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Biofilm-associated indole acetic acid producing bacteria and their impact in the proliferation of biofilm mats in solar salterns

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Abstract: Biofilm mats appear in salterns distinctively during the monsoon season when the salinity decreases below 12 percentile salinity units and within a short period cover the entire surface area of the saltern. A study was carried out in two salterns viz. Nerul and Curca to find a possible reason for the rapid proliferation of these solar biofilms. Out of the 125 bacteria isolated from these biofilms, 16 produced indole-3-acetic acid (IAA). Rapid *in-situ* assay with Salkowski reagent and HPLC analysis confirmed the IAA production. Four isolates consistently produced high IAA concentrations ranging from 9.5 to 14.2 μg/mL in the presence of 4 mg/mL tryptophan concentrations in the growth media. The IAA-producing bacteria were *Aeromonas aquariorum* (N2), *Pseudomonas alcaliphila* (N3), *Vibrio diazotrophicus* (N6) and *Pseudomonas pachastrellae* (C3). These four IAA-producing bacteria also produced other growth promoting factors like ammonia. Three isolates produced siderophores and were phosphate solubilizers. There was enhancement in the growth of *Cicer arietinum* (length of the shoot and root) under axenic conditions and of biofilm mats ($r = 0.9$, $p < 0.001$; $r = 0.8$, $p < 0.05$ and $r = 0.946$, $p < 0.01$, respectively). This is, according to our knowledge, the first report indicating IAA-producing bacteria isolated from biofilms enhancing the proliferation of these biofilm mats in the solar salterns.

Key words: indole acetic acid; bacteria; biofilms; solar salterns; plant growth promoters.

Abbreviations: IAA, indole-3-acetic acid; psu, percentile salinity unit; ZMA, Zobell marine agar; ZMB, Zobell marine broth.

Introduction

Indole-3-acetic acid (IAA) is a natural auxin produced by plants, algae, mosses, lichens and a diverse group of organisms. In addition, microorganisms also produce IAA (Muller et al. 1989; Patten & Glick 2002) especially those which live in the soil rhizosphere or as free living soil bacteria (Glick 1995) as well as bacteria associated with plants (Buggeln & Craigie 1971). Bacterial production of IAA has not only been studied regarding its physiological effects on plants, but also regarding its possible role as a phyto-hormone in plant-microbe interaction (Barbieri & Galli 1993; Xie et al. 1996; Patten & Glick 2002). A number of IAA biosynthetic pathways have been identified in bacteria and emphasized the requirement of tryptophan as a precursor. This has been confirmed by both conventional (Rahman et al. 2010) and molecular genetics methods using mutants (Idris et al. 2007).

In the aquatic system, limited studies have been carried out. Mishra & Kefford (1969) have shown that algae produced IAA associated with coral sand where as in sediment (Maruyama et al. 1989) and in sea water (Antolic et al. 1996) IAA is produced by free bacteria. Biofilms are microbial mats having organised multicellular systems with structural and functional architecture very similar to the rhizosphere, which influence metabolic processes, response to nutrients and other factors. They are found in various habitats ranging from hot springs to brackish and hypersaline coastal environments including solar salterns (Caumette et al. 1994). These habitats are classified as extreme environments because high salt concentrations are hostile to most organisms. However, many organisms, particularly prokaryotic and eukaryotic microorganisms can inhabit and survive in these environments due to their ability to cope with osmotic stress. These microorganisms have many mechanisms to protect their cells from high osmotic pressure and against the denaturing effect of salts. They play an important role as primary producers in these ecosystems (Borowitzka 1981; Oren & Seckbach 2001). They thus represent an establishment of microenvironmental conditions as the biofilm progresses from initial to more established stages (Lawrence et al. 1991).

In spite of its occurrence in various habitats, IAA-producing bacteria associated with biofilm and growth of biofilm has not been studied. We observed a rapid proliferation of biofilm mats formed on the surface layer of water (approximately 0.2–1.0 cm thick) spreading on the entire surface area of the salterns (Goa, west coast of India) specifically in the monsoon sea-

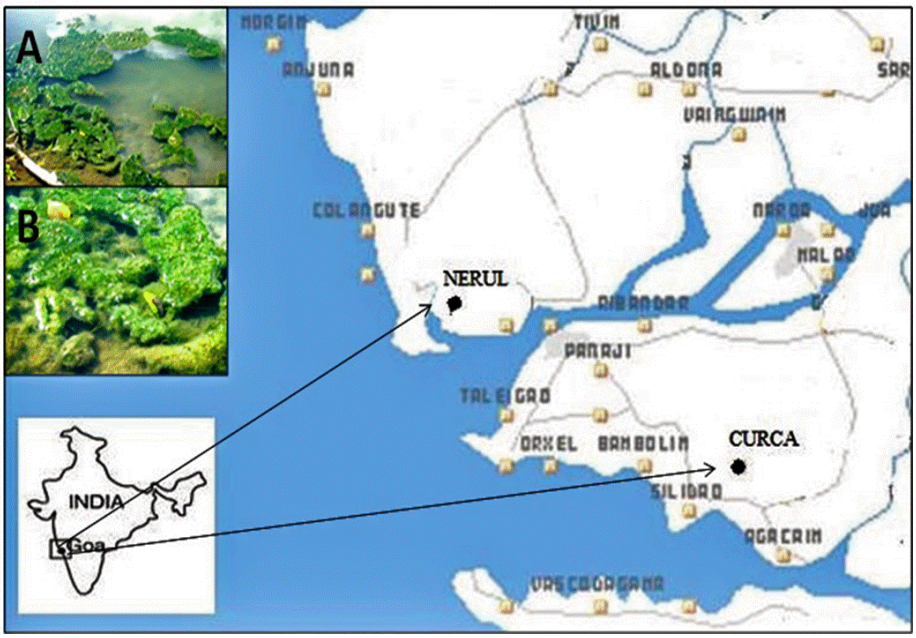

Fig. 1. Sampling sites. Inset: biofilms in solar salterns, Goa (A. Nerul & B. Curca).

son (Fig. 1) when the salinity starts to decrease. The biofilm microflora consists mainly of green algae, blue green algae, *Euglenophyceae* and diatoms. Based on the morphological characteristics, four different types of green algae viz. *Pediastrum duplex*, *Oedogonium* sp., *Cladophora* sp. and *Spirogyra exiles* were observed. The blue green algae were *Phormidium* sp. (corium), *Phorimidium* sp. (ambigeum) and *Oscillatoria* sp. Only one type of *Euglenophyceae*, i.e. *Phacus* sp., was observed. The diatoms comprised of *Pleurosigma* sp. and *Navicula* sp. The present work was carried out to find out whether IAA-producing bacteria are associated with these biofilms of solar salterns and if so, whether they are responsible for the rapid proliferation of these biofilm mats.

Material and methods

Sampling of biofilms

Biofilm samples were collected from two solar salterns, viz. Nerul (15°30′N, 73°46′E) and Curca (15°27′N, 73°52′E) in Goa, India, during the monsoon month of August. Plastic trays were inserted below the floating mats in solar salterns and the mat was cut with a pair of scissors along with the underlying water samples and carried to the laboratory in an ice box for further analysis.

Isolation of bacterial cultures

Samples of biofilm mats were cut, teased with a pair of forceps on glass slides and observed microscopically to identify the different algal flora present in the biofilm. For isolation of the bacterial cultures, biofilm sample (5 g) was weighed in 50 mL glass tubes aseptically and sonicated for 10 s alternately, with and without pulse (Vibra Cell Sonicator, USA). The supernatant (100 µL) was surface plated on Zobell marine agar (ZMA) (g/L) (peptic digest of animal tissue 5 g, yeast extract 1.0 g, ferric chloride 0.1 g, sodium chloride 19.45 g, magnesium chloride 8.8 g, sodium sulphate 3.24 g, calcium chloride 1.8 g, potassium chloride 0.55 g, sodium sulphate 3.24 g, potassium bromide 0.88 g, sodium bicarbonate 0.16 g, strontium chloride 0.034 g, boric acid 0.022 g, sodium silicate 0.004 g, sodium fluoride 0.0024 g, ammonium nitrate 0.0016 g, disodium phosphate 0.008 g, agar 2.0 g, saltern water 1 L, pH 7.6±0.2) and nutrient agar plates (peptone 5 g, NaCl 5 g, beef extract 1.5 g, yeast extract 1.5 g, prepared in salt pan water 1 L, agar 2%, pH 7.4 ± 0.2) and incubated overnight at room temperature (28 ± 2°C). Morphologically different colonies were isolated, purified and stored on slants at 4°C.

Screening for bacteria producing indole

A total of 125 bacterial colonies were isolated and preliminary screened using Kovac's reagent (isoamyl alcohol 150 mL, para-dimethylaminobenzaldehyde 10 mg, concentrated HCl 50 mL). Cultures were inoculated in tubes containing 5 mL of tryptone water (1–2%, pH 7.5) and incubated at room temperature (28±2°C) for 72 h. 0.5 mL of Kovac's reagent was added to each tube. Appearance of a pink coloration indicated the presence of indole.

Rapid in-situ assay for IAA

Rapid *in-situ* assay (Bric et al. 1991) used for the qualitative assay for IAA production for the cultures which were positive for indole production. Bacteria were inoculated into a grid pattern on LBTD4 agar plates (Luria Bertani's media supplemented with L-tryptophan 5 mM, sodium dodecyl sulphate 0.06% and glycerol 1%, prepared in sea water) with sterile tooth picks. The agar plates were overlaid with a 45 mm diameter disc of nitrocellulose membrane (Nytran, Amersham). All the plates were incubated until colonies were ∼2 mm in diameter. The nitrocellulose membrane was removed from the plates and treated with Salkowski reagent (perchloric acid 50 mL, 0.5 M ferric chloride 1.0 mL). Bacteria producing a characteristic red halo within the membrane surrounding the colony immediately were considered as positive for IAA.

Screening IAA-producing bacteria for other growth promoting factors

Phosphate solubilisation test was carried out by inoculating the cultures in a grid pattern on Pikovskaya's agar plate

(yeast extract 0.5 g, dextrose 10.0 g, calcium phosphate 5.0 g, ammonium sulphate 0.5 g, potassium chloride 0.2 g, magnesium sulphate 0.1 g, manganese sulphate 0.1 mg, ferrous sulphate 0.1 mg, agar 15 g). Plates were incubated at room temperature for 5–6 days. Appearance of clear zone around the culture indicated phosphate solubilisation. The isolates were assayed for siderophore production on chrome azurole S-ZMA plate (ZMA containing 60.5 mg of chrome azurole S, 10 mL of ferric ion solution and 72.9 mg of hexadecyl trimethyl ammonium) and incubated for 6 days. Development of a yellow-orange halo around growth was considered as positive for siderophore production. Ammonia production was tested by inoculating the cultures in autoclaved (5 mL) tryptone water (1–2%) and incubation at room temperature (28±2 °C) for 3 days. Production of ammonia was tested by Nessler's reagent (potassium iodide 50 g, mercuric chloride 22 g and 5 N sodium hydroxide). Appearance of yellow to brown colour indicated ammonia production.

Quantitative assay for IAA

Out of 16, four of IAA-producing cultures were consistent in their growth and production and were selected for quantitative and further studies. IAA was quantified for using Salkowski colorimetric assay. Bacterial cultures (10^3/mL) were grown in 10% Zobell marine broth (ZMB) at 37 °C for 5 days, centrifuged at 10,000 rpm for 10 min. The supernatant (bacterial IAA extract) (2 mL) was mixed with two drops of orthophosphoric acid and 4 mL of Salkowski reagent and incubated for 30 min and the absorbance noted at 540 nm (Shimadzu, UV mini 1240, Japan). The concentrations of IAA produced by cultures were calculated with reference to a standard graph of IAA (Sigma-Aldrich, range 0–50 µg/mL). IAA was confirmed and quantified by RP-HPLC. The culture supernatant was acidified to pH 2.5–3.0 with 1 N HCl and extracted twice with ethyl acetate (2:1 v/v). The organic phase (ethyl acetate fraction) was collected and evaporated to dryness in a Roteva Equitron (Medica Instruments MFGG, Germany) at 40 °C for 45 min. The extract was dissolved in 10 mL of methanol and centrifuged to get rid of any debris. The clear solution was applied to HPLC (Jasco, UK) having HIQ sil C18 W reverse phase column packed with silica. Samples were eluted with 30% methanol at a flow rate of 1 mL/min and analysed by UV visible spectrophotometer at 254 nm. The IAA peak was quantified in comparison to pure IAA (Sigma-Aldrich).

Effect of salinity on IAA production

IAA producers were grown in 10% ZMB and 1 mL containing 10^3 cells (estimated using a haemocytometer). They were inoculated in triplicate into 10 mL of sterile salt. The salt content was adjusted with distilled water to 0, 2, 4, 6, 8, 10, 12 and 14 percentile salinity units (psu) that were incubated at 37 °C till a visible turbidity (5 days) was seen. The tubes were centrifuged and the concentration of IAA in the supernatant was quantified spectrophotometrically as mentioned above.

Effect of tryptophan on IAA production

Bacteria (initial counts were 10^3 cells/mL estimated using a haemocytometer) were grown separately in 10% ZMB (25 mL) prepared in salt pan water in 100 mL flasks (in triplicates) supplemented with different concentrations of L-tryptophan (1, 2, 3, 4, 5 mg/mL). The incubation was carried out at 37 °C for 5 days. Quantification of IAA was done by the same method (spectrophotometry).

Effect of bacterial IAA extract on Cicer arietinum

The effect of bacterial IAA extract from the 4 cultures was initially tested on the model plant, i.e. potted plantlets of *Cicer arietinum*. The seeds were initially surface sterilized with 70% ethanol for 1–2 min in a sterile flask, rinsed three times with sterile distilled water. The seeds were subsequently sterilized with 50% chlorox (Zonrox – a commercial bleach) for 10 min in a shaker, rinsed 3–5 times in sterile distilled water to remove the chlorox and the seeds were placed on a sterile filter paper to dry. Subsequently they were inoculated in pots with 500 g sterilized soil and incubated with a photoperiod of 14 h in light and 10 h in dark per day at 28 °C for 5 days. As the seedlings grew to a length of about 3–4 inches, bacterial IAA extracts of the 4 bacteria (500 mL varying concentration (v/v) were added. Plants were watered daily. After 3 days, the plant was uprooted and observed for increase in length of shoot and root along with the lateral branching. The growth was compared to the control. Statistical analyses (Microsoft Excel 2000) were done to see the correlation between the shoot/root and bacterial extracts.

Growth enhancement studies with partially purified bacterial IAA

1. Preparation of partially purified bacterial IAA extracts. The bacteria were grown in 10% ZMB for 2 days centrifuged at 10,000 rpm for 10 min. The supernatant was mixed with Diaion HP-20 resin (Sigma-Aldrich) and eluted with HPLC-grade methanol and evaporated to dryness in a Roteva Equitron (Medica Instruments MFGG, Germany).

2. Effect of bacterial IAA in the proliferation of biofilm. Biofilm samples (2×2 cm) in triplicates were inoculated in 100 mL sterile salt pan water in 250 mL flask (18 flasks) supplemented with 0.1–0.5 µg/mL of partially purified bacterial IAA (of all the four IAA-producing bacteria). The flasks were incubated with a photoperiod of 14 h in light and 10 h in dark at 28 °C for 30 days. The weight of the biofilm was determined. Effect of the bacterial IAA and proliferation of bacterial biofilm was confirmed by correlation using statistical analyses (Microsoft Excel 2000).

3. Effect on biofilm at different salinities. Biofilm samples (2×2 cm) were inoculated into 100 mL of salt pan water with salinities ranging from 0, 2, 4, 6, 8, 10 and 12 psu, in triplicates. Partially purified 0.4 µg/mL bacterial IAA extracts of two fast growing isolates viz. N2 and N3 were added. The flasks were incubated for 30 days in a photoperiod of 12 h. Control flasks were kept with biofilm sample and 100 mL of salt pan water. After 30 days of incubation period, the biofilm was filtered on pre-weighed filter paper and dried at 50 °C for 4 h. Growth was estimated as increase in dry weight of the biofilm.

Identification of IAA-producing bacteria

Morphological and biochemical characterization of the four bacterial isolates (N2, N3, N6 and C3) was carried out. The biochemical and substrate utilization tests (47 tests) were done using Hi-assorted biochemical test kit (Hi Media, Mumbai, India KB009 and KB002). The test strip was inoculated with the culture broth of the IAA-producing isolates (grown in shaken flasks for 2 days). Colour changes in the strip were recorded subsequent to incubation for 18–20 h at room temperature (28±2 °C). Based on Bergey's manual, specific tests were also performed and the isolates were identified to the genus level. The four potential IAA-producing isolates were identified using 16S rRNA gene sequencing. The chromosomal DNA of strains were isolated according

Table 1. Substrate utilisation by IAA-producing bacteria.

Substrate	*A. aquariorum* (N2)	*P. alcaliphila* (N3)	*V. diazotrophicus* (N6)	*P. pachastrellae* (C3)
Lactose	–	–	+	–
Xylose	–	+	+	–
Maltose	+	+	+	–
Fructose	+	+	+	–
Galactose	–	–	+	–
Trehalose	+	+	+	–
L-arabinose	–	+	+	–
Mannose	–	+	+	–
Inulin	–	+	–	–
Sodium gluconate	+	+	+	–
Glycerol	+	–	+	–
Salicin	–	+	–	–
Glucosamine	–	+	+	–
Cellobiose	–	+	+	–
Citrate	+	+	+	–
Lysine	+	+	+	+
Ornithine	+	+	+	+
Nitrate reduction	+	+	+	–

to Rainey et al. (1996). The 16S rRNA gene was amplified with primers: 8–27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1500r (5'-AGAAAGGAGGTGATCCAGCCA-3'). The PCR cycling parameters included an initial denaturation at 95℃ for 4 min followed by 40 cycles of denaturation at 95℃ for 1 min, annealing at 55℃ (1 min) and amplification at 72℃ (2 min), and final extension at 72℃ (10 min). Amplified fragments were separated by electrophoresis on 1% agarose gel and the 1,490 bp of 16S rRNA gene fragment was eluted by using the QIA quick gel extraction kit (Qiagen). The purified PCR product was sequenced with four forward and three reverse primers, namely 8–27f, 357f (5'-CTCCTACGGGAGGCAGCAG-3'), 704f (5'-TAGCGGTGAAATGCGTAGA-3'), 1114f (5'-GCAACGAGCGCAACC-3'), 685r (5'-TCTACGCATTTCACCGCTAC-3'), 1110r (5'-GGGTTGCGCTCGTTG-3') and 1500r (*Escherichia coli* numbering system). The 16S rRNA gene sequence was determined by the dideoxy chain-termination method with the Big-Dye terminator kit followed by capillary electrophoresis using an ABI 310 Genetic Analyzer (Applied Biosystems, USA). The identification of phylogenetic neighbours and the calculation of pair-wise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (http://www.eztaxon.org/) (Chun et al. 2007).

Results

Out of the 125 bacteria isolated from saltern biofilm, 20% of these isolates were indole positive. All the indole positive cultures did not produce IAA. Out of the 16 IAA-producing bacteria, four isolates (N2, N3, N6 and C3) consistently produced IAA. Out of the 4 IAA-producing isolates, three isolates solubilised phosphate. Interestingly, N2, which produced maximum IAA, was also a phosphate solubilizer. All the four IAA-producing bacteria were ammonia producers, while N2, N3 and C3 produced siderophores.

Characteristics of the 4 IAA- producing bacteria

All the 4 bacteria were Gram-negative, halotolerant with length ranging from 1 to1.5 μm. They were motile, oxidase- and catalase-positive. Strains N2, N3, N6 and C3 had the ability to utilise most of the common substrates (Table 1). Strains N2, N3, N6 and C3 were identified as *Aeromonas aquariorum*, *Pseudomonas alcaliphila*, *Vibrio diazotrophicus* and *Pseudomonas pachastrellae*, respectively, with which they share highest sequence similarity. Phylogenetically they aligned most closely with *Aeromonas aquariorum* MDC47^T (100%), *Pseudomonas alcaliphila* AL15–21^T (99.167%), *Vibrio diazotrophicus* ATCC33466^T (99.213%) and *Pseudomonas pachastrellae* KMM330^T (99.553%), respectively. Though strain N3 and C3 were *Pseudomonas* sp., *P. alcaliphila* (N3) was more versatile in its sugar and amino acid utilisation pattern as compared to *P. pachastrellae* (C3).

Effect of salinity on IAA production

All the 4 strains produced low concentration of IAA which varied from 0.68±0.005 to 0.79±0.02 μg/mL. The production of IAA was salinity depended and varied with the bacteria. Maximum IAA was produced by*A. aquariorum* (N2), *P. alcaliphila* (N3) and *V. diazotrophicus* (N6) at 6 psu and subsequently it decreased with increased salinity (Fig. 2). However in *P. paschastrellae* (C3), maximum IAA production was noted at 8 psu.

Effect of tryptophan on IAA production

As seen from the Figure 3, tryptophan enhanced the production of IAA in all the strains. The maximum production of IAA was observed when supplemented with 4 mg/mL of L-tryptophan. *A. aquariorum* (N2) produced 14.27±0.09 μg/mL, *P. alcaliphila* (N3) produced 14.0±0.12 μg/mL, and *V. diazotrophicus* (N6) produced 8.93±0.02 μg/mL. However, in *P. paschastrellae* (C3) there was not much variation in the concentration of IAA (9.67±0.10 μg/mL) produced with tryptophan concentrations from 2 to 5 mg/mL. Unlike, *Pseudomonas* spp., in *V. diazotrophicus* (N6) and in*A. aquariorum* (N2), IAA production decreased drastically at 5 mg/mL tryptophan concentrations.

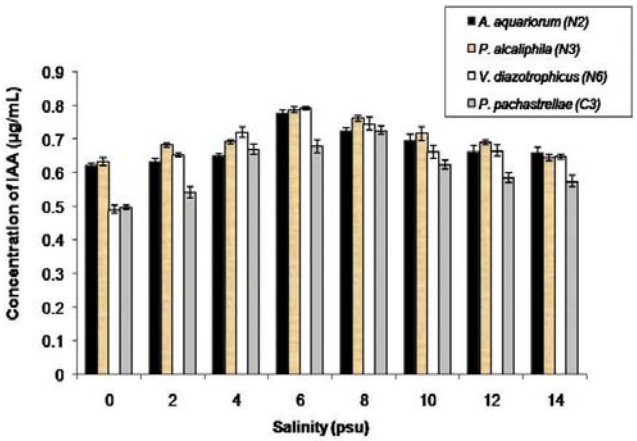

Fig. 2. Effect of salinity on IAA production (bars indicate ± standard deviation).

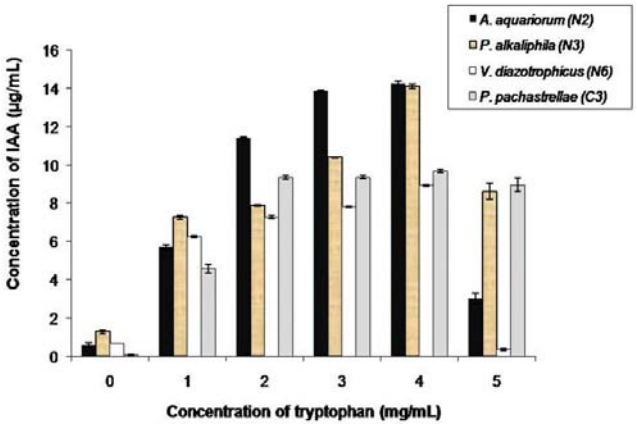

Fig. 3. Effect of tryptophan on IAA production (bars indicate ± standard deviation).

Table 2. Effect of bacterial IAA on biofilm proliferation.

Bacterial extract (IAA µg/mL)	Average weight of biofilm (weight g ± SD)
0	0.191 ± 0.004
0.1	0.243 ± 0.009
0.2	0.351 ± 0.024
0.3	0.386 ± 0.015
0.4	0.422 ± 0.009
0.5	0.418 ± 0.006

Effect of bacterial IAA extract on Cicer arietinum
The plants treated with the bacterial IAA extract showed better growth and development as compared with the control. The length of the root varied from 6.1 to 8.4 cm compared to the control (5.7 cm). In the case of shoot, the growth was twice that of the control for all the strains. Plants treated with *A. aquariorum* (N2) culture extract developed profused lateral roots which were longer and well developed as compared to the untreated control as shown in Figure 4. Correlation was observed between bacterial extract and shoot and root ($r = 0.9$, $p < 0.001$; $r = 0.8$, $p < 0.05$, respectively).

Effect of bacterial IAA extracts on biofilm growth and at different salinities
The growth of biofilm with different concentrations of partially purified IAA showed an increase in the weight of the biofilm with an increase in the concentration of IAA (Table 2). There was a significant positive correlation ($r = 0.946$, $p = 0.01$) between the bacterial IAA concentration and the weight of the biofilm (within the studied concentration) showing that the growth of the biofilm was IAA dependent. Growth of the biofilm was observed at all salinities irrespective of the addition of bacterial extracts. However, the growth was higher in bacterial extracts than the control. There was increase in growth of 13% increase in the weight of the biofilm observed at 6 psu for *A. aquariorum* (N2) and *P. alcaliphila* (N3) as the salinity increased to 6 psu. Further increase in salinity affected the growth of the biofilm (Fig. 4). At 14 psu, there was not only a decrease in growth by 8% but browning of biofilm was observed.

Discussion

IAA is the main auxin in plants controlling many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity (Moore 1989; Davies 1995; Lüthen et al. 1999). Substantial amount of work has been carried out on the IAA-producing bacteria from terrestrial sources viz. plant rhizospheres (Ahmad et al. 2008), cowdung (Manas et al. 2007) and orchid roots (Tsavkelova et al. 2005). The enhancement of plants by pure cultures producing IAA has been demonstrated (Idris et al. 2007; Shahab et al. 2009). Limited studies have been carried out from marine environments (Maruyama et al. 1989; Antolic et al. 1996; Gutierrez et al. 2009). All these studies clearly reflect the ubiquitous nature of bacteria and their significance in promoting the growth of the associated biota.

In the biofilms from marine salterns, we have also found the presence of IAA-producing bacteria belonging to γ-Proteobacteia. As established earlier, IAA production was dependent on the availability of tryptophan. In bacterial systems, the major pathway for IAA synthesis is a tryptophan-dependent pathway (Antolic et al. 1996; Nigovic et al. 2000; Woodward & Bartel 2005) which conforms with our four isolates. At tryptophan concentrations of 0 mg/mL, the IAA production for *A. aquariorum* (N2), *P. alcaliphila* (N3), *V. diazotrophicus* (N6) and *P. pachastrellae* (C3) varied from 0.58, 1.28, 0.68 and 0.05 µg/mL, respectively, whereas at higher tryptophan concentration (4 mg/mL), IAA production increased to 14.27, 14.08, 8.93 and 9.67 µg/mL, respectively, thus showing a positive effect of tryptophan on the IAA production by the bacterial isolates. Similarly tryptophan-dependent IAA production has been reported from bacteria associated with the rhizosphere IAA. Ahmad et al. (2005) have, however, reported *Azotobacter* sp. from rhizospheric soils to produce higher values compared to our studies: 7.3–32.8 µg/mL IAA with 5 mg/mL of L-tryptophan

Fig. 4. Effect of bacterial IAA extracts on root/shoot development of *Cicer arietinum*.

compared to 2.68–10.8 μg/mL in its absence. They also report a *Pseudmonas* sp. to produce 41.0–53.2 μg/mL of IAA (Ahmad et al. 2005). Such variations have been observed in type-strains viz. *A. hydrophila*, *P. aeruginosa* and *V. parahaemolyticus*, which produced almost 5–times higher concentrations of IAA with 100 mg/L of tryptophan as compared to the absence of tryptophan in the media. Such inducing effect of tryptophan on IAA production is known among various terrestrial bacteria, viz. *Pseudomonas syringae* (Hutcheson & Kosuge 1985), *Azotobacter vinelandii* (Lee et al. 1970), *Agrobacterium tumefaciens* (Liu & Kado 1979), *Azospirillum brasilense* (Tien et al. 1979), *Rhizobium leguminosarum* (Wang et al. 1982). The tryptophan-dependent IAA production was confirmed by Idris et al. (2007) in *Bacillus amyloliquefaciens*. However, the isolates varied in their intrinsic ability to produce IAA as the production varied under the same condition. It appears that the conversion of tryptophan to IAA is a general function of bacterial communities in the natural environment and biofilms from salterns are not an exception. Being from marine salterns, salinity also played a role and was one of the factors controlling the production of tryptophan-dependent IAA of these cultures as the production of IAA was maximum at 6psu. The presence of minimal IAA in the absence of tryptophan in our controls could be from the saltern water we have used for the experiment. Throughout the monsoon season we have detected average concentrations of 0.486 μg/mL of IAA in the overlying waters of solar salterns which was much higher than the values reported by Mauryauma et al. (1989) in interstitial water of eutrophicated marine sediments $0.123–0.876\times10^{-9}$ mg/mL. There seems to be two possible explanations for these high concentrations: (i) being leached from microalgae; or (ii) IAA production by the associated bacteria. The contribution from the algae to the IAA production will be minor because earlier studies have shown that living algae contribute a minor part to the total IAA production in sea water (Maruyama et al. 1989).

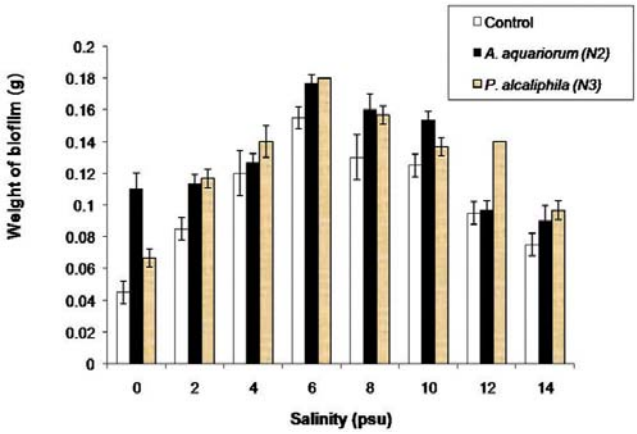

Fig. 5. Effect of biofilm proliferation with bacterial IAA extracts at different salinities (bars indicate ± standard deviation).

IAA produced by bacteria associated with biofilms has been reported as a chemical for communication between the microorganisms (Kogure et al. 1979). Our findings confirm the role of tryptophan dependent IAA in the growth of biofilms as a significant positive correlation was observed with the addition of partially purified IAA. Like IAA production, the growth of the biofilm was also salinity dependent. Growth of biofilms was maximal at 6 psu. The growth of biofilm observed in the saltern water without addition of tryptophan or bacterial IAA extract could be due to the natural occurrence of IAA found in the overlying sea water as explained above. Apart from the IAA production, other growth promoting factors were also found to be produced by the bacterial isolates, which suggest the possibility that these bacteria could be responsible in nourishing the biofilm by providing free ammonia, increasing the availability of iron (siderophores) and phosphate when normally the nutrients get diluted with the heavy precipitation during the monsoon season. Further stud-

ies are needed to confirm the role of other growth promoting factors in the surface multiplication and rapid spreading of the biofilm. Our findings demonstrate that the proliferation of the biofilms in the salterns during the monsoon season is due to the tryptophan and salinity dependent IAA biosynthesis in these biofilm bacteria, which may be synergistically augmented by the other growth promoting factors.

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