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Date of Birth: 10-Nov-1985

Education

2011-2018	Ph.D. (Biotechnology) Dept. of Biotechnology, Goa University, Goa Thesis title: Cloning and Characterization of Polysaccharide Degrading genes from selected Marine Bacteria Date of award of Ph.D.: 03-Aug-2018	
2009-2011	M.Sc. (Marine Biotechnology), Dept. of Biotechnology, Goa University	1st class (63.00%)
2006-2009	B.Sc. Zoology (Hons), Botany, Chemistry LN Mithila University, Darbhanga, Bihar	1st class (68.00 %)

Research Experience

Period	Position	Major Research Area/Techniques
Jan 2020 –Till Date	Postdoctoral fellow Department of Botany University of Delhi	Delivery of RNA-interference against tomato leaf curl virus, Nano-Biotechnology, Genomics
Jul 2019 –Dec 2019	Research Associate Bioinformatics Infrastructure Facility, ACBR, University of Delhi	Drug discovery, Machine learning, artificial intelligence, Software used: Weka, Paddler, Discovery studio etc. Database used: Binding database, Zinc database
Aug 2013 –Aug 2018	DBT-SRF Dept. of Biotechnology, Goa University, Goa	Microbial Biotechnology, Genome annotation, Study of industrially important enzymes from bacteria
Aug 2011 –Jul 2013	DBT-JRF Dept. of Biotechnology, Goa University, Goa	Genomic library preparation, Gene cloning and expression, Protein purification & characterization
Jul 2010 –May 2011	M.Sc. Dissertation Dept. of Biotechnology, Goa University, Goa	Development of a Bio-fertilizer, Isolation of Indole acetic acid producing bacteria from saltpan
May 2010 –Jun 2010	Summer Trainee Department of Botany University of Delhi	DNA isolation, PCR, Transformation, Gene cloning

Research Publications

Journal articles

1. **lmran Md.,** Pant P, Shanbhag Y, Sawant SV and Ghadi SC (2017) Genome sequence of *Microbulbifer mangrovi* DD-13 reveals its versatility to degrade multiple polysaccharides. Marine Biotechnology. 19(1): 116-124 **(IF=2.880)**

2. **Imran Md.,** Das KR and Naik MM (2018) Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat. Chemosphere**,** 215:846-857 **(IF=5.778)**

3. **Imran Md**., Saida B, Ghadi SC, Verma P and Shouche, YS (2016). The gut-associated *Klebsiella* sp. of the apple snail produces multiple polysaccharide degrading enzymes. Current science, 110(11):2170-2172 **(IF=0.725)**

4. Naik MM, **Imran Md**., Vaigankar DC, Mujawar SY, Malik, AD and Gaonkar, S. K. (2021). Genome Guided Bioprospecting of Extremely Halophilic Haloferax sp. AS1 for CAZymes, Bioremediation and Study Metabolic Versatility. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 1-9. **(IF=0.921)**

5. Jonnadula R, **Imran Md**., Vashist P, Ghadi SC (2020) Production of Agar-Derived Antioxidants and Single Cell Detritus from *Gracilaria corticata* Using Agarase from *Microbulbifer* sp. CMC-5. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 90(1):73-78. **(IF=0.921)**

6. Jonnadula R, **Imran Md**., Poduval PB and Ghadi SC (2018). Effect of polysaccharide admixture on the expression of multiple polysaccharide-degrading enzymes in *Microbulbifer* strain CMC-5. Biotechnology Reports. 17:93-96

Book Chapters

1. **Imran Md**., Poduval PB and Ghadi SC (2017). Bacterial degradation of algal polysaccharides in marine ecosystem. In: Naik MM, Dubey SK (eds) *Marine pollution and microbial remediation*. **Springer, Singapore**, pp 189–203

2. **Imran, Md** and Ghadi SC (2019). Role of Carbohydrate Active Enzymes (CAZymes) in production of marine bioactive oligosaccharides and their pharmacological applications. In: Trincone A (ed) *Enzymatic Technologies for Marine Polysaccharides*, **CRC Press (Tylor & Francis group),** pp. 357 – 374

3. **Imran, Md** and Ghadi SC (2019). Genome sequence analysis for bioprospecting of marine bacterial polysaccharide-degrading enzymes. In: Meena SN, Naik MM (eds) *Advances in biological science research*, Academic Press, **Elsevier,** pp. 21 – 34

Abstract Published (National conferences/Proceedings)

1. **Imran Md** and Ghadi SC, Preparation of a genomic library from multiple polysaccharides degrading *Pseudomonas* sp. and screening for agar degradation, National seminar on Life and life processes: Sustainable development, Dept. of Zoology, Goa University, Goa. Feb 19-21, 2015 (**Oral presentation**)

2. **Imran Md** and Ghadi SC "Carbohydrate active enzymes of *Microbulbifer mangrovi* DD-13: An insight from genome sequence, National conference of young researchers on new frontiers in life science and environment, Goa University, Goa. Mar 16-17, 2017 **(Poster presentation)**

3. **Imran Md**, Poduval PB and Ghadi SC" Cloning of Esterase gene from a bacterial strain CR-1" in the 55th Annual conference of Association of Microbiologists of India (AMI) on "Empowering Mankind with Microbial Technologies" at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, Nov. 12-14, 2014 **(Poster presentation)**

4. **Imran Md** and Ghadi SC "Cloning of agarase gene from a multiple polysaccharide degrading bacteria *Microbulbifer* strain CMC-5" in DBT-JRF regional meet at Institute of Chemical Technology (ICT), Matunga, Mumbai. Nov. 21-22, 2013 **(Poster presentation)**

Fellowship and academic achievements

2013-2016	Senior Research Fellowship from Department of Biotechnology, Govt. of India.
2011-2013	Junior Research Fellowship from Department of Biotechnology, Govt. of India.
2011	Qualified National level Biotechnology Entrance Exam (BET) for the award of DBT-JRF.
2011	Qualified Graduate Aptitude Test in Engineering (GATE-2011)
2009-2011	Studentship from Department of Biotechnology, Govt. of India during M.Sc. programme.
2009	Qualified national level Combined Biotechnology Entrance Examination (CBEE-2009) conducted by JNU New Delhi.

Participation in National Conferences/Workshop

1. National Symposium cum Workshop on "Recent Trends in Structural Bioinformatics and Computer aided Drug Design" at Alagappa University, Tamil Nadu, Feb 18-21, 2014

2. Workshop on "Fundamentals of LCMS with LCMS solution workstation" at Shimadzu Analytical (India) Pvt. Ltd. Mumbai, June 16, 2014

3. Workshop on "Intellectual Property and Innovation Management in Knowledge Economy, Jointly organised by NRDC, New Delhi and Goa University, Dec. 13, 2013

4. IVth Bharatiya Vigyan Sammelan & Expo 2015, Panji, Goa, Feb 6-8, 2015

5. Workshop entitled "Scientific writing and effective communication" Goa University, Goa, Jan 5-6, 2015

Place: University of Delhi
Date: 05-Jul-2021 Md. Imran

GOA UNIVERSITY

This is to certify that

Md. Imran

son of

Shri Md Haider Ali

and

Smt. Zareena Khatoon

from Department of Biotechnology

has been conferred the degree of

Doctor of Philosophy

in Biotechnology

having passed the qualifying examination held in August, 2018

Given under the seal of the University.

Declaration of Result : 3rd August, 2018
Taleigao Plateau - Goa

2017 - 11016 /200900019

Varun Sahni
Vice - Chancellor

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Date: 18 /12/2020

CERTIFICATE

This is to certify that Dr. Md. Imran has been awarded the Degree of Doctor of Philosophy in the subject of Biotechnology at the 31st Annual Convocation of the Goa University held on 15th December, 2018.

It is further certified that Dr. Md. Imran fulfills the conditions specified in the University Grant Commission (Minimum Standards and Procedure for Award of M.Phil/Ph.D. Degree) Regulations, 2009 and is eligible for exemption from the requirement of NET/SLET/SET for recruitment and appointment of Assistant Professor or equivalent positions in Universities /Colleges/Institutions.

This Certificate is issued at the request of Dr. Md. Imran in compliance of the UGC (Minimum Standards and Procedure for Awards of M.Phil/Ph.D. Degree) Regulations, 2009.

(Prof. Radhika Nayak)
Registrar

Mar Biotechnol
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ORIGINAL ARTICLE

Genome Sequence of *Microbulbifer mangrovi* DD-13^T Reveals Its Versatility to Degrade Multiple Polysaccharides

Md. Imran[1] · **Poonam Pant**[2] · **Yogini P. Shanbhag**[1] · **Samir V. Sawant**[2] · **Sanjeev C. Ghadi**[1]

Received: 14 September 2016 / Accepted: 12 January 2017

Abstract *Microbulbifer mangrovi* strain DD-13^T is a novel-type species isolated from the mangroves of Goa, India. The draft genome sequence of strain DD-13 comprised 4,528,106 bp with G+C content of 57.15%. Out of 3479 open reading frames, functions for 3488 protein coding sequences were predicted on the basis of similarity with the cluster of orthologous groups. In addition to protein coding sequences, 34 tRNA genes and 3 rRNA genes were detected. Analysis of nucleotide sequence of predicted gene using a Carbohydrate-Active Enzymes (CAZymes) Analysis Toolkit indicates that strain DD-13 encodes a large set of CAZymes including 255 glycoside hydrolases, 76 carbohydrate esterases, 17 polysaccharide lyases, and 113 carbohydrate-binding modules (CBMs). Many genes from strain DD-13 were annotated as carbohydrases specific for degradation of agar, alginate, carrageenan, chitin, xylan, pullulan, cellulose, starch, β-glucan, pectin, etc. Some of polysaccharide-degrading genes were highly modular and were appended at least with one CBM indicating the versatility of strain DD-13 to degrade complex polysaccharides. The cell growth of strain DD-13 was validated using pure polysaccharides such as agarose or alginate as carbon source as well as by using red and brown seaweed powder as substrate. The homologous carbohydrase produced by strain DD-13 during growth degraded the polysaccharide, ensuring the production of metabolizable reducing sugars. Additionally, several other polysaccharides such as carrageenan, xylan, pullulan, pectin, starch, and carboxymethyl cellulose were also corroborated as growth substrate for strain DD-13 and were associated with concomitant production of homologous carbohydrase.

Keywords Carbohydrate-binding module · Glycoside hydrolases · *Microbulbifer mangrovi* · Alginate lyase · Agarase

Accession No. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBankunder the accession LZDE00000000. The version described in this paper is version LZDE01000000.

Electronic supplementary material The online version of this article (doi:10.1007/s10126-017-9737-9) contains supplementary material, which is available to authorized users.

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Introduction

The cell wall of plants and seaweeds is a conglomerate of recalcitrant complex polysaccharides (CPs) fortifying against microbial intrusion and harsh environment. The eventual decay of plants and seaweeds culminates into CP-enriched litter and marine snow that promotes epiphytic association of carbohydrase-degrading bacteria. The recycling of carbon from CPs in the marine ecosystem is widely attributed to versatility of multiple polysaccharide-degrading bacteria such as *Saccharophagus degradans* and *Microbulbifer* species (Hutcheson et al. 2011; Jonnadula et al. 2009; Wakabayashi et al. 2012; Vashist et al. 2013). Besides their innate role in degradation of CP from litter, the multiple polysaccharide-degrading bacteria have already been exploited for degradation of algal wastes and are being acclaimed as a prospective option for generating reducing sugars from complex polysaccharides for biofuel production (Kang and Kim 2015; Kim et al. 2013; Wargacki et al. 2012).

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Review

Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat

Md. Imran [a, *, 1], Kirti Ranjan Das [a, 1], Milind Mohan Naik [b, **, 1]

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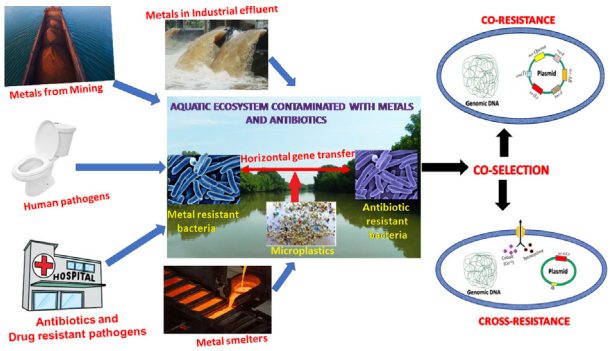
HIGHLIGHTS

- Bacterial human pathogens co-selects multi-antibiotic resistance in metal polluted environment.
- Microplastics act as a hotspot for co-selection of metal-driven multi-antibiotic resistant human pathogens.
- Microplastics are potential vector for spread of human pathogens in marine environment.

GRAPHICAL ABSTRACT



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ABSTRACT

Misuse/over use of antibiotics increases the threats to human health since this is a main reason behind evolution of antibiotic resistant bacterial pathogens. However, metals such as mercury, lead, zinc, copper and cadmium are accumulating to critical concentration in the environment and triggering co-selection of antibiotic resistance in bacteria. The co-selection of metal driven antibiotic resistance in bacteria is achieved through co-resistance or cross resistance. Metal driven antibiotic resistant determinants evolved in bacteria and present on same mobile genetic elements are horizontally transferred to distantly related bacterial human pathogens. Additionally, in marine environment persistent pollutants like microplastics is recognized as a vector for the proliferation of metal/antibiotics and human pathogens. Recently published research confirmed that horizontal gene transfer between phylogenetically distinct microbes present on microplastics is much faster than free living microbes. Therefore, microplastics act as an emerging hotspot for metal driven co-selection of multidrug resistant human pathogens and pose serious threat to humans which do recreational activities in marine environment and ingest marine derived foods. Therefore, marine environment co-polluted with metal, antibiotics, human pathogens and microplastics pose an emerging health threat globally.

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0045-6535/

The gut-associated *Klebsiella* sp. of the apple snail produces multiple polysaccharide degrading enzymes

Md. Imran[1], Bhukya Saida[2], Sanjeev C. Ghadi[1,*], Pankaj Verma[3] and Yogesh S. Shouche[3]

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***Pila globosa*, an edible variety of apple snail is a common inhabitant of lentic ecosystem and feeds on plant detritus. The tissue extract of gastrointestinal tract from *Pila globosa* demonstrated the presence of carboxymethyl cellulase, xylanase, alginate lyase and pectinase activity. Culture-dependent method was used to isolate carboxymethyl cellulose (CMC) degrading bacteria from the gastrointestinal tract of apple snail. Morphologically identical colonies were obtained on M9 gelrite plates containing CMC as carbon source. One such bacterial isolate was purified by streaking and designated as strain PG-1. Bacterial strain PG-1 degraded CMC, pectin, starch and alginate. The 16S rDNA sequence of strain PG1 was 99% identical to *Klebsiella oxytoca*. Phylogenetic analysis using maximum likelihood revealed the clustering of strain PG1 with the clade belonging to *Klebsiella oxytoca* type strain.**

Keywords: Apple snail, *Klebsiella*, *Pila globosa*, polysaccharide degrading bacteria.

PILA GLOBOSA, commonly referred to as apple snail, inhabit lentic ecosystem and is widely distributed in Asia. Besides being a human delicacy, apple snail is used to feed shrimps and fishes[1]. The apple snail soup is also used in traditional medicine for bringing relief against asthma, tuberculosis and stomach disorders[2]. Snails feed on algal or plant detritus and reportedly degrade cellulose, laminaran, and mannan[3,4]. The capability of snails to degrade polysaccharide is linked to presence of cellulase, amylase, alginate lyase and mannanase in the digestive fluids of marine gastropods[5–10]. All molluscs persistently ingest bacteria from soil, sediments and water resulting in unique microbiota in the gut region[11]. Kiran *et al.*[12] studied bacterial diversity in different regions of gastrointestinal tract (GI) of giant african snail (*Achatina fulica*) and reported the presence of various bacterial genera including *Citrobacter*, *Kluyvera*, *Acinetobacter*, *Escherichia*, *Shigella, Salmonella*, *Acidovorax*, *Staphylococcus*, *Bacillus*, *Enterococcus*, *Lactococcus*, *Kurthia* and *Exiguobacterium* in the whole gut region. The microbiota in GI are believed to play an important role in degrading plant detritus by providing the bacterial host with a battery of polysaccharide hydrolysing enzymes (carbohydrases)[12].

In the present study, we report the isolation and identification of autochthonous multiple polysaccharide degrading *Klebisiella* sp. from the GI of *P. globosa* using culture dependent method. Furthermore, the endogenous carbohydrase activities from the extract of GI were compared with carbohydrase produced by *Klebsiella* sp.

P. globosa was collected from the paddy fields of Goa, India ($n = 10$). After washing with tap water, the snails were surface-sterilized by dipping in 70% ethanol for 8 minutes. The snails were air-dried in laminar flow hood and rinsed thrice with sterile distilled water. After removal of outer shell, GI was dissected aseptically, cut into smaller pieces and homogenized in 20 mM *Tris* Cl (pH 7.0) at 4°C using Potter Elvehjem. The homogenate was centrifuged at 17,000 *g*, 4°C for 15 min. The supernatant was saturated with 80% ammonium sulphate and the protein precipitate was collected by centrifugation. The protein pellet was resuspended in 20 mM *Tris* Cl (pH 7) and later dialysed against the same buffer for 24 h. The partially purified enzyme extract was stored at –20°C and immediately used to determine CMCase, amylase, alginate lyase, pectinase and xylanase activities by measuring the amount of reducing sugar by DNSA method[13]. Alternatively, the pieces of gastrointestinal tract were aseptically added to the sterile M9 medium containing 0.2% of CMC and grown on the orbital shaker for 72 h at 30°C ($n = 3$). The broth was serially diluted and plated on M9 medium containing 0.75% gelrite and 0.2% CMC. The plates were incubated at 30°C for 24 h to check for bacterial growth. To evaluate the degradation of CMC, pectin, starch, alginate and xylan as a carbon source, strain PG-1 was inoculated in M9 medium broth supplemented with 0.2% of individual polysaccharides. After incubation for 48 h, the culture was centrifuged and CMCase, pectinase, α-amylase, alginate lyase and xylanase activities in the culture supernatant were determined by DNSA method[13]. CMC and pectin were resuspended in 0.1 M citrate buffer (pH 5) whereas other polysaccharides were resuspended in 20 mM *Tris* Cl (pH 7). The CMCase activity was determined at 45°C whereas other carbohydrase were assayed at 30°C. Glucose was used as reference sugar. One unit is defined as micromole of reducing sugar released per minute at respective temperature.

Genomic DNA from bacterial strain PG-1 was isolated by Maloy method[14]. 16S rDNA sequence was amplified using the two universal primer 27F and 1525R. The amplified PCR product was purified using QIAquick PCR purification kit (Qiagen Inc, Hilden, Germany). Sequencing reaction was carried out in a 96 well PCR plate using Big Dye terminator cycle sequencing kit using ABI-PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). Homologous sequences similar to that of strain PG-1 were retrieved from Ribosomal

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RESEARCH ARTICLE

Genome Guided Bioprospecting of Extremely Halophilic *Haloferax* sp. AS1 for CAZymes, Bioremediation and Study Metabolic Versatility

Milind Mohan Naik[1] · Md. Imran[2] · Diviya C. Vaigankar[1] · Sajiya Yusuf Mujawar[1] · Alisha D. Malik[1] · Sanket K. Gaonkar[1]

Received: 21 April 2020 / Revised: 30 October 2020 / Accepted: 6 January 2021

Abstract An extremely halophilic archaeon was isolated from brine sample collected from a salt pan and designated as AS1. The strain AS1 formed a coherent cluster with the species of genus *Haloferax* and shared the clade of *Haloferax alexandrinus* in a neighbour joining phylogenetic tree. The draft genome of strain AS1 comprised of 3,778,087 bp with a G + C content of 66.10%. A total 3,797 (100%) ORFs were predicted in the genome of strain AS1 and 3,740 (98.5%) ORFs were annotated as protein coding genes. Using BLAST2GO programme, gene ontology terms were assigned to the 2305 (60.75%) predicted genes from strain AS1. Further, a total of 1,404 (36.97%) genes from strain AS1, were annotated in 24 KEGG categories using KEGG automated annotation server (KAAS). Our analysis indicates that the genome of strain AS1 harboured 58 genes specific for carbohydrate-active enzymes (CAZymes). Additionally, strain AS1 contain genes involves in degradation of sodium benzoate, chloroalkane and selenite reduction. Furthermore, genome of strain AS1 harboured a set of genes that encode enzymes required for the operation of reductive acetyl CoA pathway and also grew under anaerobic condition when supplied with CO_2 as a sole source of carbon in minimal media. Genome guided bioprospecting is an efficient and holistic approach to study extremozymes produced, bioremediation potential and metabolic versatility of haloarchaea in extreme hypersaline environment.

Keywords Archaea · Extremely halophilic · Cazymes · Biodegradation · Reductive acetyl CoA pathway · Bioprospecting

Significance Statement Genome Guided bioprospecting is an efficient and holistic approach for bioprospecting of extremozymes, bioremediation potential in hypersaline environment and study metabolic versatility of haloarchaea *Haloferax* sp. strain AS1.

Milind Mohan Naik and Md. Imran contributed equally.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40011-021-01232-3.

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Introduction

Salt pans are important man-made hypersaline ecosystems formed as a result of evaporation of sea/estuarine water along the coastlines and are often situated in estuarine environment [1]. Salt pans are inhabited by salt loving extremophiles which belong to three domains of life-archaea, bacteria and fungi [1–3]. Among extremophiles, haloarchaea of the class *Halobacteria* and domain Archaea have unique adaptation to survive at high NaCl (1.8–5.5 M NaCl) and low a_w with currently 52 genera been reported from various hypersaline environments [3]. Under class *Halobacteria* include genera *Halobacterium*, *Haloferax, Halococcus, Haloarcula, Halorubrum, Halosarcina, Haloquadratum, Natrinema, Natrobacterium* [4]. Salt-loving archaea are also isolated from extreme hypersaline environments such as Great Salt Lake of Utah, Dead sea and Sambar lake [2].

Microbial bioprospecting mainly involves discovery and commercialization of novel products such as enzymes,

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RESEARCH ARTICLE

Production of Agar-Derived Antioxidants and Single Cell Detritus from *Gracilaria corticata* Using Agarase from *Microbulbifer* sp. CMC-5

RaviChand Jonnadula[1] · Md. Imran[1] · Poonam Vashist[1] · Sanjeev C. Ghadi[1]

Received: 28 September 2018 / Revised: 26 November 2018 / Accepted: 7 February 2019

Abstract *Microbulbifer* strain CMC-5 reportedly degrades multiple polysaccharides including agar. In present study, the agarase from the culture supernatant of *Microbulbifer* strain CMC-5 pregrown in artificial sea water-based medium containing agar was tenfold concentrated by ultrafiltration. The bacteriological agar-derived oligosaccharides obtained by agarase treatment were observed to depict reducing power, lipid peroxidation inhibition and hydroxyl radical scavenging activities. The oligosaccharides produced from agar by agarase treatment for 6 h demonstrated higher antioxidative activities as compared to agar oligosaccharides produced by 12-h agarase treatment. Further, the agar oligosaccharides demonstrated concentration-dependent augmentation of antioxidative activities. Since agar is a recognized safe food additive, the agar-derived oligosaccharides can be blended with food to augment its antioxidative property. Additionally, the agarase from strain CMC-5 in conjunction with Onozuka R-10 degraded *Gracilaria corticata* thalli to produce single cell detritus that can be used as aquaculture feed material reducing the dependency on artificial feeds.

Keywords Agarase · Agar oligosaccharides · Hydroxyl radical scavenging activity · Lipid peroxidation *Microbulbifer* · Reducing power

Significance Statement In the present study, enzymatically produced agar oligosaccharides depict broad range antioxidative properties that could be used as safe food additives. Additionally, agarase produces SCD from *Gracilaria* thalli that may be used as feed in aquaculture.

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Introduction

Bacterial agarases are routinely used to recover high molecular weight DNA from agarose gel and for isolation of labile bioactive compounds from seaweeds. Additionally, agar-derived oligosaccharides are reported to inhibit bacterial growth, decrease rate of starch degradation and improve the quality of food when used as additives [1]. Bacteria belonging to genus *Microbulbifer* degrade multiple polysaccharides and are a potential source of polysaccharide-degrading enzymes with unique specificity that can be exploited to produce novel oligosaccharides [2–4]. *Microbulbifer* strain CMC-5 is one such bacterial strain that was isolated from the decomposing seaweeds and degrades agar, alginate, carrageenan, xylan, carboxymethyl cellulose and chitin [5].

Excessive reactive oxygen species (ROS) formed during oxidative stress instigates progressive cell damage leading to stroke, cancer, neurodegenerative diseases, etc. Besides the role played by cellular enzymes in combating ROS, the natural antioxidants present in fruits and vegetables promote protection against ROS. Due to possible carcinogenic nature of synthetic antioxidants, their use as a food additive is restricted. Agar, obtained from red seaweeds, is an acceptable natural safe food additive and is widely used in jelly, sweets, processed cheese, pudding, ice cream, etc. Agar is commonly used as thickener, emulsifying agent or as a stabilizer and has no calorific value. Besides its role as a mild laxative agent, no medicinal property has been attributed to agar. In the recent past, the antioxidant properties observed in red and brown seaweed extracts have been primarily attributed to the presence of carotenoids and sulfated polysaccharides [6, 7]. Additionally, agar oligosaccharides prepared by acid hydrolysis of agar

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Short Communication

Effect of polysaccharide admixtures on expression of multiple polysaccharide-degrading enzymes in *Microbulbifer* strain CMC-5

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ARTICLE INFO

Keywords:
Polysaccharide degrading enzymes
Single cell detritus

ABSTRACT

Microbulbifer strain CMC-5 produces agarase, alginate lyase, xylanase, carboxymethyl cellulase and carrageenase. The extracellular production of the above carbohydrases was investigated by growing *Microbulbifer* strain CMC-5 in a sea water based medium containing homologous/heterologous polysaccharides as a single substrate or as a combination of mixed assorted substrate. Presence of singular homologous polysaccharides in the growth medium induces respective carbohydrase at high levels. Any two polysaccharides in various combinations produced high level of homologous carbohydrase and low level of other heterologous carbohydrase. All five carbohydrases were consistently produced by strain CMC-5, when carboxymethyl cellulose was included as one of the substrate in dual substrate combination, or in presence of mix blends of all five polysaccharides. Interestingly, thalli of *Gracilaria* sp. that contain agar and cellulose predominantly in their cell wall induces only agarase expression in strain CMC-5.

1. Introduction

Insoluble complex polysaccharides (ICPs) such as agar, alginate, carrageenan, xylan and chitin are primarily responsible for maintaining structural integrity in marine organisms. Occasionally, ICPs are combined in heterogeneous proportions to generate a complex recalcitrant polysaccharide framework that is difficult to degrade. Likewise, the cell wall of *Gracilaria* sp. consist of an agarose matrix embedded in a mesh of cellulose network [1,2]. Multiple polysaccharide degrading marine bacteria from decomposing sea grasses and seaweeds have been isolated and participate in recycling of carbon from ICPs [3–6].

In marine ecosystems, the ICPs degrading bacteria would be concurrently exposed to multiple polysaccharides. Although, production of homologous polysaccharide degrading enzymes in presence of respective individual polysaccharides such as cellulose, xylan, agar, alginate and carrageenan have been studied, [7–11] the effect of these individual or mixed polysaccharides on expression of homologous and other heterologous polysaccharide degrading enzymes (carbohydrases) have not been extensively studied. The only other reported studies demonstrating expression of heterologous carbohydrases in presence of individual polysaccharides was from *Saccharophagus degradans* 2–40, a multiple polysaccharide degrader [12].

Microbulbifer strain CMC-5 isolated previously from decomposing seaweeds and degrading multiple polysaccharides was used in the present study [6]. The objective was to determine the expression of different carbohydrases when cellulose or agar or carrageenan or xylan or alginate was provided as single or as a combination of mixed assorted substrates. Additionally, expression of carbohydrases in strain CMC-5 was also studied using an ecological simulation by providing seaweed thalli (*Gracilaria* sp.) as a biomass whose cell wall naturally consists of agar blended with cellulose.

2. Material and methods

2.1. Growth condition

Starter culture of *Microbulbifer* strain CMC-5 (MTCC 9889) was prepared by growing in artificial sea water (ASW) medium [13] containing 0.2% of single or dual polysaccharides or polysaccharides mix containing all the five polysaccharides as carbon substrate and supplemented with 0.05% yeast extract. The polysaccharides used in present study were low melting point (LMP) agarose, CMC (Na- salt), alginate (sodium salt, polyguluronic and polymannuronic acid mixture), carrageenan (mixture from Irish moss) and xylan (from oat spelts). The culture was incubated at 30 °C on orbital shaker at 130 rpm for 24 h. 0.1% of the starter culture was aseptically transferred to a freshly prepared ASW medium containing single or dual or mix polysaccharides and supplemented with yeast extract under conditions mentioned above. After 48 h, the culture supernatant was obtained by centrifugation at 6360 × *g* for 15 min at 4 °C and immediately used to

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