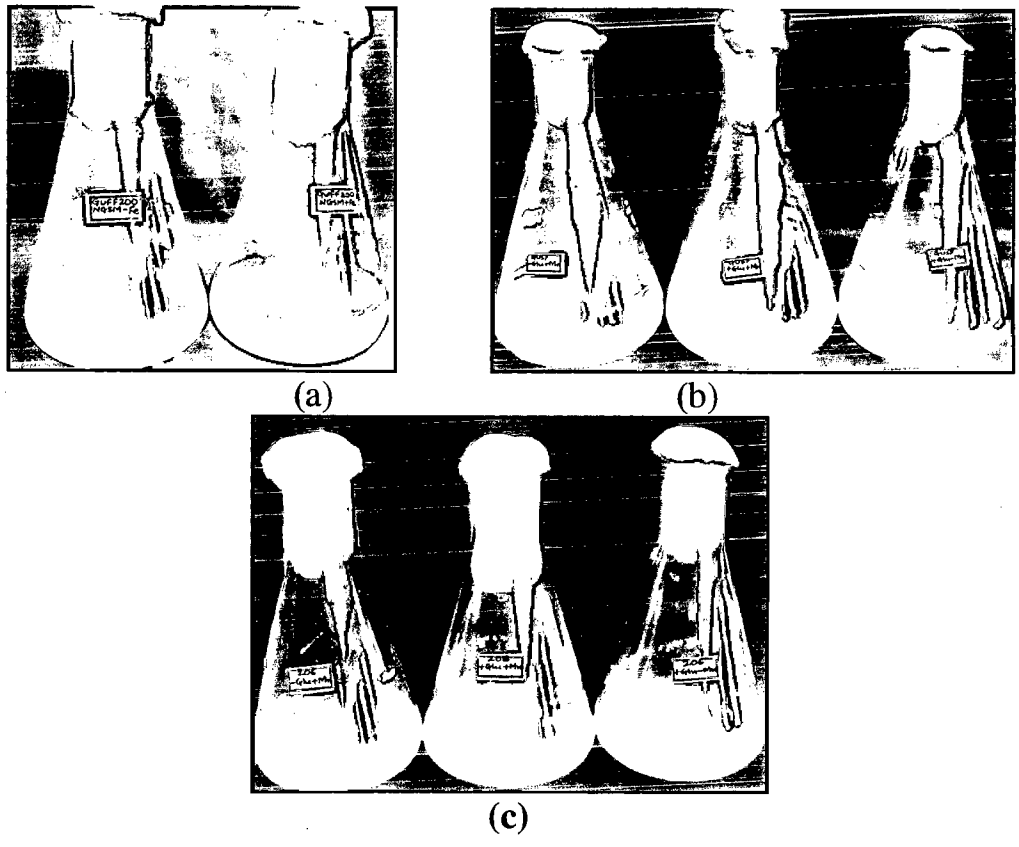


Plate 22: Growth of (a) $GUFF_{200}$ and (b) $GUFF_{206}$ (c) GUSF MTCC 3265 in NGSM containing Fe^{2+} and Mn^{2+} along with controls respectively

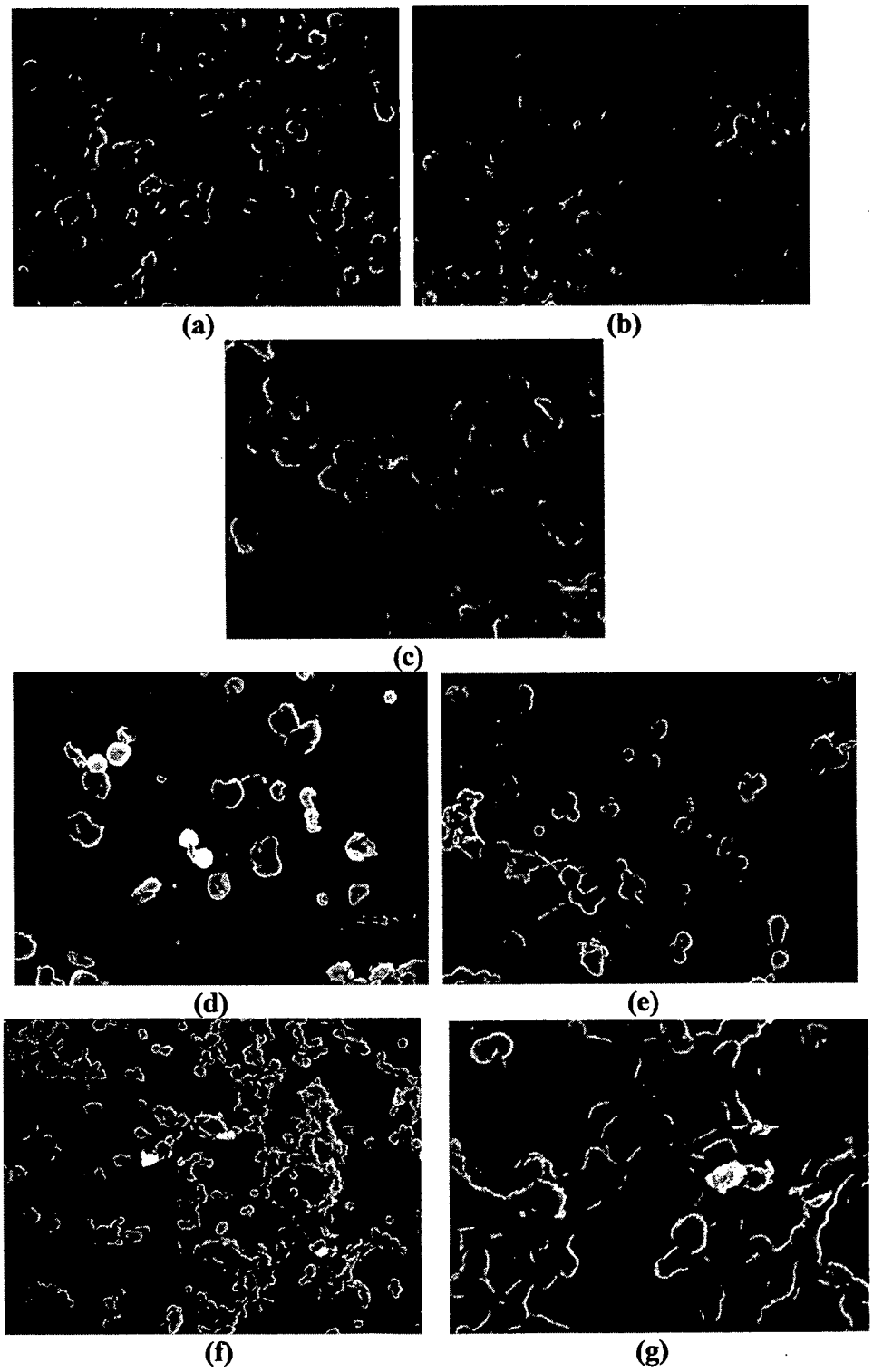
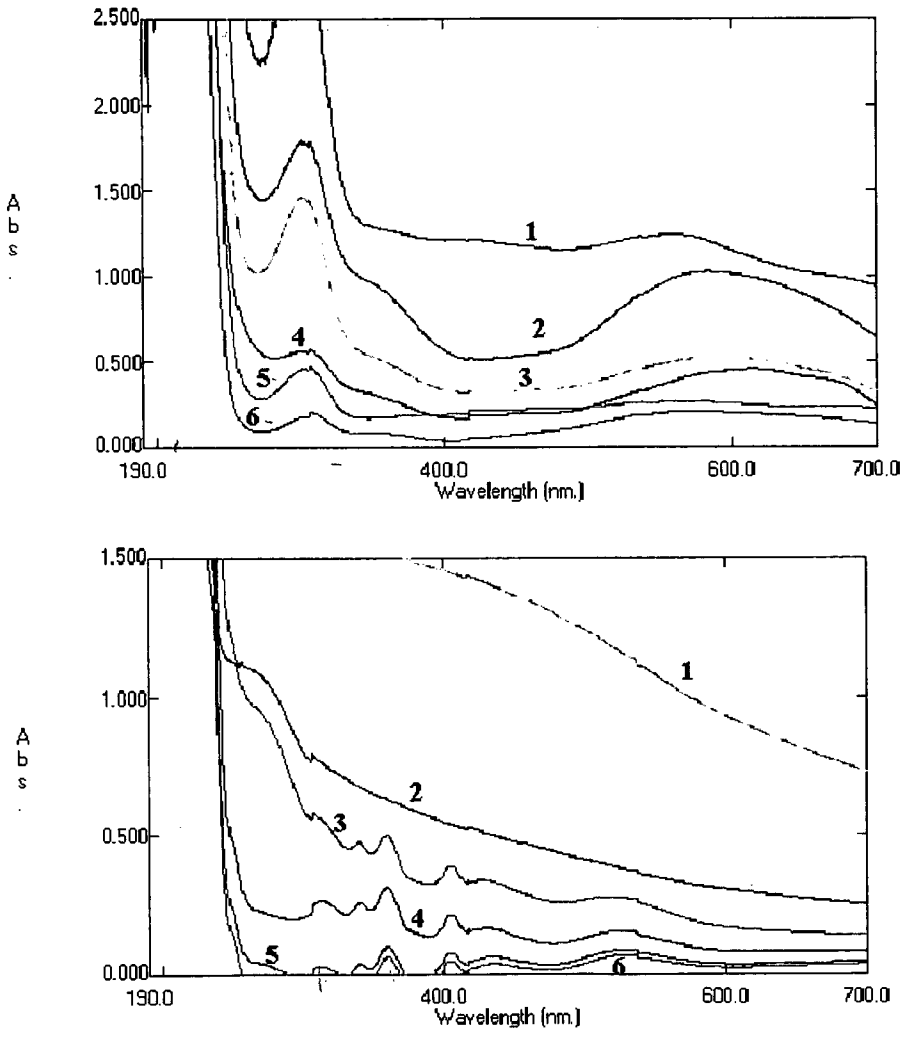


Plate 23 : Scanning electron micrographs of gold-plated cells grown in liquid culture NSM medium, pH 7.0 with (a) glucose 5,000x, glucose in presence of 2 mM Fe^{2+}- (b) 5,000x, (c) 9,500x of $GUFF_{200}$; (d) glucose 4,500x, glucose in presence of 2 mM Mn^{2+}- (e) 4,500x (f) 2,500x (g) 8,000x of GUSF MTCC 3265

Biosorption of Mn^{2+} metal by haloarchaeon GUSF (MTCC 3265)

Mn^{2+} metal sorption by whole cells of haloarchaeon GUSF (MTCC 3265) is as indicated in **Fig. 12.**



1-50 µl cells in 15% NaCl
2-5 µl cells in 15% NaCl
3-concentrated whole cells
4-control
5-1000 µl cells
6-500 µl cells

Fig. 12: Biosorption of Mn^{2+} metal by whole cells of haloarchaeon GUSF (MTCC 3265)

CHAPTER IV

DISCUSSION

Various ecosystems have been studied extensively around the globe for abiotic and living components (Oren, 1994;Ventosa *et al* 1998; Oren, 2002). On the Indian front also, salt pans have been explored (Sequeira, 1992; Kerkar, 2004). The salt pans are econiches subjected to water of a riverine/estuarine system, particularly in coastal regions where such waters are evaporated to obtain salt (Sequeira, 1992).

Goa, on the west coast of India has two rivers, namely, Mandovi and Zuari (have sources in the Western Ghats), forming interconnecting canal, of a tidal estuarine system (Qasim and Sengupta, 1981) with marine, brackish, limnetic and migratory elements, fauna whose abundance is determined by several environmental factors which get adapted and evolved to either euryhaline grow at 0% to 30% NaCl and eurycoecious (Parulekar *et al*, 1975). Mandovi has many tributaries and receives a greater run-off than the Zuari which has only a few tributaries. The two rivers are influenced by the seawater inflow caused by the tidal action upto a considerable distance inland (Qasim and Sengupta, 1981). The riverine flow is seasonal during the pre-monsoon season, the flow of freshwater is minimal, is regulated by the tides, with salinity of Mandovi- (29-34 ‰) and Zuari- (31-35 ‰) ends of the estuary.

Temperature, salinity and oxygen are related to tidal rhythm (Singbal, 1973). Variations in the hydrographic conditions were minimum during the pre-monsoon season. Suspended matter in the middle and upstream of the Zuari is reported to be greater than Mandovi because of strong tidal currents, upstream (Cherian *et al*, 1974, Varma *et al*, 1975).

The temperature of water increases from January to May, followed by a decline during the monsoon months and again an increase thereafter. Both the estuaries and the Cumbarjua canal behaved in a similar fashion in temperature changes which varied between 31.02 and 24.8 °C – the difference in annual variation being 5-7 °C (Qasim and Sengupta, 1981).

The estuarine land on these river reaches, used for salt pans in Goa, is observed to undergo three seasonal phases : submerged (June to October), Shallow (November) and Salt Farming (January to May) (Furtado, 1994). During summer, the riverine waters are taken on to this land, these waters at pH 6.8 to 7.5, with evaporation of water and formation of brine, the waters overlaying–the salt crystals reach to 7.5; correspondingly, the pH of sediments ranged from 8.0 to 8.33. The Shah and Rathod (1990) report on salt pans have reported crude salt of Goa to consist of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{+} ions, and $CaSO_4$, $MgSO_4$, $MgCl_2$, NaCl.

Similar pH values are reported in case of a marine saltern (Ramos-Cormenzana, 1990). Sediments were mostly brownish grey but at a depth of 15 cm and below, sediments were observed to be brownish black identical to an observation made earlier, by Kerkar, 2004 emitting H_2S odour for Goan salt pans and by Ramos-Cormenzana, 1990 for salt pans from Spain, Italy, Yugoslavia. The colour with H_2S odour is attributed to sulfate reduction (Sorensen *et al*, 2004) occuring in this econiche.

Approximately 32 - 35 % of NaCl concentration is noted in pans known as crystallizers, having precipitated crude solar salt (Litchfield *et al*, 2000) favoured by temperatures of 40^0 - 45 ^{0}C. Marine salterns are also reported to have identical salinities and temperature (Ramos-Cormenzana, 1990). Occasional, orange-red colouration seen on surface of waters in crystallizer pans of salt pans reflect en mass growth of extreme halophiles, namely haloarchaea, such **halobacterial blooms** associated with hypersaline brines are as reported by Oren (1994) where salt concentrations approach saturation.

Very few quantitative studies on the microbial ecology of salterns have been reported, and most of them deal with plate counts, followed by isolation and characterization of the bacteria present (Rodriguez-Valera *et al*, 1981, 1985; Marquez *et al*, 1987, Oren *et al*, 1993). A simple age-old enrichment technique like most probable number (MPN) was used during the present study along with plate counts for obtaining viable populations of halophilic microorganisms. The MPN technique although laborious, as it employs many tubes of liquid media to establish necessary dilutions to reach a point of extinction. Its greatest advantage is that a single organism can be enriched and recovered. Further, earlier studies from this laboratory have reported consistent, repeatable results with haloarchaea using the technique (Raghavan and Furtado, 2004).

As it is well established that no one medium composition or set of growth conditions can provide the growth requirements of the entire "viable" bacterial flora (Javor, 1984 ; Bull *et al*, 2000). Hence various media were used. For instance, the NB medium was used for non-halophiles, HS for moderate halophiles and NTYE for extreme halophiles. Growth in MPN tubes was differentiated as : growing as pellicle (P), facultatively anaerobic growing throughout the column of media (C) and those growing submerged (B) at the bottom of the tube (Fernandes and Furtado, 2003).The bacterial counts in sediments, 2 - 3 orders higher than the bacterial counts of brines, we suggest that the variations in counts is possibly due to availability of nutrients in sediments, brought by the tidal water and accumulated at the base of salt pans or due to paucity of essential nutrients in brine.

The overall counts of $8.0x10^{9}$ cfu/ml corresponding to fungi, cyanobacteria, yeast, eubacteria and haloarchaea for six salt pans. There are no earlier reports on microbial counts

from Goan salt pans, however, bacterial counts in the range of $1.0x10^5$ to $2.1x10^8$ cfu/ml have been reported for estuaries of Goa (Lokabharathi *et al*, 1977).

In synthetic media, the count $3.6x10^4$ cfu/ml, it is noteworthy that these media did not have any supplements of 0.5 % vitamins and 0.01 % of nutrient broth / sugar / organic acid / glycine betaine and amino acid media reported for brine samples of saltern of Australia by Burns *et al*, 2004.

Counts in sediment samples were higher on PM than that obtained for brine samples. The equal numbers of moderate halophiles in HS media, both in brines and sediments of salt pans could possibly be due to the chemical composition of media, containing 12.5 % NaCl. Counts of 100 times less than that obtained on PM could be that extreme halophiles are more in number as it was the salt farming stage.

Two hundred and thirty four cultures were isolated and are maintained in the Haloarchaea repository. This study has indicated that salt pans sustain not only halophilic fungi- yeast, cyanobacteria, but also non-halophilic eubacteria besides the extreme halophiles / haloarchaea. How non-halophiles survive saturation concentrations of NaCl merits scientific investigation. That halophilic eubacteria and archaebacteria co-exist in hypersaline environments, have been reported by Ramos – Cormenzana (1990). Cyanobacterial mats are also reported to be present in shallow ponds of marine salterns (Ramos – Cormenzana, 1990).

Growth potential of haloarchaeal cultures at different pH and Mg^{2+} concentration range of 0.07 to 790 mM enabled to refer the isolated haloarchaeal cultures to i) neutrophilic ii) weakly alkaline iii) acidic iv) normal pH range v) broad pH range.

Haloarchaeal cultures have been referred thus in Bergey's (Gibbons, 1974) and Prokaryotes (Tindall, 1992). Several workers have reported such isolates from salterns of Israel, China, Spain, California.

To our knowledge, this is the first time salt pans have been investigated in such details for the microbiota not only in Goa but in India. The similarity of groups isolated in their affiliation to diverse types of haloarchaea reported globally is of high significance to the haloarchaeal studies in India, a group of extreme microorganisms much sought for their enzymic potential and industrial implication.

The significant less viable counts of fungi to that compared to bacteria probably indicate that the pH - neutral to slightly alkaline may be less favourable to fungi, as compared to that of bacteria.

There have been few reports on occurrence of fungi in hypersaline environments. Even the number of marine fungi is low (about 800 described species) in comparison to the number of terrestrial fungi which is more than 60 times as high (Kis – Papo *et al*, 2001). Biodiversity of fungal life drastically decreases with increasing salt concentrations.

With most of the fungal species being halotolerant, tolerating NaCl concentrations of 20 % NaCl and eighteen fungi halophilic as they grow at 20 % NaCl and not at 0 % NaCl and is it because these fungi have adapted to the NaCl concentrations present in salt pans or are they inherently requiring high salt. This result is similar to that found for a study of halotolerant and halophilic fungal species reported for some hypersaline waters (Buchalo *et al*, 1998; Gunde – Cimerman *et al*, 2000; Kis – Papo *et al*, 2001, 2003; Mejanelle *et al*, 2001) and soils (Steiman *et al*, 1997).

Highly saline conditions in waters and soils exert a strong selective pressure on the biota, favouring the development of halotolerant and halophilic forms (Kis – Papo *et al*, 2001). Filamentous fungi are highly adaptable organisms (Kis – Papo *et al*, 2003).

Fungi belonged to 4/2/4/7/8 of *Paecilomyces, Acremonium, Humicola, Aspergillus,and Talaromyces* which also are frequently isolated from hypersaline environments as reported by Steiman *et al*, 1997; Kis-Papo *et al*, 2001. The fungal community composition found in the present study was strikingly similar to that found for two hypersaline soil and water of Mono Lake area, California (Steiman *et al*, 2004); *Penicillium* sp., *Mucor* sp., *Aspergillus* sp., *Acremonium* sp. along with other fungi of Aswan soil and *Penicillium* sp., *Aspergillus* sp., *Acremonium* sp., *Paecilomyces* sp. and *Trichoderma, Fusarium, Cladosporium, Cunninghamella, Botrytis, Helminthosporium, Mucor, Absidia, Alternaria* and *Gliocadium.* The genus *Aspergillus* was isolated throughout the period of our study and, therefore probably is indigenous to the salt pans. For those which occurred only in submerged/shallow season, may have originated from mycelia/spores drifted from land into the water.

Black yeasts, a group of rare extremophilic eukaryotes (Kogej *et al*, 2005) are grouped in the halophilic genus *Hortaea.* We isolated one such black yeast tentatively identified as *Hortaea* sp. in the present study, also reported for hypersaline environments, where yeasts belonged to halophilic members of the genera *Hortaea werneckii* and *Aureobasidium pullulans* (Gunde – Cimerman *et al* 2000; Kogej *et al*, 2005).

Differentiation of bacteria

234 isolates were assigned to the two groups of bacteria, haloarchaea and eubacteria ; based on a study using characteristic chemotaxonomic markers as growth at 0%, 20% NaCl ; 700

U/ml of penicillin, 20 μg/ml of Kanamycin. High salt requirement, resistance to Kanamycin, Penicillin enabled to sorting of bacterial isolates as Halobacteriales (Tindall, 1992; Torreblanca *et al*, 1986) from halophilic eubacteria whose growth is inhibited.

; bile salt;; response to distilled water; GDEM, the type of pigment – $C_{40\text{-}50}$ carotenoids, chlorophyll, menaquinones. Occurrence of spots at R_f 0.2/0.6 in methanolysates of individual isolates, is attributable to glycerol diether moieties (GDEM), and to methyl esters of non–hydroxylated fatty acids (FAME) (Minnikin *et al*, 1975) and hence isolates were segregated as archaebacterial halophiles containing GDEM (Ross *et al*, 1981; Torreblanca *et al*, 1986). and eubacterial halophiles lacking FAME. Although, diphytanyl glycerol ether analogues of phosphatidyl glycerol and phosphatidyl glycerophosphate are present as major components in all strains of haloarchaea, there is considerable variability in the ocurrence and distribution of the glycolipid and the derivatives of sulfated glycolipid (Kushwaha *et al*, 1982).

The haloarchaeal community in brine, sediment and water samples of the various salt pans studied were tentatively identified as belonging to genera *Halobacterium* sp., *Haloarcula* sp., *Halococcus* sp., *Haloferax* sp., *Halobaculum* sp., *Natrialba* sp., *Natrinema* sp., *Natronomonas* sp., *Natronococcus,* sp. and halotolerant haloarchaea, their numbers were 11 / 15 / 62 / 9 / 2 / 12 / 4 / 2 / 3 / 54 respectively.

$GUFF_{41,42,43}$-*Haloferax*, $GUFF_{54,180}$-*Halococcus* sp., $GUFF_{94}$-halotolerant haloarchaea of Arambol salt pan brine and sediment samples containing $GUFF_{44}$, $GUFF_{55}$, $GUFF_{96}$, $GUFF_{181}$, $GUFF_{107,155,161}$ corresponded to *Haloferax* sp., *Halobacterium* sp., *Natrialba* sp., *Haloarcula* sp.and halotolerant haloarchaea respectively.

Isolates of Agarwada salt pan brine, $GUFF_{34,186}$, $GUFF_{35,88,148}$, $GUFF_{37}$, $GUFF_{58,59}$, $GUFF_{87}$, $GUFF_{86}$ corresponded to *Haloarcula* sp., *Halobacterium* sp., *Natrialba* sp., *Halococcus* sp., *Natrinema* sp. and halotolerant haloarchaea respectively; while $GUFF_{38}$, $GUFF_{39,93}$,

$GUFF_{187,188}$, $_{189}$, $GUFF_{89,90}$, $_{165}$ contained in sediment were identified to be *Haloferax* sp., *Halococcus* sp., *Haloarcula*, sp. and halotolerant haloarchaea respectively.

Brine and sediment samples of Arpora salt pan containing $GUFF_{19}$, $GUFF_{20,22}$, $GUFF_{51,52,67,68,136,199,205}$, $GUFF_{177}$, $GUFF_{81,97,98,114,115,134,135,137,159,160,178,200}$ and $GUFF_{23}$, $GUFF_{25,26}$, $GUFF_{53,99,102,179,201}$, $GUFF_{100,\ 103,139}$ were tentatively identified as *Halobacterium* sp., *Haloferax* sp., *Halococcus*, *Haloarcula* sp., halotolerant haloarchaea and *Haloarcula* sp., *Natrialba* sp., *Halococcus* sp. and halotolerant haloarchaea respectively.

Brine of Nerul salt pan contained $GUFF_{27,28,56,69,116,117,162,182,202}$-*Halococcus* sp.,$GUFF_{82}$-*Natrinema* sp., $GUFF_{184}$-*Natrialba* sp., $GUFF_{206}$-*Haloarcula* sp., $GUFF_{30,85}$-halotolerant haloarchaea while sediment harboured $GUFF_{32}$-*Natrialba* sp.,, $GUFF_{33}$-*Natrinema* sp.,, $GUFF_{57}$-*Halococcus* sp., $GUFF_{104}$-*Halobacterium* sp., $GUFF_{185}$-*Haloarcula* sp., and $GUFF_{105,\ 106,118}$, $_{166,203,207}$-*halotolerant haloarchaea* respectively.

Water samples of Ribandar and Siridao salt pans contained $GUFF_{5,\ 8,\ 74,\ 110,\ 158}$ / $GUFF_{13}$-halotolerant haloarchaea, $GUFF_{6,\ 47}$/$GUFF_{63,\ 64,111}$-*Halococcus* sp. Respectively. Brine sample of Ribandar salt pan harboured $GUFF_{1,156}$-halotolerant haloarchaea, $GUFF_{2,45,46,70,71,120,168,170,196,197,204}$-*Halococcus* sp., $GUFF_{72}$-*Halorubrum* sp., $GUFF_{60,169}$-*Halobacterium* sp., besides unidentified nitrogen fixing bacteria $GUFF_{193,194,195}$ while sediment of Ribandar salt pan harboured $GUFF_{3,171}$-*Haloarcula* sp., $GUFF_{4,61}$-*Halobacterium* sp., $GUFF_{73,172}$-*Halococcus* sp., and $GUFF_{17,21,112,122,125,127,167}$-halotolerant haloarchaea contained sediment. Siridao salt pan brine harboured $GUFF_{7}$- *Natrinema* sp., $GUFF_{9}$-*Halobacterium* sp., $GUFF_{10}$-*Haloferax* sp., $GUFF_{48,62,75,173,174,175,198,208,209}$-*Halococcus* sp., $GUFF_{77}$-halotolerant haloarchaea, $GUFF_{129}$-*Natrialba* sp., $GUFF_{157}$-*Haloarcula* sp., while sediment contained $GUFF_{12,131,225,226,231}$-*Natrialba* sp., $GUFF_{14,49,50,65,66,113,176}$-*Halococcus* sp., $GUFF_{16,18}$-*Haloarcula* sp., and $GUFF_{24,31,36,78,108,109,183}$-halotolerant haloarchaea, $GUFF_{224,234}$-

Halobaculum sp., $GUFF_{227}$-*Haloferax* sp., $GUFF_{228,229}$-*Natronomonas* sp. and $GUFF_{230,232,233}$-*Natronococcus* sp.

Halococcus sp..and halotolerant haloarchaea were found to be dominant in water, brine as well as sediments of Ribandar and Siridao salt pans.

Haloarchaeal composition for Ribandar salt pan was $GUFF_{5,\ 8,\ 74,\ 110,\ 158}$-halotolerant haloarchaea, $GUFF_{6,\ 47}$ -*Halococcus* sp. for water, $GUFF_{60}$-*Halobacterium* sp. for brine and $GUFF_{172}$-*Halococcus* sp. for sediment during monsoon while that for post monsoon was $GUFF_{17,21,112,167}$-halotolerant haloarchaea, $GUFF_{61}$-*Halobacterium* sp.

Siridao salt pan contained $GUFF_{13}$-halotolerant haloarchaea, $GUFF_{63,\ 64,111}$-*Halococcus* sp. in water and $GUFF_{24,31,183}$-halotolerant haloarchaea in sediment during monsoon while $GUFF_{50,65,66,113,176}$-*Halococcus* sp., $GUFF_{16,18}$-*Haloarcula* sp., $GUFF_{36,78}$-halotolerant haloarchaea, in sediment during post monsoon.

Of the 53 eubacteria, the numbers of *Halomonas, Gluconobacter, Proteus. Acinetobacter / Pseudomonas / Planococcus / Alcaligenes / Alteromonas, Micrococcus / Klebsiella* and *Salmonella* were 5 / 4 / 3 / 11 / 6 / 2 / 1 respectively.

The eubacterial community of salt pans consisted of $GUFF_{79}$-*Pseudomonas* sp., $GUFF_{152,153}$-*Alcaligenes* sp. in brine and $GUFF_{95}$-*Gluconobacter* sp. in sediment of Arambol salt pan. Agarwada salt pan had $GUFF_{147}$- *Alcaligenes* sp., $GUFF_{146}$-*Alteromonas* sp. in brine and $GUFF_{40,\ 91}$-*Pseudomonas* sp., $GUFF_{92}$-*Halomonas* sp., $GUFF_{149}$-*Alcaligenes* sp., $GUFF_{164}$-*Acinetobacter* sp. in sediment.

Brine sample of Arpora salt pan contained $GUFF_{80}$-*Halomonas* sp. while $GUFF_{101}$-*Acinetobacter* sp. and $GUFF_{138}$-*Alteromonas* sp. in sediment.

Nerul salt pan harboured $GUFF_{83,84}$-*Gluconobacter* sp., $GUFF_{140}$- *Pseudomonas* sp., $GUFF_{141,142}$-*Alteromonas* sp. in brine and $GUFF_{143,145}$- *Alteromonas* sp. and $GUFF_{144}$-*Alcaligenes* sp. in sediment.

Ribandar salt pan water had $GUFF_{121,124}$- *Acinetobacter* sp., $GUFF_{216}$-*Klebsiella* sp. ; brine had $GUFF_{119}$-*Planococcus* sp., $GUFF_{210,211}$-*Micrococcus roseus, M. .varians* respectively, while $GUFF_{212}$- *Micrococcus halobius,* $GUFF_{11,15}$-*Halomonas* sp., $GUFF_{128}$- *Alcaligenes* sp., $GUFF_{150}$- *Planococcus* sp., $GUFF_{217}$-*Klebsiella* sp. and $GUFF_{219,220}$-*Proteus* sp. *alomonas* in sediment.

Siridao salt pan water contained $GUFF_{123}$- *Acinetobacter* sp., $GUFF_{218}$-*Proteus* sp. and $GUFF_{221}$-*Salmonella* sp.; $GUFF_{76}$-*Gluconobacter* sp., $GUFF_{130}$-*Alcaligenes* sp., $GUFF_{213,214}$-*Micrococcus halobius,* ; sediment had $GUFF_{29}$-*Halomonas* sp., $GUFF_{126,\ 132,\ 151,163}$-*Alcaligenes* sp., $GUFF_{133}$-*Gluconobacter* sp., $GUFF_{154}$-*Planococcus* sp. and $GUFF_{222,223}$-*Proteus* sp., $GUFF_{215}$- *Micrococcus* sp.

Monsoon eubacterial community of Ribandar and Siridao salt pans was $GUFF_{121,124}$ / $GUFF_{123}$- *Acinetobacter* sp. in water ; and $GUFF_{11}$ / $GUFF_{29}$-*Halomonas* sp., $GUFF_{128}$ / $GUFF_{126}$- *Alcaligenes* sp. in sediment.

$GUFF_{15}$-*Halomonas* sp., $GUFF_{150}$- *Planococcus* sp. of Ribandar salt pan while $GUFF_{151,163}$-*Alcaligenes* sp. and $GUFF_{154}$-*Planococcus* sp. of Siridao salt pan constituted community of post monsoon. *Alcaligenes* sp. was found to be the dominant genus in all the salt pans under study.

The natural brines of salt farming stage in which the extreme halophilic archaea demonstrated the greatest growth potential also supported growth of halophilic eubacteria. This was observed during the study when isolates were plated on nutrient-rich media containing 25% NaCl. Thus, although the NaCl – crystallizing ponds of solar salterns are optimal

environments for halobacteria, they are also entirely hostile for eubacteria. Moderately halophilic eubacterial species like *Halomonas* sp., *Pseudomonas* sp., *Alteromonas* sp., *Micrococcus* sp., *Acinetobacter* sp., *Alcaligenes* sp. have been reported in saline soils near Alicante, Spain; La Malá saltern near Granada; Salar de Atacama, Chile (Ventosa *et al*, 1998a).

Vreeland and Huval isolated many strains with a range of salt tolerances, such as halo-versatile types [0 to > 17 % (w/v) NaCl], moderate halophiles [2 to 20 % (w/v) NaCl] and extreme halophiles [12 to 32 % (w/v) NaCl] from brines formed by dissolution of salt deposits by surface waters (McGenity *et al*, 2000).

Studies on Na^+, Mg^{2+} requirements and sensitivities of 178 isolates, showed that both Na^+ and Mg^{2+} concentrations could affect the distribution of individual strains.
Some isolates demonstrated an extremely high Mg^{2+} requirement for optimal growth, a characteristic previously described only in the Dead Sea strain *Halobacterium sodomense* (Oren, 1983). Most other isolates grow with varying quantities of Mg^{2+} in the range of 0.07 mM to 10 mM, while some are tolerant of higher concentrations.

The extremely halophilic, non-alkaliphilic cocci from several saltpans located in Goa using different culture media and isolation conditions, were assigned to *Halococcus* sp. They were able to produce acid from glucose and used a large number of organic compounds as the sole source of C and energy as those previously described by Montero *et al*, 1988.

Most of the isolates could grow well in nearly all the substrates under aerobic conditions. They grew fermentatively on carbohydrates (determined by the acidification of the medium). Most of the isolates reduced nitrate.

Among the bacterial isolates, several major groups can be recognized according to the substrates they metabolise: isolates that oxidize a broad range of substrates and isolates that are largely confined to the oxidation of carbohydrate/pyruvate/acetate/amino acid. The salt pans harbour mixed populations of those that are versatile and specialist strains of bacteria. Low molecular weight carboxylic acids, particularly the products of glycolysis and the tricarboxylic acid cycle supported the growth of nearly all halophiles. The inability of some species of *Haloferax, Haloarcula, Natrialba, Natrinema* and *Halococcus* to metabolise the substrate tested may be due to : 1) an inability to take up the substrate from the medium or 2) there may be an enzymatic block in the degradative pathway.

However, all the enzymes of the tricarboxylic acid cycle have been demonstrated in halobacteria (Javor, 1984).

In the ionic environments in which the moderate halophiles coexist with the extreme halophiles, the two types of microorganisms apparently can compete for many of the same organic substrates.

The abundance of extremely halophilic cocci in the salt pans, during salt farming, submerged (when salinity is lowered) and shallow stages, is explained by the ability of these bacteria to remain viable in dilute solutions as reported by Rodriguez – Valera *et al*, 1979, 1982.

Both *Halobacterium, Haloarcula* lyse in dilute solutions. However, like all *Halococci*, do not lyse in low salt concentrations, so they could survive in waters of submerged stage.

Haloarchaeal isolates fermented a variety of sugars to acid products, a property shared by other Dead Sea isolates, such as the *Halobacterium* of the Dead Sea and *H. volcanii* (Oren,

1983) and additional strains isolated from other habitats, such as *H. saccharovorum* (Tindall et al,1980) and *H. vallis-mortis* (Gonsalez *et al* , 1978).

Nutrients and salt requirements when tested by Javor (1984), demonstrated that moderate halophiles & strains of extreme halophiles – *Haloarcula* & *Halococcus*, grew on most of the substrates tested in the experiment. *Halobacterium* has been isolated & reported from Marine saltern (Rodriguez-Valera *et al*, 1983), *Halococcus* has been reported from Marine saltern (Ramos-Cormenzana, 1990), *Haloarcula* & *Haloferax* have been isolated & reported from Marine saltern (Torreblanca *et al*, 1986).

The general characteristics of these hypersaline environments seem to affect the types of halophilic bacteria (Ramos-Cormenzana, 1990). The fact that extreme halophiles have been isolated from sea water (Rodriguez-Valera *et al*, 1979) suggest that these organisms could probably also have been isolated from freshwater rivers, at least in those with a relatively high salt content (Ramos-Cormenzana, 1990).

The most unexpected finding of this study is the presence of members of the family Halobacteriaceae at the lands (which are used as salt pans during summer) during monsoons. Halobacteria were readily obtained from MPN tubes, isolated from dilutions upto 10^{-7}, could grow at 0% salinity. This 0% salinity provides an unsuitable ionic environment for members of the family Halobacteriaceae , whose cells generally lyse at NaCl concentrations lower than 8 to 10% (Tindall, 1992; Elshahed *et al*, 2004). The halobacterial isolates belonged to the genus *Haloferax,* members of which are known to have the lowest salt optimum of and the minimum NaCl requirement for retaining cell wall integrity among the halophilic archaea (Oren, 2002). The ability to isolate *Halococcus* sp. is due to their relative ease of

isolation compared to that of other halobacterial species and is not necessarily indicative of their numerical abundance or relative ecological significance in the waters during monsoons. A similar or lower salt requirement for other Halobacteriales in waters during monsoons may be possible.

This work indicates that proliferation of members of the Halobacteriales are not restricted to hypersaline ecosystems such as salt lakes, salterns and the Dead Sea (Tindall, 1992), but can also inhabit, tolerate and adapt to lower salinity environments as those prevalent during monsoons. Indeed, few culture dependent or independent surveys have either isolated Halobacteriales (Rodriguez – Valera *et al*, 1979; McGenity *et al*, 1998) or encountered 16S rRNA Halobacteriales clones (Munson *et al* , 1997; Takai *et al*, 2001) in low-salt environments such as low salinity salterns, black smoker chimney structures, sea water and coastal sea marshes.

The abundance and viability of Halobacteriales in the waters indicates their ability to adapt and survive in waters of lands during monsoons.

The pigments of isolates studied showed various peaks at 275, 285.5, 298 λ; 331, 348, 367 λ; 335, 418, 438, 463 λ; 424, 448, 477 λ; 371, 388, 471, 498, 532 λ; 490 λ; 472 λ; 285 λ; these peaks represent pigments dehydrosqualene, phytofluene, α-carotene, β-carotene, carotenoids , bacterioruberins, lycopene, phytoene, respectively. Such pigments are reported in *Halobacterium* sp. The pigments with peaks at 204 λ, 216 λ, 224 λ, 237 λ, 249 λ, 314 λ, 321 λ, 403 λ, 579 λ, 589 λ, 599 λ, 632 λ, 640 λ, 654 λ, 697 λ, cannot be identified with any known pigment

SEM of extremely halophilic $GUFF_{13}$ revealed rod-shaped spore formers? Identity is not known. Extreme halophiles described as Gram-positive rod-shaped spore formers were isolated from primary Permian Zechstein salt cores (McGenity *et al*, 2000).

SEM revealed a variety of morphological types : squares, triangles, rectangular types; cocci, rods, spherical, cup-shaped cells, pleimorphic, C-shaped cells, cricket–ball shaped cells. Such morphotypes except C-shaped cells and cricket–ball shaped cells are known to be reported (Grant and Larsen, 1989).

The fixation and degradation of C & N_2 by haloarchaea is explained by the ability of these microbiota to play a role in C & N_2 bio-geo chemical recycling. This has also been reported by Truper and Galinski (1986).

The growth and tolerance of haloarchaea in media containing metals such as 2 mM Fe^{2+} and 2 mM Mn^{2+} clearly indicate that these metal-resistant organisms could be used as bioassay indicator organisms (Nieto *et al*, 1989) in polluted hypersaline environments.

Mining plays an important role in the economy of Goa and nearly 10 million tonnes of iron and manganese ores are exported from the Marmugoa harbour. Much of the mining activities are carried out along the banks of the two river systems - Mandovi and Zuari and the Cumbarjua canal.

The range in surface sedimentary iron values of between 2.2 and 49.7% are somewhat higher than that reported from other Indian estuaries like Cauvery, Ganges, Krishna, Godavari, Narmada and Tapti. During the pre-monsoon period, higher concentrations of iron are observed throughout the estuary which may be attributed to high spillage during transportation of iron and ferromanganese ores down the Mandovi estuary (Alagarsamy,2006). Kamat and Sankaranarayanan (1975) observed a high value (5 to 44 mg l^{-1}) of particulate iron in the Mandovi estuary and low value in the nearshore water (0.65 to 12.7mg l^{-1}).

Compared with other seasons, the Mn concentrations in the sediment were generally highest during the pre-monsoon period. Values showed an increase from the mouth to the freshwater upper regions as the water concentrations varied in the range from 6.2 to 102.2μg l^{-1} (Zingde *et al*, 1976).The high Mn concentrations observed for both water and sediment are related to high levels of manganese in the surrounding ore bearing landmass (Zingde *et al*, 1976) as the rivers flowing through the ore bearing (iron and manganese) region might be picking up the element (Sankaranarayanan and Reddy, 1973).

CHAPTER V

SUMMARY

Survey of records on salt pans in Goa showed 72 active salt pans of a total 200 salt pans distributed in the 5 talukas along the Tiracol, Sal, Mandovi, Zuari, Talpona, Galgibaga riverine system. Salt pans located in villages of Arambol (A) and Agarwada (Ag) of Pernem Taluka; Arpora (Ar), Nerul (N) & Pilerne (P) of Bardez Taluka; were studied in 2003, while Ribandar (R), Batim (B), Siridao (S) of Tiswadi Taluka were studied in the years 2001, 2003 & 2005 during the salt farming season which extends from the month of January to the month of May. Two salt pans Ribandar and Siridao salt pans were studied over various climatic seasons of the year 2001. These lands which serve the purpose of salt crystallization/(**salt pans)** in summer are filled with monsoon waters and run-offs hence referred as **submerged**, while during post-monsoons, they have stagnant water to approximately 10-15 cm above the sediment and hence referred as **shallow water stage**, in this study.

Colour of the soil in lands used for soil farming ranged from brownish grey to brownish black. Texture of the soil was mostly clay loam. Moisture content of the soil varied from 0.258 to 0.275/g of soil. The sediments had 8.0 to 8.33 pH while brine was neutral.
At salt farming stage, salinity range was found between 39.0 to 41.0 %. Na^+ content was 269 ppm per ml, and 594 ppm per gram in brine of Batim and sediment of Siridao respectively. Batim brine showed the presence of 32 ppm per ml., while Siridao sediment showed the presence of 28 ppm per gram of K^+. Brine of Batim showed the presence of 246 ppm per ml, while Siridao sediment showed 346 ppm per gram of Ca^{2+}. One gram of crude solar salt of Arambol, Agarwada, Ribandar, Siridao salt pans showed the presence of 2.5 ppm of iodine, while iodine was absent in salt from salt pans of Arpora & Nerul.

Enumeration and types of microbes

The MPN technique yielded important insights in recovering the members of the salt pan econiche. Growth in MPN tubes consisted of aerobic pellicle formers (P), facultatively anaerobic bacteria growing throughout the column of media (C) and those growing submerged (B) at the bottom of the tube, could be recorded.

Pigmented colonies of bacterial isolates were obtained during the period of study. Total viable counts of fungi in brine & sediment of salt pan were obtained on nutrient rich media as NTYE, HS and NA, pH 7.0 and synthetic media with a sole C source as glucose, starch and casein as cfu/ml.

Forty two fungal isolates purified by sub-culturing were designated as $GUFFf_1$ to $GUFFf_{42}$ and maintained in laboratory repository. Certain fungi were thermotolerant as they could grow upto 50^0C. Most of the fungal isolates were halotolerant while eighteen were halophilic. The different fungal cultures were identified as *Paecilomyces, Acremonium, Humicola, Aspergillus, Talaromyces. Aspergillus* and *Penicillium*. One species of black yeast was isolated and identified as *Hortaea* sp.

234 bacterial isolates obtained on various media of pH 5, 7.0 & pH 10.5, containing 20% NaCl in synthetic (NSM) media while 25% crude solar salt in nutrient rich media,.designated as $GUFF_1$-$GUFF_{234}$, were grouped as haloarchaea, cyanobacteria and eubacteria in numbers of 178, 3, and 53 respectively using characteristic

chemotaxonomic markers. The haloarchaeal community in brine, sediment and water samples of the various salt pans based on study of pH, Mg^{2+}, penicillin, kanamycin, bile salt, biochemical characteristics pigments, GDEM, lipids, were tentatively identified as belonging to genera : 11 of *Halobacterium* sp., 15 of *Haloarcula* sp., 62 of *Halococcus* sp., 9 of *Haloferax* sp., 2 of *Halobaculum* sp., 12 of *Natrialba* sp., 4 of *Natrinema* sp., 2 of *Natronomonas* sp., 3 of *Natronococcus* sp. and 54 of halotolerant haloarchaea. *Halococcus* sp..and halotolerant haloarchaea were found to be dominant in water, brine as well as sediments of Ribandar and Siridao salt pans. Of the 53 eubacteria, *Halomonas*, *Gluconobacter, Proteus. Acinetobacter/Pseudomonas/Planococcus/Alcaligenes/ Alteromonas, Micrococcus/Klebsiella* and *Salmonella* were 5/4/3/11/6/2/1 respectively. *Alcaligenes* sp. was found to be the dominant eubacteria in salt pans.

Wet mount, Gram character and microscopic features, indicated that cyanobacteria mostly belonged to *Synechococcus* sp, *Spirulina* sp and *Nodularia* sp.

Pigment analysis of the isolates showed that the peaks represent several pigments: dehydrosqualene, phytofluene, α-carotene, β-carotene, carotenoids, bacterioruberins, lycopene, phytoene, a characteristic of haloarchaea.

Annual climatic changes did influence the physico-chemical status of brine and sediment of salt pan and salt pan econiche biota as well. The haloarchaea could fix as well as degrade C & N_2 containing compounds as indicated by the presence of growth in high numbers in these media.

Members of Enterobacteriaceae family as *Salmonella, Proteus, Klebsiella* are prevalent during the non salt farming seasons.

The haloarchaea could grow and tolerate in media containing metals such as Fe^{2+} and Mn^{2+}.

Table 1 : Composition of various media for culturing microorganisms.

Media comp (g/L)	NTYE	*Halobacterium. sodomense*	*Natronobac terium. pharaonis*	Purple Membrane	N. Agar	NGSM
$MgSO_4.7H_2O$	20	-	2.0/0.07	20	-	20
KCl	5	-	2	2	-	4
$CaCl_2.2H_2O$	0.2	0.1	-	-	-	1
$NaHCO_3$	-	-	-	-	-	0.2
Yeast extract	3	1	-	5	-	-
NH_4Cl	-	-	-	-	-	2
Tryptone	5	-	-	-	-	-
$FeCl_3$ $6H_2O$	-	-	-	-	-	0.005
Crude solar salt	250	125	250	250	*5	200
KH_2PO_4	-	-	-	-	-	0.5
D/W (ml)	1000	1000	1000	1000	1000	900
Beef extract	-	-	-	-	3	-
pH adjustment	7 1 N NaOH	7 1 N NaOH	8.5/10.5 1 N KOH	7.2-7.4 1 N NaOH	7.2-7.4 1 N NaOH	7 1 N KOH
$MgCl_2.6H_2O$	-	160	-	-	-	13
K_2SO_4	-	5	-	-	-	-
Casaminoacids	-	1	15	-	-	-
Soluble starch	-	2	-	-	-	-
Na citrate	-	-	3	3	-	-
Peptone	-	-	-	-	10	-
2 % Glucose (ml)	-	-	-	-	-	100
Glutamic acid	-	-	2.5	-	-	-

* synthetic

Table 2: Composition of various media used for biochemical characterization of isolates

Media comp(g/L)	Indole production medium	H_2S & motility medium	Gelatin hydrolysis medium	Gelatin agar	Christensen's urea medium	Glucose peptone basal medium
Tryptone	10	-	-	-	-	-
NaCl	5	-	5	5	5	-
D/W (ml)	1000	1000	1000	1000	1000	1000
pH	7.4	7.4	7.4	7.4	6.8-6.9	7.0
Peptone	-	20	10	10	1	5
10% Glucose (ml)	-	0.1	-	0.5	10	-
Sodium thiosulphate	-	0.8	-	-	-	-
Agar	-	4	-	15	20	-
Beef extract	-	-	3	3	-	-
Gelatin	-	-	4	40	-	-
KH_2PO_4	-	-	-	0.05	2	-
K_2HPO4	-	-	-	0.15	-	5
0.2% Phenol red (ml)	-	-	-	-	6	-
1% Bromothymol blue (ml)	-	-	-	-	-	-
KNO_3	-	-	-	-	-	-
20% Urea (ml)	-	-	-	-	0.5	-
10% Starch	-	-	-	-	-	-
Ferrous sulphate	-	-	-	-	-	-
$MgSO_4$	-	-	-	-	-	-
NH_4HPO_4	-	-	-	-	-	-
Na citrate	-	-	-	-	-	-
Glucose	-	-	-	-	-	-
Lactose	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-
Yeast extract	-	-	-	-	-	-

Table 3: Composition of various reagents used for biochemical identification of isolates.

(g/L)	Gram's Stain	Gram's iodine	Safranine	$HgCl_2$	Methyl red	Oxidase	O'Meara's	Kovac's	Sulphanilic acid
Crystal violet	20	-	-	-	-	-	-	-	-
Methylated spirit (rectified)	200 ml	-	-	-	-	-	-	-	-
Ammonium oxalate	8	-	-	-	-	-	-	-	-
D/W (ml)	800	1000	100	100	200	100	100	-	41.36
Iodine	-	10	-	-	-	-	-	-	-
KI	-	20	-	-	-	-	-	-	-
Safranine	-	-	0.500	-	-	-	-	-	-
$HgCl_2$	-	-	-	15	-	-	-	-	-
Conc. HCl (ml)	-	-	-	20	-	-	-	50	-
Methyl red	-	-	-	-	0.1	-	-	-	-
Ethanol (ml)	-	-	-	-	200	-	-	-	-
NNNN-tetra methyl-p-phenylene diamine dihydrochloride	-	-	-	-	-	1 g	-	-	-
KOH	-	-	-	-	-	-	40	-	-
Creatine	-	-	-	-	-	-	0.3	-	-
Isomyl alcohol/ amyl alcohol (ml)	-	-	-	-	-	-	-	150	-
p-dimethyl-1-amino benzaldehyde	-	-	-	-	-	-	-	10	-
5 N CH_3COOH (ml)	-	-	-	-	-	-	-	-	0.8
α- naphthylamine	-	-	-	-	-	-	-	-	58.64
Sulphanilic acid								-	0.5

BIBLIOGRAPHY

Aguiar, R. and Furtado, I. 1996. Growth of *Halobacterium* strain R_1 on sodium benzoate. In : Perspectives in Microbiology. Ed.: R. S. Kahlon. Natl. Agri.Tech. Inf. Centre. India. 78-79.

Alagarsamy, R. 2006. Distribution and seasonal variation of trace metals in surface sediments of the Mandovi estuary, West coast of India. Estuarine, Coastal and Shelf Science. 67: 333-339.

Alvey, S., Yang, C.H., Buerkert, A. and Crowley D.E. 2005. Bacterial ecology of ancient Saharan salt-enrichment ponds at Teguidda-n-Tessoumt. J. of Plant Nutrition and Soil Science 168: 489-495.

Amat, F., Hontoria, F., Ruiz, O., Green, A. J., Sanchez, M. I., Figuerola, J. and Hortas, F. 2005. The American brine shrimp as an exotic invasive species in the Western Mediterranean. Biological Invasions. 7: 37-47.

Anderson, J. P. E. and Domsch, K. H. 1975. Measurement of bacterial and fungal contribution to respiration of selected agriculture and forest soils. Can. J. of Microbiol. 21:314-22.

Anshuman, K.P. and Dave, B. P. 2005. Diversity of extremely halophilic archaea isolated from salterns around Bhavnagar. Proceedings of the Indian National Science Academy, Part B, Biological Sciences. 75:59-64.

Asker, D. and Ohta, Y. 2002. *Haloferax alexandrinus* sp. nov., an extremely halophilic canthaxanthin-producing archaeon from a solar saltern in Alexandria (Egypt). International J. of Systematic and Evolutionary Microbiol. 52:729-738.

Atlas, R. M. 1982. Enumeration and estimation of microbial biomass. In: Experimental microbial ecology. (Ed. by Richard G. Burns and J. Howard Slater). Blackwell Scientific Publication, Great Britain.

Bancroft, K., Paul, E. A. and Wiebe, W. J. 1976. The extraction and measurement of adenosine triphosphate from marine sediments. Limnology and Oceanography. 21:473-80.

Bebout, B. M., Hoehler, T. M., Thamdrup, B., Albert, D., Carpenter, S. P., Hogan, M., Turk, K. and Des-Marais, D. J. 2004. Methane production by microbial mats under low sulfate concentrations. Geobiology. 2: 87-96.

Ben-Amotz, A. and Avron, M. 1983. Accumulation of metabolites by halotolerant algae and its industrial potential. Ann. Rev. Microbiol. 37: 95-119.

Betlach, M.C. and Shand, R. F. 1991. Expression of the bop gene cluster of *Halobacterium halobium* is induced by low oxygen tension and by light. J. of Bacteriol. 173:4692-4699.

Bickel-Sandkoetter, S., Gaertner, W. and Dane, M. 1996. Conversion of energy in halobacteria: ATP synthesis and phototaxis. Arch. Microbiol. 166: 1-11.

Bloch, David. 1996. MRBLOCH SALT ARCHIVE.

Blaurock, A. E. and Stoeckenius, W. 1971. Structure of the purple membrane. Nature New Biol. 233.

Bouchotroch, S., Quesada, E., Del- Moral, A. and Bejar, V. 1999. Taxonomic study of exopolysaccharide-producing, moderately halophilic bacteria isolated from hypersaline environments in Morocco. Systematic and Appl. Microbiol. 22: 412-419.

Bowden, W. B. 1977. Comparison of two direct-count techniques for enumerating aquatic bacteria. Appl. and Environ. Microbiol. 33:1229-32.

Braganca, J. M. and Furtado, I. 2006. Microbial system for detection of Cadmium (II) ions. International workshop on biosensors for environmental analysis at BITS, Pilani- Goa campus, India in collaboration with Lund University, Sweden. Institute for advanced studies in basic sciences, Zanjan, Iran, University of Calcutta, India(Feb. 21- 23, 2005).

Brown, A. D.1963. The peripheral structure of Gram-negative bacteria. IV. The cation-sensitive dissociation of the cell membrane of the halophilic bacterium *Halobacterium halobium*. Biochim. Biophys.Acta 75 : 425-435.

Brown, L.S., Varo, G., Hatanaka, M., Sasaki, J., Kandori, H., Maeda, A., Friedman, N., Sheves, M., Needleman, R. and Lanyi, J. K. 1995. Complex extracellular domain regulates the deprotonation and reprotonation of the retinal Schiff base during the bacteriorhodopsin photocycle. Biochemistry. 34:12903 – 12911.

Brumlik, J. M., Szymajda, U., Zakowska, D., Liang, X., Redkar, J. R., Patra, G. and Vecchio, D. G. V. 2001. Use of long-range repetitive element polymorphism – PCR to differentiate *Bacillus anthracis* strains. Appl. and Environ. Microbiol. 67: 3021-3028.

Buchalo, A. S., Nevo, E., Wasser, S. P., Oren, A. and Molitoris, H. P. 1998. Fungal life in the extremely hypersaline water of the Dead Sea: first records. Proc. R. Soc. Lond. B. 265:1461-1465.

Bull, A. T., Ward, A. C. and Goodfellow, M. 2000. Search and discovery strategies for biotechnology: the paradigm shift. Microbiol. and Molecular Biol. Reviews. 64:573-606.

Burns, D. G., Camakaris, H. M., Janssen, P. H. and Dyall-Smith, M. L. 2004. Combined use of cultivation-dependent and cultivation-independent methods indicates that members of most Haloarchaeal groups in an Australian crystallizer pond are cultivable. Appl. and Environ. Microbiol. 70:5258-5265.

Butinar, L., Santos S., Spencer-Martins, I., Oren, A. and Gunde-Cimerman, N. 2005a. Yeast diversity in hypersaline habitats. FEMS Microbiol. Letters. 244:229-234.

Butinar, L., Sonjak, S., Zalar, P., Plemenitas, A. and Gunde-Cimerman, N. 2005b. Melanized halophilic fungi are eukaryotic members of microbial communities in hypersaline waters of solar salterns. Botanica Marina. 48:73-79.

Butinar, L., Zalar, P., Frisvad, J. C. and Gunde-Cimerman, N. 2005c. The genus *Eurotium*-members of indigenous fungal community in hypersaline waters of salterns. FEMS Microbiol. Ecol. 51:155-166.

Buttner, P. M., Cruz-Perez, P. and Stetzenbach, D. L. 2001. Enhanced detection of surface-associated bacteria in indoor environment by Quantitative PCR. Appl. and Environ. Microbiol. 67: 2504-2570.

Caldwell, D. E. 1977. Accessory pigment fluorescence for quantitation of photosynthetic microbial populations. Can. J. of Microbiol. 23:1594-7.

Calo, P., De Miguel, T., Sieiro, C., Velazquez, J. B. and Villa, T. G. 1995. Ketocarotenoids in halobacteria: 3-hydroxy-echinenone and trans-astaxanthin. J. Appl. Bacteriol. 79: 282-85.

Campos- Ramos, R., Maeda- Martinez, A. M., Obregon-Barboza, H., Murugan, G., Guerrero-Tortolero, D. A. and Monsalvo-Spencer, P. 2003. Mixture of parthenogenetic and zygogenetic brine shrimp *Artemia* (Branchiopoda: Anostraca) in commercial cyst lots from Great Salt Lake, UT, USA. J. of Experimental Marine Biol. and Ecology. 296:243-251.

Cayol, J. L., Ducerf, S., Patel, B. K. C., Garcia, J. L., Thomas, P. and Ollivier, B. 2000. *Thermohalobacter berrensis* gen. nov., sp. nov., a thermophilic, strictly halophilic bacterium from a solar saltern. International J. of Systematic and Evolutionary Microbiol. 50:559-564.

Cherian, T., Rao, L. V. G. and Varma, K. K. 1974. Hydrography and suspended sediment load of oceanic and estuarine waters adjoining Marmugao harbour during early summer. Indian J. of Marine Sciences. 3:99-104.

Clegg. J. S., Jackson, S. A., Van-Hoa, N. and Sorgeloos, P. 2000. Thermal resistance, developmental rate and heat shock proteins in *Artemia franciscana*, from San Francisco Bay and Southern Vietnam. J. of Experimental Marine Biol. and Ecol. 252:85-96.

Coates, D. A. 1977. Enhancement of the sensitivity of the Limulus assay for the detection of Gram-negative bacteria. J. of Appl. Bacteriol. 42:445-9.

Cohen, Y., Krumbein, W.and Shilo, M. 1977. Solar lake (Sinai). 3. Bacterial distribution and production. Limnology and Oceanography. 22:621-34.

Collins, M. D.., Ross, H. N. M., Tindall, B. J. and Grant,W. D. 1981. Distribution of quinones in halophilic bacteria. J. Appl. Bacteriol. 50:550-565.

Cruickshank, R. 1965. Medical Microbiology – A guide to the Lab Diagnosis and Control of Infection. 11th ed. The English Language Book Society and E. & S. Livingstone Ltd.

Daley, R. J. and Hobbie, J. E. 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnology and Oceanography. 20:875-82.

DasSarma, S. and Arora, P. 2001. Halophiles. Encyclopedia of Life sciences, Nature Publishing Group / www. els. net.

De Rosa, M., Gambacorta, A., Nicolaus, B., Ross, H. N. M., Grant, W. D. and Bu'lock, J. D. 1982. An asymmetric archaebacterial diether lipid from alkaliphilic halophiles. J. Gen Microbiol. 128:244-348.

De Rosa, M., Gambacorta, A., Nicolaus, B., Ross, H. N. M. and Grant, W. D. 1983. A $C_{25:25}$ diether core lipid from archaebacterial haloalkaliphiles. J. Gen. Microbiol. 129:2333-2337.

Diaz- Munoz, G. and Montalvo- Rodriguez, R. 2005. Halophilic black yeast *Hortaea werneckii* in the Cabo Rojo solar salterns: Its first record for this extreme environment in Puerto Rico. Carribean J. of Science. 41:360-365.

Dittmer, J. C. and Lester, R. L. 1964. A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. J. Lipid Res. 5:126-127.

Dunn, R., McCoy, J., Simsek, M., Majumdar, A., Chang, S. H., RajBhandary, U. L. and Khorana, H.G. 1981. The bacteriorhodopsin gene. Proc. Natl. Acad. Sci. USA. 78:6744–6748.

Dyall-Smith, M., Tang, S. L. and Bath, C. 2003. Haloarchaeal viruses: how diverse are they? Res. in Microbiol. 154:309–13.

Edgerton, M. E. and Brimblecombe, P. 1981. Thermodynamics of halobacterial environments. Can. J. Microbiol. 27:899-909.

Egan, H., Kirk, R. S. and Sawyer, R. 1981. Pearson's Chemical Analysis of Foods. 8th ed.

Elazari-Volcani, B. 1972. In: Bergey's Manual of Determinative Bacteriology. 7th ed. P. G. Breed, E. G. D. Murray and N. P. Smith, Williams and Wilkins, Baltimore. pp. 207-212.

Ellis, M. B. 1971. Dematiaceous Hyphomycetes.Published by the Commonwealth Mycological Institute, Kew, Surrey, England, reprinted 1977, 2nd reprint 1980.

Elshahed, M. S., Najar, F. Z., Roe, B. A., Oren, A., Dewers, T. A. and Krumholz, L. R. 2004. Survey of archaeal diversity reveals an abundance of halophilic archaea in a low-salt, sulfide- and sulfur-rich spring. Appl. Environ. Microbiol. 70:2230-2239.

Fernandes, C. F. E. and Furtado, I. 2003. Evaluation of diversity of extremely halophilic archaea in saltpans of Goa, India', abstracted at 44th AMI Conference, Dharwad.

Fliermans, C. B. and Schmidt, E. L. 1975. Autoradiography and immunofluorescence combined for autecological study of single cell activity with *Nitrobacteria* as a model system. Appl. Microbiol. 30:674-7.

Franzmann, P. D., Stackebrandt, E., Sanderson, K., Volkmabb, J. K., Cameron, D. E., Stevenson, P. L. and McMeekin, T. A. 1988. *Halobacterium lacusprofundi* sp. nov., a halophilic bacterium isolated from Deep Lake, Antarctica. Syst. Appl. Microbiol. 11:20-27.

Fu, W. and Oriel, P. 1999. Degradation of 3-phenyl propionic acid by *Haloferax* sp. D. 1227. Extremophiles. 3:45-53.

Furtado, I. 1994. Information kit produced by WWF-India, Goa Division and the International Institute of Rural Reconstruction.

Garabito, M. J., Arahal, D. R., Mellado, E., Marquez, M. C. and Ventosa, A. 1997. *Bacillus salexigens* sp. nov., a new moderately halophilic *Bacillus* sp. International J. of Systematic Bacteriol. 47:735-741.

Garabito, M. J., Marquez, M. C. and Ventosa, A. 1998. Halotolerant *Bacillus* diversity in hypersaline environments. Can. J. of Microbiol. 44:95-102.

Gibbons, N. E. 1974. Halobacteriaceae. In: Bergey's Manual of Determinative Bacteriology. 8th ed., R. E. Buchanan and N. E. Gibbons, Williams and Wilkins, Baltimore. pp. 269-273.

Gochnauer, M. B., Leppard, G. G., Komaratat, P., Kates, M., Novitsky, T. and Kushner, D. J. 1975. Isolation and characterization of *Actinopolyspora halophila*, gen. et sp. nov., an extremely halophilic actinomycete. Can. J. Microbiol. 21:1500-1511.

Gonzalez, C., Gutierrez, C. and Ramirez, C. 1978. *Halobacterium vallismortis* sp. nov. an amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. Can. J. Microbiol. 24:710-715.

Grant, W.D. and Ross, H.N.M. 1986. The ecology and taxonomy of halobacteria. FEMS Microbiol. Rev. 39:9-5.

Grant, W. D. and Larsen, H. 1989. Extremely halophilic archaebacteria. In: Staley, Bryant, Pfennig and Holt(Editors), Bergey's Manual of Systematic Bacteriology, 1st Ed., Vol. 3, The Williams & Wilkins Co., Baltimore, pp. 2216- 2219.

Grant, W.D., Gemmell, R. T. and McGenity, T. J. 1998. Halobacteria: the evidence for longevity. Extremophiles. 2: 279-287.

Grasshoff, K. 1983. In "Methods of seawater analysis", ed. by K. Grasshoff, M. Ehrdardt and K. Krembling (Verlag Chemie, Weinneim) 419.

Gunde- Cimerman, N., Zalar, P., de-Hoog, S. and Plemenitas, A. 2000. Hypersaline waters in salterns: natural ecological niches for halophilic black yeasts. FEMS Microbiol. Ecology. 32: 235-240.

Harris, J. E., McKee, T. R., Wilson, R. C. and Whitehouse, U. G. 1972. Preparation of membrane filter samples for direct examination with an electron microscope. Limnology & Oceanography. 17:784-7.

Hartmann, R., Sickinger, H.D. and Oesterhelt, D. 1980. Anaerobic growth of halobacteria. Proc. Natl. Acad.Sci. (Wash.) 77:3821-3825..,

Herbert, D., Phipps, P. J. and Strange, R. E. 1971. Chemical analysis of microbial cells. In: Methods in Microbiology (Eds J. R. Norris & D. W. Ribbons), Vol. 5B. pp. 209-344. Academic Press, London.

Henderson, R. and Unwin, P. N. T. 1975. Three dimensional model of purple membrane obtained by electron microscopy. Nature. 257.

Hill, I. R. and Grey, T. R. G. 1967. Application of the fluorescent antibody technique to an ecological study of bacteria in soil. J. of Bacteriol. 93:1888-96.

Hirschler-Rea, A., Matheron, R., Riffaud, C., Moune, S., Eatock, C., Herbert, R.A., Willison, J. C. and Caumette, P. 2003. Isolation and characterization of spirilloid purple phototrophic bacteria forming red layers in microbial mats of Mediterranean salterns: Description of *Halorhodospira neutriphila* sp. nov. and emendation of the genus *Halorhodospira*. International J. of Systematic and Evolutionary Microbiol. 53:153-163.

Hobbie, J. E., Holm-Hansen, O., Packard, T. T., Pomeroy, L. R., Sheldon, R. W., Thomas, J. P. and Wiebe, W. J. 1972. A study of the distribution and activity of micro-organisms in ocean water. Limnology and Oceanography. 17:544-55.

Hobbie, J. E., Daley, R. J. and Jasper, S. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl. and Environ. Microbiol. 33:1225-8.

Holm-Hansen, O. 1969. Determination of microbial biomass in ocean profiles. Limnology and Oceanography. 19:31-4.

Holm-Hansen, O. and Booth, C. R. 1969. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnology and Oceanography. 11:510-19.

Holm-Hansen, O., Sutcliff, W. H. and Sharpe, J. 1968. Measurement of deoxyribonucleic acid in the ocean and its ecological significance. Limnology and Oceanography. 13:507-14.

Hubbard, J. S. and Rinehart, C. A. 1976. Bacteriorhodopsin formation in *Halobacterium halobium.* Can. J. Microbiol. 22:1274-1281.

Hungate, R. E. 1969. A roll tube method for cultivation of strict anaerobes. In: Methods of Microbiology (Eds. J. R. Norris & D. W Ribbons) vol. 3B, pp. 117-32, Academic Press, London.

Imhoff, J. F. and Rodriguez–Valera, F. 1984. Betaine is the main compatible solute of halophilic eubacteria. J. Bact. 160:478-479.

Imhoff, J. F. and Truper, H. G. 1977. *Ectothiorhodospira halochloris* sp. nov., a new extremely halophilic phototrophic bacterium containing bacteriochlorophyll b. Arch. Microbiol. 114:115-121.

Jae-Chang, C. and Tiedje, J. M. 2001. Bacterial species determination from DNA-DNA hybridization by using genome fragments and DNA microarrays. Appl. and Environ. Microbiol.. 67: 3677-3682.

Jannasch, H. W. and Jones, G. E. 1959. Bacterial population in sea water as determined by different methods of enumeration. Limnology and Oceanography. 4:128-39.

Javor, B. J. 1984. Growth potential of halophilic bacteria isolated from solar salt environments: carbon sources and salt requirements. Appl. and Env. Microbiol. 48:352-360.

Jones, J. G. 1970. Studies on freshwater bacteria: effect of medium composition and method on estimates of bacterial population. J. of Appl. Bacteriol. 33:679-87.

Jones, P. C. and Mollison, J. E. 1948. A technique for equivalent estimation of soil microorganisms. J. of Gen. Microbiol. 2:54-69.

Jones, J. G. and Simon, B. M. 1975. An investigation of errors in direct counts of aquatic bacteria by epifluorescence microscopy, with reference to a new method for dyeing membrane filters. J. of Appl. Bacteriol. 39:317-29.

Jones, J. G. and Simon, B. M. 1975. Some observation on the fluorometric determination of glucose in fresh water. Limnology and Oceanography. 20:882-7.

Kamat, S. B. and Sankaranarayanan, V. N. 1975. Concentration of particulate iron in estuarine and coastal waters of Goa. Ind. J. of Marine Sciences. 4:34-38.

Kamekura, M. and Kates, M. 1988. Lipids of halophilic archaebacteria.p.25-54. In: F. Rodriguez-Valera (ed.), Halophilic bacteria. II. CRC Press, Boca Raton, FL.

Kamekura, M., Dyall-Smith, M. L., Upasani, V., Ventosa, A. and Kates, M. 1997. Diversity of alkaliphilic halobacteria: proposals for transfer of *Natronobacterium vacuolatum, Natronobacterium magadii* and *Natronobacterium pharaonis* to *Halorubrum, Natrialba* and

Natronomonas gen. nov. respectively as *Halorubrum vacuolatum* comb. nov., *Natrialba magadii* comb. nov. and *Natronomonas pharaonis* comb. nov. respectively. Int. J. Syst. Bacteriol. 47: 853-857.

Kates, M. 1993. Biology of halophilic bacteria, Part II. Membrane lipids of extreme halophiles: biosynthesis, function and evolutionary significance. Experientia. 49:1027- 1036.

Kerkar, S. 2004. Studies on bacteria of the dissimilatory reductive processes of the sulphur cycle from the salt pans of Goa. Ph. D. Thesis. Dept. of Biotech. Goa University.

King, J. D. and White, D. C. 1977. Muramic acid as a measure of microbial biomass in estuarine and marine samples. Appl. and Environ. Microbiol. 33:777-83.

Kis-Papo, T., Grishkan, I., Oren, A., Wasser, S. P. and Nevo, E. 2001. Spatiotemporal diversity of filamentous fungi in the hypersaline Dead Sea. Mycol. Res. 105:749-756.

Kis-Papo, T., Oren, A., Wasser, S.P. and Nevo, E. 2003. Survival of filamentous fungi in hypersaline Dead Sea water. Microbial Ecol. 45:183-190.

Kitajima, T., Hirayama, J., Ihara, K., Sugiyama, Y., Kamo, N. and Mukohata, Y. 1996. Novel Bacterial Rhodopsins from *Haloarcula vallismortis*. Biochemical and Biophysical Research Communications. 220.

Kogej, T., Ramos, J., Plemenitas A., and Gunde-Cimerman, N. 2005. Halophilic fungus *Hortaea werneckii* and the halotolerant fungus *Aureobasidium pullulans* maintain low

intracellular cation concentrations in hypersaline environments. Appl. and Environ. Microbiol. 71:6600-6605.

Kogure, K., Simidu, U. and Taga, N. 1979. A tentative direct microscopic method for counting living marine bacteria. Can. J. of Microbiol. 25:415-20.

Kubitschek, H. E. 1969. Counting and sizing micro-organisms with the Coulter Counter. In: Methods in Microbiology (Eds J. R. Norris & D. W. Ribbons), Vol. 1, pp. 593-610. Academic Press, London.

Kushner, D.J. 1968. Halophilic bacteria. Adv. In Appl. Microbiol. 10:73-99.

Kushner, D.J. 1985. The Halobacteriaceae. In:The Bacteria, a treatise on structure and function, Vol. 8, ed. C.R. Woese and R.S. Wolfe, Academic Press, London, 171-214.

Kushwaha, S.C., Pugh, E.L., Kramer, J. K. G. and Kates, M. 1972. Isolation and identification of dehydro-squalene and C_{40}–carotenoid pigments in *Halobacterium cutirubrum*. Biochimica et Biophysica Acta. 160:492-506.

Kushwaha, S. C., Juez–Perez, G., Rodriguez-Valera, F., Kates, M. and Kushner, D.J. 1982. Survey of lipids of a new group of extremely halophilic bacteria from salt ponds in Spain. Can. J. of Microbiol. 28:1365-1372.

Lanyi, K. J. 1978. Light energy conversion in *Halobacterium halobium*. Microbiological Reviews. 42:682-706.

Larsson, K., Wenbull, C. and Cronberg, G. 1978. Comparison of light and electron microscopic determinations of the number of bacteria and algae in lake water. Appl. and Environ. Microbiol. 35:397-404.

Larsen, H. 1984. Family V. Halobacteriaceae, In: Bergey's Manual of Systematic Bacteriology, Vol. 1. ed. N. R. Krieg, Williams and Wilkins, Baltimore. 261-267.

Le Conte, John. 1999. How to make salt from sea-water : Electronic Edition, 1st edition, Academic Affairs Library, UNC-CH. University of North Carolina at Chapel Hill.

Litchfield, C. D., Irby, A., Kis-Papo, T. and Oren, A. 2000. Comparisons of the polar lipid and pigment profiles of two solar salterns located in Newark, California, U. S. A. and Eilat, Israel. Extremophiles. 4:259-265.

Lokabharathi, P. A., Nair, S. and Karanth, N. G. K. 1977. Microbial flora and dehydrogenase activity of beaches. Ind. J. of Marine Sciences. Vol.6:91-94.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. of Biol. Chem. 193:263-75.

MacKay, M. A., Norton, R. S. and Borowitzka, L. J. 1984. Organic osmoregulatory solutes in cyanobacteria. J. Gen. Microbiol. 130: 2177 – 2191.

Madigan, M. T. and Marrs, B. L. 1997. Extremophiles. Scientific American. 276:66-71.

Marquez, M.C., Ventosa, A. and Ruiz-Berraquero, F. 1987. A taxonomic study of heterotrophic halophilic and non-halophilic bacteria from a solar saltern. J. Gen. Microbiol. 133: 45-56.

Martin, E. L., Reinhardt, R.L., Baum, L.L., Becker, M. R., Shaffer, J. J. and Kokjohn, T. A. 2000. The effects of ultraviolet radiation on the moderate halophile *Halomonas elongata* and the extreme halophile *Halobacterium salinarum*. Can. J. of Microbiol. 46:180-187.

Marwan, W. and Oesterhelt, D. 2000. Archaeal vision and bacterial smelling. ASM News. 66.

Mayer, R. J. 2002. Morphology and biometry of three populations of *Artemia* (Branchiopoda: Anostraca) from the Dominican Republic and Puerto Rico. Hydrobiologia. 486:29-38.

McGenity, T. J., Gemmell, R. T. and Grant, W. D. 1998. Proposal of a new halobacterial genus *Natrinema* gen. nov., with two species *Natrinema pellirubrum* nom. nov. and *Natrinema pallidum* nom. nov. Int. J. Syst. Bacteriol. 48: 1187-1196.

McGenity, T. J., Gemmell, R. T., Grant, W. D. and Stan-Lotter, H. 2000. Origins of halophilic microorganisms in ancient salt deposits. Environmental Microbiol. 2:243-250.

Mejanelle, L., Lopez, J. F., Gunde-Cimerman, N. and Grimalt, J. O. 2001. Ergosterol biosynthesis in novel melanized fungi from hypersaline environments. J. of Lipid Res. 42: 352-359.

Meyer-Reil, L. A. 1978. Autoradiography and epifluorescence microscopy combined for determination of number and spectrum of actively metabolizing bacteria in natural waters. Appl. and Environ. Microbiol. 36:506-12.

Millar, W. N. and Casida, L. E. Jr. 1970. Evidence for muramic acid in soil. Can. J. of Microbiol. 16:299-304.

Minnikin, D.E., Alshamaony, L. and Goodfellow, M. 1975. Differentiation of *Mycobacterium, Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. J. of Gen. Microbiol. 88:200-204.

Montalvo-Rodriguez, R., Ruiz-Acevedo, A. and Lopez-Garriga, J. 1997. New isolates of extremely halophilic archaebacteria (Halobacteria) from Puerto Rico and the Caribbean. Caribbean J. of Science. 33:98-104.

Montalvo-Rodriguez, R., Vreeland, R. H., Oren, A., Kessel, M., Betancourt, C. and Lopez-Garriga, J. 1998. *Halogeometricum borinquense* gen. nov., sp. nov., a novel halophilic archaeon from Puerto Rico. International J. of Systematic Bacteriol. 48:1305-1312.

Montalvo-Rodriguez, R., Lopez-Garriga, J., Vreeland, R. H., Oren, A., Ventosa, A. and Kamekura, M. 2000. *Haloterrigena thermotolerans* sp. nov., a halophilic archaeon from Puerto Rico. International J. of Systematic and Evolutionary Microbiol. 50:1065-1071.

Montero, C. G., Ventosa, A., Rodriguez-Valera, F. and Ruiz-Berraquero, F. 1988. Taxonomic study of non-alkaliphilc halococci. J. Gen. Microbiol. 134: 725-732.

Mounes, S., Manach, N., Hirschler, A., Caumette, P., Willison, J. C. and Matheron, R. 1999. *Haloanaerobacter salinarius* sp. nov., a novel halophilic fermentative bacterium that reduces glycine-betaine to trimethylamine with hydrogen or serine as electron donors: emendation of the genus *Haloanaerobacter*. International J. of Systematic Bacteriol. 49:103-112.

Moune, S., Eatock, C., Matheron, R., Willison, J. C., Hirschler, A., Herbert, R. and Caumette, P. 2000. *Orenia salinaria* sp. nov., a fermentative bacterium isolated from anaerobic sediments of Mediterranean salterns. International J. of Systematic and Evolutionary Microbiol. 50:721-729.

Mouné, S., Caumette, P., Matheron, R. and Willison, J. 2003. Molecular sequence analysis of prokaryotic diversity in the anoxic sediments underlying cyanobacterial mats of two hypersaline ponds in Mediterranean salterns. FEMS Microbiol. Ecol. 44:117-130.

Munson, M. A., Nedwell, D. B. and Embley, T. M. 1997. Phylogenetic diversity of Archaea in sediment samples from a coastal salt marsh. Appl. Environ. Microbiol. 63:4729-4733.

Mukohata, Y. 1994. Comparative studies on ion pumps of the bacterial rhodopsin family. Biophysical Chem. 50:191-201.

Mwatha, W.D. 1993. *Natronobacterium vacuolata* sp. nov., a haloalkaliphilic archaeon isolated from Lake Magadi, Kenya. Int. J. Syst. Bacteriol. 43:401-404. Erratum: Int. J. Syst. Bacteriol. 43:867.

Nazareth, S. W. 2005. Halotolerant fungi with heavy metal resistance and exibiting alkalo-thermotolerant enzyme activity. 'Microbial Diversity, 2005, International Conference, Microbial Diversity: Current Perspectives and potential Applications', April 16-18, New Delhi-India, pg.237.

Nieto, J. J., Fernandes-Castillo, R., Marquez, M. C., Ventosa, A., Quesada, E. and Ruiz-Berraquero, F. 1989. Survey of metal tolerance in moderately halophilic eubacteria. Appl. and Environ. Microbiol. 55: 2385-2390.

Norton, C.F. and Grant, W.D. 1988. Survival of halobacteria within fluid inclusions of salt crystals. J. Gen. Microbiol. 134:1365-1373.

Nuebel, U., Bateson, M. M., Madigan, M. T., Kuhl, M. and Ward, D. M. 2001. Diversity and distribution in hypersaline microbial mats of bacteria related to *Chloroflexus* sp. Appl. and Environ. Microbiol. 67: 4365-4371.

Onions, A. H. S., Allsopp, D. and Eggins, H. O. W. 1981. Smith's introduction to industrial mycology. 7th ed.. Edward Arnold (Publishers) Ltd.

Oren, A. 1983. *Halobacterium sodomense* sp. nov., a Dead Sea *Halobacterium* with an extremely high magnesium requirement. Int. J. Syst. Bact. 33:381-386.

Oren, A. 1990. Estimation of the contribution of halobacteria to the bacterial biomass and activity in solar salterns by the use of bile salts. FEMS Microbiol. Ecol. 73:41-48.

Oren, A. 1993. Availability, uptake and turnover of glycerol in hypersaline environments. FEMS Microbiol. Ecol. 12:15-23.

Oren, A. 1994. The ecology of the extremely halophilic archaea. FEMS Microbiol. Reviews. 13: 415-440.

Oren, A. 1999. Microbiological studies in the Dead Sea: future challenges towards the understanding of life at the limit of salt concentrations. Hydrobiologia. 405:1-9.

Oren, A. 1999. Bioenergetic aspects of halophilism. Microbiol. and Molecular Biol. Reviews. 63:334-348.

Oren, A. 2002. Molecular ecology of extremely halophilic archaea and bacteria. FEMS Microbiol. Ecol. 39:1-7.

Oren, A. and Gurevich, P. 1993. Characterization of the dominant halophilic archaea in a bacterial bloom in the Dead Sea. FEMS Microbiol. Ecol. 12:249-256.

Oren, A., Ventosa, A. and Grant, W. D. 1997. Proposed minimal standards for description of new taxa in the order Halobacteriales. Int. J. Syst. Bacteriol. 47: 233-238.

Oren, A. and Rodriguez-Valera, F. 2001. The contribution of halophilic bacteria to the red coloration of saltern crystallizer ponds. FEMS Microbiol. Ecol. 36:123-130.

Oren, A. 2002. Halophilic microorganisms and their environments. In:The Cellular and Molecular Biology, Book Review, by R. Wegmann, Fouding President.

Oslon, B. H. 1978. Enhanced accuracy of coliform testing in seawater by a modification of the most probable number method. Appl. and Environ. Microbiol. 36:438-44.

Parulekar, A. H., Rajamickam, V. and Dwivedi, S. N. 1975. Benthic studies in Goa estuaries: biomass and faunal composition in the Zuari estuary. Ind. J. of Marine Science. 4:202-204.

Pasic L., Bartual, S. G., Ulrih, N. P., Grabnar, M. and Velikonja, B. H. 2005. Diversity of halophilic archaea in the crystallizers of an Adriatic solar saltern. FEMS Microbiol. Ecol. 54:491-498.

Patriquin, D. G. and Doberiner, J. 1978. Light Microscopy observations of tetrazolium-reducing bacteria in the endorhizosphere of maize and other grasses in Brazil. Can. J. of Microbiol. 24:734-42.

Pearl, H. W. and Shimp, S. L. 1973. Preparation of filtered plankton and detritus for study with scanning electron microscopy. Limnology and Oceanography. 18:802-5.

Petrovic, U., Gunde-Cimerman, N. and Plemenitas, A. 1999. Salt stress affects sterol biosynthesis in the halophilic black yeast *Hortaea werneckii*. FEMS Microbiol. Letters. 180 : 325-330.

Post, F. J. 1977. The microbial ecology of the Great Salt Lake. Microb. Ecol. 3: 143-165.

Postgate, J. R. 1969. Viable counts and viability. In: Methods in Microbiology (Ed. J.R. Norris and D. W. Ribbons), Vol. 1:611-28. Academic Press, London.

Pugsley, A. P. and Evison, L. M. 1975. A fluorescent antibody technique for the enumeration of faecal streptococci in water. J. of Appl. Bacteriol. 38:63-5.

Qasim, S. Z. and Sen Gupta, R. 1981. Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. Estuarine, Coastal and Shelf Science. 13: 557-578.

Quesada, E., Ventosa, A., Rodriguez-Valera, F. and Ramos-Cormenzana, A. 1982. Types and properties of some bacteria from hypersaline soils. J. Appl. Bacteriol. 53: 155-161.

Quesada, E.,Ventosa, A., Rodriguez-Valera, F., Megias, L. and Ramos-Cormenzana, A. 1983. Numerical taxonomy of moderately halophilic Gram-negative bacteria from hypersaline soils. J. Gen. Microbiol. 129: 2649-57.

Quesada, E., Bejar, M. V.,Valderrama, M. J. , Ventosa, A., Ruiz-Berraquero, F. and Ramos-Cormenzana, A. 1987. Numerical taxonomy of moderately halophilic Gram-negative non-motile eubacteria. Syst. Appl. Microbiol. 9: 132-7.

Quesada, E., Valderrama, M. J., Bejar, V., Ventosa, A., Ruiz-Berraquero, F. and Ramos-Cormenzana, A. 1987. Numerical taxonomy of moderately halophilic Gram-negative nonmotile eubacteria. Syst. Appl. Microbiol. 9: 132-137.

Raghavan, T. M. and Furtado, I. 2004. Current Science, 86, 8, 1065-1067.

Ramos-Cormenzana, A. 1990. Ecological distribution and biotechnological potential of halophilic microorganisms. In: Microbiology of Extreme Environments and its potential for biotechnology (Ed.: M. S. Da Costa, J. C. Duarte, R. A. D. Williams, Elsevier Science Publishers Ltd., London and New York). From FEMS Symposium No. 49.

Ramsay, A. J. 1974. The use of autoradiography to determine the proportion of bacteria metabolizing in an aquatic habitat. J. of Gen. Microbiol. 80:363-73.

Reed, R. H., Richardson, D.L., Warr, S. R. C. and Stewart, W. D. P. 1984. Carbohydrate accumulation and osmotic stress in cyanobacteria. 130:1- 4.

Robb, F. T., Place, A. R., Sowers, K. R., Schreier, H. J., DasSarma, S. and Fleischmann, E. M. 1995. Archaea-A Laboratory manual. Spring Harbor Laboratory Press.

Rodriguez-Valera, F., Ruiz-Berraquero, F. and Ramos-Cormenzana, A. 1979. Isolation of extreme halophiles from sea water. Appl. Environ. Microbiol. 38:164-165.

Rodriguez-Valera, F., Ruiz-Berraquero, F. and Ramos-Cormenzana, A. 1980. Isolation of extremely halophilic bacteria able to grow in defined organic media with single carbon sources. J. Gen. Microbiol. 119:535-538.

Rodriguez-Valera, F., Ruiz-Berraquero, F. and Ramos-Cormenzana, A. 1981. Characteristics of the heterotrophic bacterial populations in hypersaline environments of different salt concentrations. Microb. Ecol. 7: 235-243.

Rodriguez-Valera, F., Ventosa, A., Quesada, E. and Ruiz-Berraquero, F. 1982. Some physiological features of a *Halococcus* sp. at low salt concentrations. FEMS Microbiol. Lett. 15:249-252.

Rodriguez-Valera, F., Juez, G. and Kushner, D. J. 1983. *Halobacterium mediterranei* species nov., a new carbohydrate-utilising extreme halophile. Syst. Appl. Microbiol. 369-381.

Rodriguez-Valera, F., Ventosa, A., Juez, G. and Imhoff, J.F. 1985. Variation of environmental features and microbial populations with salt concentrations in a multi-pond saltern. Microb. Ecol. 11: 107-115.

Rodriguez-Valera, F. and Lillo, J. A. G. 1992. Halobacteria as producers of polyhydroxyalkanoates. In: Bacterial polyhydroxyalkanoates-Poly-betahydroxyalkanoic acid. H. G. Schlegel, A. Steinbuchel. ed. 181-186.

Ross, H. N. M., Collins, M. D., Tindall, B. J. and Grant, W. D. 1981. A rapid procedure for the detection of archaebacterial lipids in halophilic bacteria. J. Gen. Microbiol. 123:75-80.

Ross, H. N. M and Grant, W. D. 1985. Nucleic acid studies on halophilic archaeabacteria. J. Gen. Microbiol. 131:165-173.

Ross, H. N. M., Grant, W. D. and Harris, J. E. 1985. lipids in archaebacterial taxonomy. P.289-300. In:M. Goodfellow and D. E. Minnikin (ed.), Chemical methods in bacterial systematics. Society for Appl. Bacteriol. Technical Series 20, Academic Press, London.

Rotthschild, L. J. and Mancinelli, R. L. 2001. Life in extreme environments. Nature. 409: 1092-1101.

Rowe, R., Todd, R. and Waide, J. 1977. Microtechnique for most-probable-number analysis. Appl. and Environ. Microbiol. 33:675-80.

Sankaranarayanan, V. N. and Reddy, C. V. G. 1973. Copper content in the inshore and estuarine waters along the central west coast of India. Current Science. 42: 223-224.

Schmidt, E. L., Bankole, R. O. and Bohlool, B. B. 1968. Fluorescent antibody approach to study of Rhizobia in soil. J. of Bacteriol. 95:1987-92.

Sequeira, F. 1992. Microbiological study of salt pans in Goa. M.Sc. Dissertation Thesis. Department of Microbiology. Goa University.

Shah, H. N. and Rathod, R. D. 1990. Survey report on the salt production in Goa. Government Central Salts and Marine Chemical Research Institute, Bhavnagar.

Shaikh, M. R. 1996. Study of halophilic nitrogen fixing cyanobacteria from saline paddy fields of Goa. M. Sc. Dissertation.

Shirokitamura and Doi, Y. 1994. Staining method of poly(3-hydroxyalkanoic acid) producing bacteria by Nile Blue. Biotechnology Techniques. 8:345-350.

Siakotos, A. N. and Rosser, G. 1965. J. American Oil Chemists Soc. 42 : 913.

Singbal, S. Y. S. 1973. Diurnal variations of some physico-chemical factors in the Zuari estuary of Goa. Ind. J. of Marine Sciences. 2:90-93.

Skinner, F. A., Jones, P. C. and Mollison, J. E. 1952. A comparison of a direct and a plate counting technique for quantitative estimation of soil micro-organisms. J. of Gen. Microbiol. 6:261-71.

Soliman, G. S. H. and Truper, H. G. 1982. *Halobacterium pharaonis* sp. nov., a new, extremely haloalkaliphilic archaebacterium with low magnesium requirement. Zbl. Bakt. Hyg. I. Abt. Orig. C 3:318-329.

Soppa, J., Otomo, J., Straub, J., Tittor, J., MeeBen, S. and Oesterhelt, D. 1989. Bacteriorhodopsin mutants of *Halobacterium* sp. GRB-Characterization of mutants. J. of Biol. Chem. 264:13049–13056.

Sorensen, K. B., Canfield, D. E. and Oren, A. 2004. Salinity responses of benthic microbial communities in a solar saltern (Eilat, Israel). Appl. and Eviron. Microbiol. 70:1608-1616.

Stan Lotter, H., McGenity, T. J., Legat, A., Denner, E. B. M., Glaser, K., Stetter, K. O. and Wanner, G. 1999. Very similar strains of *Halococcus salifodinae* are found in geographically separated Permo-Triassic salt deposits. Microbiol.145:3565–3574.

Steensland, H. and Larsen, H. 1968. A study of cell envelopes of Halobacteria. J. Gen. Microbiol. 55 : 325-336.

Steiman, R., Guiraud, P., Sage, L. and Seigle-Murandi, F. 1997. Soil mycoflora from the Dead Sea Oases of Ein Gedi and Einot Zuqim (Israel). Antonie van Leeuwenhoek. 72:261-270.

Steiman, R., Ford, L., Ducros, V., Lafond, J. L. and Guiraud, P. 2004. First survey of fungi in hypersaline soil and water of Mono Lake area (California). Antonie van Leeuwenhoek. 85: 69-83.

Stoeckenius, W. and Bogomolni, R. A. 1982. Bacteriorhodopsin and related pigments of Halobacteria. Ann Rev. Biochem. 52:587-616.

Strayer, R. F. and Tiedje, J. M. 1978. Application of fluorescent-antibody technique to the study of a methanogenic bacterium in lake sediments. Appl. and Environ. Microbiol. 35:192-8.

Tateno, M., Ihara, K. and Mukohata, Y. 1994. Novel ion pump Rhodopsins from *Haloarcula* form family independent from both the Bacteriorhodopsin and Archaeorhodopsin families/tribes. Archives of Biochem. and Biophys. 315:127 – 132.

Takai, K., Komatsu, T., Inagaki, F. and Horikoshi, K. 2001. Distribution of archaea in a black smoker chimney structure. Appl. Environ.Microbiol. 67: 3618-3629.

Tepsic, K., Gunde-Cimerman, N. and Frisvad, J. C. 1997. Growth and mycotoxin production by *Aspergillus fumigatus* strains isolated from a saltern. FEMS Microbiol. Letters. 157: 9-12.

Thacker, L. 1977. Isolation of *Legionella pneumophila* from non epidemic-related aquatic habitats. Appl. and Environ. Microbiol. 37:1239-42.

Thiery, A. and Peunte, L. 2002. Crustacean assemblage and environmental characteristics of a man-made solar saltwork in southern France, with emphasis on anostracan (Branchiopoda) population dynamics. Hydrobiologia. 486:191-200.

Ticknor, O. L., Kolsto, A.B., Hill, K. K., Keim, P., Laker, T. M., Tonks, M. and Jackson, J. P. 2001. Fluorescent amplified fragment length polymorphism analysis of Norwegian *Bacillus cereus & B. thuringiensis* soil isolates. Appl. and Environ. Microbiol. 67: 4863-4873.

Tindall, B.J., Mills, A.A. and Grant, W.D. 1980. An alkaliphilic red halophilic bacterium with a low magnesium requirement from a Kenyan soda lake. J. Gen. Microbiol.116:257-260.

Tindall, B. J., Ross, H. N. M. and Grant, W. D. 1984. *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., two new genera of haloalkaliphilic archaebacteria. Syst. Appl. Microbiol. 5: 41-57.

Tindall, B. J. 1992. The Family Halobacteriaceae. In: A. Balows, H. G. Trüper, M. Dworkin, W. Harder and K. H. Schleifer (eds.). In : The Prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications. 2nd edition. Springer-Verlag, New York. pp. 768-808.

Tomioka, H., Takahashi, T., Kamo, N. and Kobatake, Y. 1986. Flash spectrophotometric identification of a fourth rhodopsin-like pigment in *Halobacterium halobium.* Biochemical and Biophysical Research Communications. 139.

Tomlinson, G. A. and Hochstein, L. I. 1976. *Halobacterium saccharovorum* sp. nov., a carbohydrate-metabolising, extremely halophilic bacterium. Can. J. Microbiol. 22: 587-591.

Tomlinson, G. A., Jahnke, L. L. and Hochstein, L. I. 1986. *Halobacterium denitrificans* sp. nov., an extremely halophilic denitrifying bacterium. Int. J. Syst. Bacteriol. 36 : 66-70.

Torreblanca, M., Rodriguez-Valera, F., Juez, G., Ventosa, A., Kamekura, M. and Kates, M. 1986. Classification of non-alkaliphilic halobacteria, based on numerical taxonomy and polar lipids composition, and description of *Haloarcula* gen. nov., and *Haloferax* gen. nov. Syst. Appl. Microbiol. 8: 89-99.

Toteja, G. S., Mukherjee, A., Mittal, R. and Saxena, B. N. 1990. Manual Methods of Analysis for Adulterants and Contaminants in Foods.

Toumi, N., Ayadi, H., Abid, O., Carrias, J. F., Sime-Ngando, T., Boukhris, M. and Bouain, A. 2005. Zooplankton distribution in four ponds of different salinity : a seasonal study in the solar salterns of Sfax (Tunisia). Hydrobiologia. 534:1-9.

Trebaol, G., Manceau, C., Tirilly, Y. and Boury, S. 2001. Assessment of the genetic diversity among strains of *Xanthomonas cynarae* by randomly amplified polymorphic DNA analysis (RAPD) and development of specific characterized amplified regions for the rapid identification of *X. cynarae*. Appl. and Environ. Microbiol. 67: 3379-3380.

Truper, H. G. and Yentsch, C. S. 1967. Use of glass fiber filters for the rapid preparation of *in vivo* absorption. Spectra of photosynthetic bacteria. J. of Bacteriol. 94:1255-6.

Truper, H. G. and Galinski, E. A. 1986. Concentrated brines as habitats for microorganisms. Experientia..42. Birkhauser Verlag, CH-4010 Basel/Switzerland.

Upsani, V. and Desai, S. 1990. Sambar salt lake chemical composition of the brines and studies on haloalkaliphilic archaebacteria. Arch. Microbiol. 154: 589-593.

Varma, K. K., Rao, L. V. G. and Cherian, T. 1975. Temporal and Spatial variations in hydrographic conditions of Mandovi Estuary. Ind. J. of Marine Sciences. 4: 11-17.

Ventosa, A. 1990. Taxonomy of halophilic bacteria. In: Microbiology of Extreme Environments and its potential for biotechnology (Ed.: M. S. Da Costa, J. C. Duarte, R. A. D. Williams, Elsevier Science Publishers Ltd., London and New York). From FEMS Symposium No. 49.

Ventosa, A., Quesada, E., Rodriguez–Valera, F. and Ramos–Cormenzana, A. 1982. Numerical taxonomy of moderately halophilic Gram–negative rods. J. Gen. Microbiol. 128: 1959-1968.

Ventosa, A., Marquez, M. C., Garabito, M. J. and Arahal, D. R. 1998. Moderately halophilic gram-positive bacterial diversity in hypersaline environments. Extremophiles. 2: 297-304.

Ventosa, A., Nieto, J. J. and Oren, A. 1998. Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev. 62:504-544.

Villar, M., De Ruiz Holgado, A. P., Sanchez, J. J., Trucco, R. E. and Oliver, G. 1985. Isolation and characterization of *Pediococcus halophilus* from salted anchovies (*Engraulis anchoita*). Appl. Environ. Microbiol. 49:664-666.

Vreeland, R. H., Litchfield, C. D., Martin, E. L. and Elliot, E. 1980. *Halomonas elongata*, a new genus and species of extremely salt–tolerant bacteria. Int. J. Syst. Bact. 30:485-495.

Watson, S. W., Novitsky, T. J., Quinby, H. L. and Valois, F. W. 1977. Determination of bacterial number and biomass in the marine environment. Appl. and Environ. Microbiol. 33: 940-6.

Weisser, J. and Truper, H. G. 1985. Osmoregulation in a new haloalkaliphilic *Bacillus* from the Wadi Natrun (Egypt). Syst. Appl. Microbiol. 6:7-11.

Xu, X., Wu, M. and Huang, W. 2005. Isolation and characterization of a novel strain of *Natrinema* containing a bop gene. Journal of Zhejiang Univ. Science B. 6: 142-146.

Yeon, S. H., Jeong, W. J. and Park, J. S. 2005. The diversity of culturable organotrophic bacteria from local solar salterns. J. of Microbiol. 43:1-10.

Zalar, P., de- Hoog, G. S., Schroers, H. J., Frank, J. M. and Gunde-Cimerman, N. 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and Wallemiales, cl. et ord. nov.) Antonie van Leeuwenhoek. 87:311-328.

Zheng- Bo, Sun- Shichun, Ma-Lin. 2004. The occurrence of an exotic bisexual *Artemia* species, *Artemia franciscana*, in two coastal salterns of Shandong. Province, China. J. of Ocean University of China. 3:171-174.

Zhou, P. Xu-Yi., Xiao, C., Ma, Y. and Liu, H. 1994. New species of *Haloarcula*. Acta. Microbiol. Sin. 34:89-95.

Zimmermann, R., Iturriaga, R. and Becker-Birck, J. 1978. Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. Appl. and Environ. Microbiol. 36:926-35.

Zingde, M. D., Singbal, S. Y. S., Moraes, C. F. and Reddy, C. V. G. 1976. Arsenic, copper, zinc and manganese in the marine flora and fauna of coastal and estuarine waters around Goa. Ind. J. of Marine Sciences. 5: 212-217.

http : // www.saltinstitute.org/36.html

LIST OF PRESENTATIONS

1. Fernandes, C. F. E. and Furtado, I. 2003. Evaluation of diversity of extremely halophilic archaea in saltpans of Goa, India', abstracted at 44th AMI Conference, Dharwad.

2. Fernandes, C. F. E. and Furtado, I. 2005. Occurrence & isolation of non-halophilic eubacteria from brines of salt pans of Goa, India, abstracted at 'Microbial Diversity, 2005, International Conference, Microbial Diversity Current Perspectives and potential Applications', April 16-18, New Delhi-India, pg.90.

3. Fernandes, C. and Furtado, I. 2005. Culturable haloarchaeal diversity of salt pans of Goa, India, abstracted at 'International Marine Biotechnology Conference at St. John's New Foundland, Canada, June 7-12, pg. 300.