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Characterization of *Microbulbifer* Strain CMC-5, a New Biochemical Variant of *Microbulbifer elongatus* Type Strain DSM6810^T Isolated from Decomposing Seaweeds

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Abstract A Gram-negative, rod-shaped, non-spore forming, non-motile and moderate halophilic bacteria designated as strain CMC-5 was isolated from decomposing seaweeds by enrichment culture. The growth of strain CMC-5 was assessed in synthetic seawater-based medium containing polysaccharide. The bacterium degraded and utilized agar, alginate, carrageenan, xylan, carboxymethyl cellulose and chitin. The strain was characterized using a polyphasic approach for taxonomic identification. Cellular fatty acid analysis showed the presence of iso-$C_{15:0}$ as major fatty acid and significant amounts of iso-$C_{17:1\omega 9c}$ and $C_{18:1\omega 7c}$. Phylogenetic analysis based on 16S rDNA sequence indicated that strain CMC-5 is phylogenetically related to *Microbulbifer* genus and 99% similar to type strain *Microbulbifer elongatus* DSM6810^T. However in contrast to *Microbulbifer elongatus* DSM6810^T, strain CMC-5 is non-motile, utilizes glucose, galactose, inositol and xylan, does not utilize fructose and succinate nor does it produce H_2S. Further growth of bacterial strain CMC-5 was observed when inoculated in seawater-based medium containing sterile pieces of *Gracilaria corticata* thalli. The bacterial growth was associated with release of reducing sugar in the broth suggesting its role in carbon recycling of polysaccharides from seaweeds in marine ecosystem.

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Introduction

Recalcitrant polysaccharides such as cellulose, agar, alginate, xylan, carrageenan and chitin are commonly referred to as insoluble complex polysaccharides (ICPs). In marine ecosystems, ICPs are represented in a variety of organisms such as seaweeds, fungi, zooplankton and crustaceans [15, 16]. In order to escape oligotrophic conditions in oceans, marine bacteria often tend to evolve association with surrounding biota containing ICPs. The cell walls of seaweeds constitute wide varieties of polysaccharides and hence extend a unique micro-niche sustaining growth of diverse bacterial communities [14]. Bacteria degrading ICPs are one of the most unique groups amongst these bacterial communities and are primarily responsible for recycling of organic carbon from the recalcitrant ICPs [31].

In 1997, the genus *Microbulbifer* was first proposed by Gonzalez et al., for rod-shaped, strictly aerobic marine bacteria showing the presence of iso-$C_{15:0}$ as one of the major cellular fatty acid [11]. On the basis of chemotaxonomic and 16S rDNA studies, *Pseudomonas elongatus* DSM 6810^T was reclassified as *Microbulbifer elongatus* DSM 6810^T [32]. Although M*icrobulbifer* genus falls into γ-subclass of *Proteobacteria*, phylogenetic analysis based on 16S rDNA sequence studies revealed that it has less than 91% sequence identity to all previously described members of γ-*Proteobacteria* [2]. Several new species have been isolated from diverse niches such as lignin-rich pulp wastes, red sand stone, intertidal sediments, salt marshes and marine solar saltern [11, 27, 32–35]. One of the prominent features of these bacteria is their ability to degrade more than one ICPs, as determined in strains of *M. hydrolyticus*, *M. arenaceous*, *M. elongatus*, *M. salipaludis* and *M. celer* [11, 27, 32, 33, 35]. *Microbulbifer*

degradans 2–40, the only strain reported to degrade 10 ICPs has been reclassified as *Saccharophagus degradans* [7].

During routine screening for agarolytic bacteria from decomposing seaweeds, a multiple polysaccharide degrading bacterial strain CMC-5 was isolated. This strain was subjected to polyphasic taxonomy study including 16S rDNA gene sequence analysis. The results based on phylogenetic tree conclusively suggest that the strain is closely related to *Microbulbifer elongatus*. However strain CMC-5 differed with respect to morphological and biochemical characteristics when compared to *Microbulbifer elongatus* type strain DSM6810^T. Further in vitro study on growth of bacterial strain CMC-5 in seawater-based medium containing sterile seaweed thalli of *Gracilaria corticata* demonstrate its capability to decompose seaweed thalli. The reducing sugars released during decomposition of seaweed were accompanied by growth of bacterial strain CMC-5.

Materials and Methods

Isolation of Multiple Polysaccharide Degrading Bacteria

Seaweeds (*Gracilaria corticata* and *Sargassum tenneri-mum*) collected from Anjuna coast of Goa, India (15°35′65″N, 73°49′182″E), in January 2004 were kept for decomposition at room temperature (30 ± 2°C) for 40 days in natural sea water collected from the vicinity of seaweeds. The bacterial flora from decomposing seaweeds was serially diluted and screened on artificial sea water medium (ASW) containing (g/l) Tris base: 6.05; $MgSO_4$: 12.32; KCl: 0.74; $(NH_4)_2HPO_4$: 0.13; NaCl: 17.52; $CaCl_2$: 0.14; agar 2.0%; pH 7 [10]. The plates were incubated at room temperature for 72 h. The bacterial isolates obtained were purified further by streaking the colonies on the same medium. One of the bacterial isolate designated as strain CMC-5 showed extensive pit formation on ASW agar plates and degraded multiple polysaccharides. Hence bacterial strain CMC-5 was chosen for further studies.

Culture of Bacterial Strain CMC-5

The strain CMC-5 was routinely grown in ASW medium at 30 ± 2°C for 48 h. When grown in liquid broth, ASW medium was supplemented with 0.2% agarose and incubated on orbital shaker at 130 rpm whereas for growth on solid medium 2% agar was added to ASW medium. All subsequent growth conditions were same as above.

Phenotypic Characterization

Phenotypic characterization was performed as mentioned by Smibert and Krieg [26]. Cell morphology was studied by scanning electron microscopy with bacterial cells grown in ASW broth for 48 h. Motility was examined in ZoBell marine broth medium by hanging drop method [26]. Growth at different temperatures was tested by streaking cultures on ASW agar plates and incubating the plates at 4, 10, 20, 37 and 42°C, respectively. Requirement of NaCl for bacterial growth was tested using modified ASW medium (without NaCl). NaCl was added in the range of 0–10% to modified ASW medium and bacterial growth was spectrophotometrically determined (OD_{600}) for 48 h. Utilization of different carbon compounds were tested using BIOLOG GN2 plate (Biolog Inc., Hayward, CA) as per manufacturer's instructions with slight modification which included addition of 1.5% NaCl to the inoculating fluid. Sensitivity of strain CMC-5 to various antibiotics was tested by disc diffusion method on Muller–Hinton agar plates. Quantitative analysis of cellular fatty acids was carried out according to the instructions of Microbial Identification system (MIDI Inc., Newark, USA). The G + C content of genomic DNA was determined spectrophotometrically (Lambda 35, Perkin-Elmer) using the thermal denaturation method [18].

Degradation and Utilization Studies of Various Polysaccharides

Degradation of agar (bacteriological, purified), carboxymethyl cellulose (low viscosity, sodium salt), chitin (from Crab shells), xylan (from Oat spelt), alginate (sodium salt, polyguluronic and polymannuronic acid mixture) and carrageenan (from Irish moss) were tested. Individual polysaccharide (1%) was added to ASW agar medium and strain CMC-5 was streaked on the culture plate and incubated for 72 h. The degradation of individual polysaccharide was detected by the various plates screening method using dyes or precipitating agents specific for detection of respective polysaccharides [9, 12, 24]. Utilization of polysaccharide as carbon substrate was determined by inoculating the strain in ASW medium supplemented with one of the above polysaccharides (agar replaced by low melting agarose) at a final concentration of 0.2% and incubated on orbital shaker. The growth was assessed spectrophotometrically at 600 nm after 48 h.

In Vitro study on Decomposition of Algal Thalli by Bacterial Strain CMC-5

The ability of bacterial strain CMC-5 to degrade *Gracilaria corticata* thalli was studied. Bacterial strain CMC-5 was

grown in 25 ml of ASW medium containing 0.2% glucose for 24 h at room temperature at 130 rpm. The bacterial inoculums for the experiment were prepared by centrifuging the culture, washing the cell pellet twice with sterile ASW medium and resuspending in 25 ml of sterile ASW medium. Axenic culture of *Gracilaria corticata* was prepared and maintained as mentioned by Chen and McCracken [5]. Fifty milligrams (wet weight) of axenic thalli were cut into small pieces (3–4 mm) and aseptically transferred to conical flasks containing sterile ASW medium. 0.1% of the above bacterial inoculum was added to ASW medium containing sterile pieces of *Gracilaria* thalli and incubated at room temperature at 130 rpm for 8 days. ASW medium containing axenic seaweed or bacterial inoculum were kept as control for 8 days. Samples were collected from the above flask at regular intervals. Growth of bacterial strain CMC-5 was determined by measuring optical density at 600 nm whereas reducing sugars released by algal thalli degradation were estimated from culture supernatant by the dinitrosalicylic acid (DNSA) method using D-galactose as standard [19].

Phylogenetic Analysis of 16S rDNA

Chromosomal DNA was isolated according to Maloy [17]. 16S rDNA sequence was amplified using two universal primers 27F and 1525R [22]. The amplified PCR product was purified using QIAquick PCR purification kit (QIAGEN Inc., Calif, USA) according to the supplier's instructions. Sequencing of purified PCR product was performed using Big Dye Terminator Kit according to manufacturer's instructions. The sequence was determined by using an ABI-PRISM 3730 automated sequencer (Applied Biosystems Inc., Foster City, USA). Homologous sequences most similar to that of strain CMC-5 were retrieved from Ribosomal Database Project (RDP). Multiple sequence alignment of 16S rDNA sequence was carried out using Clustal W program [30]. Phylogenetic tree was constructed by the maximum likelihood method using PHYLIP package [8]. Evolutionary distance matrices were calculated according to Kimura-2 parameter using DNADIST program in PHYLIP package. Bootstrap analysis was done with 1000 resamplings using SEQBOOT program of PHYLIP package.

Culture Collection and Nucleotide Sequence Accession Number

The bacterial strain CMC-5 after identification has been deposited in MTCC, Chandigarh, as MTCC 9889 whereas the 16S rRNA gene sequence of strain CMC-5 determined in this study is accessible from GenBank database under accession no. EU121671.

Results and Discussion

Isolation and Characteristics of Strain CMC-5

During preliminary screening for agarolytic bacteria from decomposing seaweeds, several bacterial isolates were obtained on ASW agar plates. Most of the bacterial isolates obtained during present study were observed to degrade more than one polysaccharide. The microbial community associated with different biota has already been exploited for isolating multiple polysaccharide degrading bacteria [3, 25]. An unknown unique bacterium degrading seven different polysaccharides was obtained from reproductive tissue of fronds of *Fucus distichus* [23]. Similarly, *Saccharophagus degradans* strain 2–40 isolated from degrading salt marsh grass *Spartiana alterniflora* was found to degrade 10 different polysaccharides [1, 7]. Even non-marine sources such as terrestrial rhizosphere have served as a source for isolating several strains of *Paenibacillus* sp. which showed multiple polysaccharide degrading activity [13]. Several strains of *Microbulbifer* degrading more than one polysaccharides have been reported from various niches [11, 27, 32–35].

One of the bacterial isolate designated as strain CMC-5 obtained during present study was chosen for further characterization as it was observed to degrade multiple polysaccharides. Cells of strain CMC-5 are short rods, Gram-negative and non-motile. The cells measured 0.18–0.22 μm in width and 0.9–1.2 μm in length. The bacterial colonies are 3–4 mm in diameter, cream coloured, regular with undulate margin and depicted convex elevation when grown on ZoBell marine agar. Diffusible brown pigment was observed on peptone agar plate. The bacterial strain formed deep craters on ASW agar medium with a clearance zone around the colonies and did not require any growth factors or any other nitrogen sources for its growth. The bacterium did not grow on nutrient agar plates unless it was amended with 2% NaCl. The strain grew optimally in the range of 2–4% NaCl. Growth was observed up to 8% NaCl; however, no growth was observed at 10% NaCl. No growth was observed at 4, 10, 20 and 42°C. Growth was observed at 30°C within 48 h whereas growth was ascertained at 37°C only after 72 h. The phenotypic characteristics of strain CMC-5 are as described in Table 1. The DNA G + C content was estimated to be 65.6 mol% (done in duplicate). An average value of 59 mol% has been reported for most *Microbulbifer* strain whereas a value of 63.2 mol% has been reported for *Microbulbifer halophilus* [11, 27, 28, 32, 34].

Degradation and Utilization of Polysaccharides

Various dyes and precipitating agents have been widely used to determine polysaccharide degradation using medium-based agar plate containing individual polysaccharides

Table 1 Biochemical properties of *Microbulbifer* strain CMC-5

Biochemical tests	Reaction
Arginine decarboxylase	+
Casein hydrolysis	−
Catalase	+
DNase	−
Esculin hydrolysis	+
Gelatin hydrolysis	+
Lysine decarboxylase	+
MR-VP test	+
Oxidase	+
Sodium malonate utilization	−
Urease	−
Antibiotics	
Penicillin G, co-trimaxazole, erythromycin, tetracycline, ampicillin, gentamycin, kanamycin, tetracycline, streptomycin, nitrofurantoin	Resistant
Chloramphenicol	Sensitive
Substrate utilization	
Tween 40, tween 80, adonitol, L-arabinose, D-arabitol, cellobiose, D-galactose, gentiobiose, α-D-glucose, *m*-inositol, D-lactose, α-D-lactulose, maltose, D-mannitol, D-mannose, D-melibose, xylitol, *N*-acetyl-D-glucosamine, acetic acid, D-glucuronic acid, α-ketoglutaric acid, D,L-lactic acid, hydroxyl-L-proline, propionic acid, L-alanine, L-alaninamide, L-leucine, L-serine, L-histidine, L-threonine, 2-aminoethanol, glycerol, D,L-α-glycerol phosphate, -D-glucose phosphate, D-glucose-6-phosphate.	+
α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, *i*-erythritol, D-Saccharic acid, D-fructose, D-fucose, *β*-methyl-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, pyruvic acid methyl ester, succinic acid mono-methyl ester, malonic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, glycyl-L-glutamic acid, D-galacturonic acid, D-glucoronic acid, D-glucosamine acid, D-serine, α-hydroxybutyric acid, *β*-hydroxybutyric acid, *γ*-hydroxybutyric acid, *ρ*-hydroxybutyric acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, sebacic acid, succinic acid, bromosuccinic acid, quinic acid, glucuronamide, D-alanine, L-alanylglycine, L-aspergine, L-aspartic acid, L-glutamic acid, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D,L-carnitine, *γ*-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethyamine, putrescine, 2,3-butanediol, α-D-glucose phosphate.	−

[24]. Based on above studies, strain CMC-5 was observed to degrade six different polysaccharides such as agar, CMC, carrageenan, chitin, alginate and xylan (Table 2). An increase in cell mass as evident by increase in OD_{600} substantiated that strain CMC-5 utilized the above polysaccharides as a source of carbon and energy (Table 2). Simultaneously a reduction in broth viscosities in all tested polysaccharides after 48 h indicated degradation of polysaccharide.

Table 2 Polysaccharides degradation and utilization by bacterial strain CMC-5

Polysaccharides	After 48 h	
	Degradation	Utilization
Agar	+	*†[c]
Alginic acid	+	†[c]
Xylan	+	†[b]
Carrageenan	+	†[b]
CMC	+	†[a]
Chitin	+	†[a]

+, Degraded (detected by plate screening method); †[a] $0.19 > O.D_{600} > 0.1$; †[b] $0.29 > O.D_{600} > 0.2$; †[c] $0.39 > O.D_{600} > 0.3$ († utilization correlated with bacterial growth as determined by measuring optical density at 600 nm). * Agarose used as carbon substrate

16S rDNA Gene Sequence and Phylogenetic Tree

The almost complete 16S rDNA sequence of 1517 bases of strain CMC-5 was obtained. The sequence has highest similarity with *γ*-subclass of *Proteobacteria*, in particular to the genus *Microbulbifer*. It shares a similarity of 99% with *M. elongatus* DSM 6810^T (AF500006), 98% with *M. salipaludis* SM-1^T (AF479688), 96% with *M. arenaceous* RSBr-1^T (AJ510266) and *M. celer* ISL-39^T (EF486352), 97% with *M. hydrolyticus* IRE-31^T (U58338) and 95% with *M. maritamus* TF-17^T (AY377986). Phylogenetic tree was constructed using sequences of closely related genera. The phylogenetic tree constructed using maximum-likelihood algorithm showed that this strain formed a coherent

cluster with the clade that comprises type strains of *Microbulbifer* genus supported by bootstrap confidence level of 99% (Fig. 1). Similar tree topology was observed when trees were constructed using neighbour joining and maximum parsimony algorithms. As evident from phylogenetic tree, strain CMC-5 was observed to be closely related to *Microbulbifer elongatus* DSM 6810^T.

Comparison of Characteristics of Strain CMC-5 with Other *Microbulbifer* Species

Biochemical studies of strain CMC-5 did not suggest any possible leads towards identification hence chemotaxonomic approach to identify bacterial strain was undertaken. During FAME analysis, iso-$C_{15:0}$, $C_{18:1\omega7c}$ and iso-$C_{17:1\omega9c}$ were detected as major fatty acids (Table 3). Although the fatty acid profile did not match with any known bacterial genera in the database, presence of iso-$C_{15:0}$ as one of the major fatty acid has been reported to be a characteristic feature of all *Microbulbifer* strains [32]. The fatty acid profile of strain CMC-5 is similar to those reported for type strains of *M. hydrolyticus*, *M. elongatus*, *M. maritamus*, *M. salipalidus* and *M. celer* except for variations in the proportion of $C_{16:0}$ fatty acid [11, 32–35]. Thus on the basis of chemotaxonomic analysis and phylogenetic analysis, it can be concluded that strain CMC-5 should be placed in the genus *Microbulbifer*.

Although *Microbulbifer* strain CMC-5 showed 99% sequence similarity with *M. elongatus* type strain DSM 6810^T, there are some differences. Strain CMC-5 was isolated from decomposing seaweeds and forms cream coloured colonies on Zobell marine agar plates whereas *M. elongatus* DSM 6810^T isolated from intertidal sand and bottom sediment forms yellowish orange colour colonies

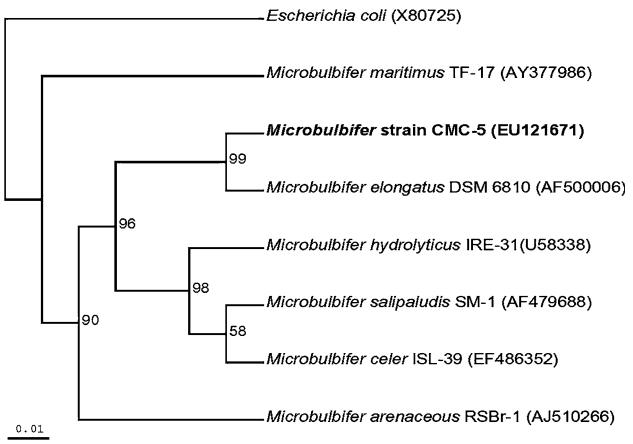

Fig. 1 Maximum likelihood tree showing phylogenetic position of *Microbulbifer* strain CMC-5 and the type strains of *Microbulbifer* based on 16S rDNA. % of bootstrap values from 1000 replications are shown at branch points

Table 3 Percentage cellular fatty acid composition of strain CMC-5

Fatty acid	Percentage
Straight chain	
$C_{10:0}$	1.0
$C_{14:0}$	0.51
$C_{15:0}$	1.06
$C_{16:0}$	5.07
$C_{17:0}$	1.51
$C_{18:0}$	0.66
Branched	
iso-$C_{11:0}$	5.78
iso-$C_{15:0}$	20.88
iso-$C_{15:1}$	1.31
iso-$C_{16:0}$	0.24
iso-$C_{17:0}$	9.30
anteiso-$C_{17:0}$	0.36
iso-$C_{17:1\omega9c}$	20.22
Unsaturated	
$C_{17:1\omega8C}$	1.79
$C_{18:1\omega7C}$	14.07
Hydroxy	
$C_{10:0}$3-OH	0.64
iso-$C_{11:0}$3-OH	6.61
Summed feature-3 ($C_{16:1\omega7C}$, iso-$C_{15:0}$ 2-OH)[#]	6.60
Summed feature-7 ($C_{19:1\omega6c}$, $C_{19:0}$cyclo)[#]	0.24

[#] Summed feature represent groups of fatty acids that could not be resolved by GC with the MIDI system

[21]. Further like most *Microbulbifer* strains, strain CMC-5 is non-motile whereas type strain DSM 6810^T is motile [11, 27, 32–35]. Also in contrast to *Microbulbifer elongatus* DSM6810^T, strain CMC-5 utilizes glucose, galactose, inositol and xylan but does not utilize fructose and succinate nor does it produce H_2S [6, 32]. The proportion of $C_{16:0}$ fatty acid in strain CMC-5 was very low when compared to type strain DSM 6810^T [32]. Thus *Microbulbifer* strain CMC-5 is morphologically and biochemically different from *Microbulbifer elongatus* type strain DSM6810^T. Table 4 depicts an overall comparison of *Microbulbifer* strain CMC-5 with other reported *Microbulbifer* strains.

Microbulbifer strains have been previously reported from intertidal sediments, salt marshes, lignin rich pulp wastes, red sand stone and marine solar saltern [11, 27, 32–35]. Recently *Microbulbifer variabilis* and *Microbulbifer epialgicus* strains have been isolated from marine algae and sea grass [20]. However the present report on isolation of *Microbulbifer* strain CMC-5 is atypical as it has been isolated from decomposing seaweeds after 40 days. Unlike other reported *Microbulbifer* strains, *Microbulbifer* strain

Table 4 Different characteristic of *Microbulbifer* strain CMC-5 as compared to other known *Microbulbifer* species. Strain species: 1. *Microbulbifer hydrolyticus*; 2. *Microbulbifer arenaceous*; 3. *Microbulbifer maritimus*; 4. *Microbulbifer salipaludis*.; 5. *Microbulbifer celer*; 6. *Microbulbifer elongatus*; 7. *Microbulbifer* strain CMC-5

Characteristics	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7 [g]
Source	Lignin-rich pulp waste	Red sand stone	Intertidal sediment	Salt marsh	Marine solar saltern	Intertidal sand/ seawater	Decomposing seaweeds
Cell morphology (length/ shape)	1.1–1.7 µm Rods	3–7 µm Rods	3–6 µm Rods	1.2–3 µm Rods	0.8–3.5 µm Rods	3–6 µm Cocci or rods	0.9–1.2 µm Rods
Motility	−	−	−	−	−	+	−
Colour of colonies on marine agar	Cream	Brown	Yellowish brown	Greyish yellow	Greyish yellow	Yellowish brown	Cream
H_2S production	ND	ND	ND	ND	ND	+	−
Utilization of							
Glucose	+	+	−	+	−	−	+
Galactose	−	+	−	−	−	−	+
Hydrolysis of							
Agar	−	−	−	w	−	+	+
Chitin	+	+	−	−	ND	+	+
Gelatin	+	+	+	−[e]	−	+	+
Fatty acids (%)							
iso-$C_{15:0}$	24.4	ND	25.9	19.4	21.7	20.7	20.8
$C_{16:0}$	11.4	ND	8.7	16.3	12.6	7.1	0.2

ND Not described, *w* weakly positive, + positive to the reaction, − negative to the reaction. [a,b,c,d,e,f] Described in [11, 27, 32–35], respectively; [g] present study

CMC-5 has been observed to degrade and utilize carrageenan (Table 2).

In Vitro Algal Thalli Decomposition by *Microbulbifer* Strain CMC-5

The present study intended to study the possible role of association of *Microbulbifer* strain CMC-5 with decomposing seaweeds. The degradation of *Gracilaria* thalli by strain CMC-5 was evident by influx of reducing sugars in the culture broth which were utilized by strain CMC-5 to promote its growth (Fig. 2). Control ASW medium with axenic seaweed thalli (without bacterial inoculum) and ASW medium (lacking seaweed thalli) with bacterial inoculum did not show any increase in OD at 600 nm nor any reducing sugars were detected in the culture broth up to 8 days. Thus release of reducing sugar and growth of strain CMC-5 in the presence of *Gracilaria* thalli suggest that strain CMC-5 decomposes seaweed thalli by hydrolysing the cell wall polysaccharides leading to production of reducing sugars and other nutrients.

The presence of heterogeneous polysaccharides with diversified structures in the cell wall of seaweeds offer unique niche for supporting growth of microbial community. *Pseudoalteromonas* sp. and *Halomonas* were isolated as dominant strains during degradation of brown algae *Fucus evanescens*. It was proposed that *Pseudoalteromonas*

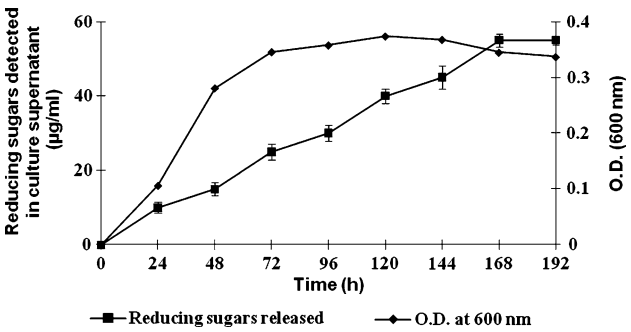

Fig. 2 Growth of *Microbulbifer* strain CMC-5 and reducing sugars released during in vitro decomposition of seaweed

species play a major role in initial stages of algal degradation as it produced various polysaccharases and proteinases whereas *Halomonas* which produce caesinase and DNase utilized the degradation products of polysaccharides [14]. Similarly *Gracilibacillus* strain A7 isolated from wakame seaweed compost has been reported to be widely used for disposal of seaweed waste and production of valuable products from seaweed wastes [29]. Further single cell detritus (SCD) have been prepared from *Laminaria saccharina* with the help of two bacterial strains having high cellobiosic, proteolytic and alginolytic activities and are being used as feed material for clam *Ruditapes decussates* [4]. Thus *Microbulbifer* strain CMC-5 seems to be a

promising strain having the potential to be exploited for degradation of algal cultural wastes as well as production of SCD.

The isolation of multiple polysaccharide degrading bacteria from decomposing seaweeds indicates a major role in carbon recycling of ICPs from seaweeds. Since *Microbulbifer* species have been isolated from sediments and intertidal region, bacteria such as strain CMC-5 could be opportunistic bacteria from sea which might inhabit or invade the nutrient-rich seaweed environment and degradation of polysaccharides from seaweed cell wall would result in leaking of nutrients on which bacteria such as strain CMC-5 would proliferate. The antibiotic profile of strain CMC-5 as reported in Table 1 indicates resistance to most commonly used antibiotics which can be a cause of concern especially in seaweed culture as the role of this bacteria as seaweed pathogen is yet unknown.

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