



CHARACTERIZATION OF HETEROTROPHIC BACTERIA FROM THE SEDIMENT OF MARINE CAGE FARM OF POLEM, GOA

Mamatha. H. Hazari and Jagannath L. Rathod*

Department of Post Graduate Studies and Research in Marine Biology, Karnatak University's P.G. centre, Kodibag, Karwar 581303, Karnataka, India

*Corresponding Author Email: jagannathrathod9@gmail.com

ABSTRACT

Present study aimed at characterising the sediment bacterial population from the cage farm of Polem, Goa with reference to phenotypic and biochemical characterization. Total of 9 isolates were selected for the present study based on the colony morphology and were identified by conventional biochemical and molecular techniques as *Bacillus firmus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus aryabhatai*, *Acinetobacter* sps, *Vibrio harveyi*, *Vibrio algenolyticus*, *Ferrimonas balearica* and *Oceanomonas smirnovii*. The study suggests that the sediment bacteria from the cage farm can be used as biological indicator of farming practices.

KEY WORDS

Cage farm, Bacteria, Sediment, Polem

INTRODUCTION

Marine ecosystem is highly dynamic, providing a high diversity of ecological niches. Biodegradation of organic matter and energy transfer in marine sediment is mainly influenced by the presence of heterotrophic bacteria [1] and the structure of microbial assemblage is sensitive to changes in environmental conditions and trophic state [2]. Further, it is thought that marine sediment harbours around 55–85% of all microbial life [3]. A large number of studies are conducted on the role of heterotrophic bacteria in releasing the organic substrate and inorganic nutrients into the higher trophic levels [4,5].

Marine finfish culture is rapidly growing food sector in the Asia Pacific. Previous studies shown that disturbance in coastal areas due to cage farming practices might determine changes in the community structure and activity of benthic bacteria [4]. Knowledge on the microbial community composition may give important information about the health of the ecosystem, since bacteria responds quickly to the abiotic changes in the environment [5]. Measurement

of microbial assemblages in the sediment has been recently proposed as tools for monitoring the impact of intensive aquaculture activities [4]. Although bacterial communities have been frequently studied, recent studies show that lots of bacterial communities need to be still characterized in marine environment [6,7,8]. Further, the information on the total culturable heterotrophic bacterial population from the cage farms in India is insufficient. The aim of this work was to isolate and identify the bacterial population from sediment of cage farm by conventional methods and 16s rDNA sequencing.

MATERIALS AND METHODS

Study area

Present study was carried out in marine fish farm located in Southern part of Goa, Central west coast of India (Polem village 14° 54' 21.12" N ;74° 04' 32.20" E). The cage farm consisting of 25 no's of floating circular GI cages of 6m diameter with Asian seabass, pompano and cobia cultured in it.

Isolation of bacteria and biochemical characterization

Sediment samples were collected from the bottom of the cage farm during September 2014 to May 2016. Sediment samples were collected in sterile polythene bags by using Van veer grab sampler (0.042m²) and transported to the laboratory in an ice cold condition. Microbiological analysis of the culturable heterotrophic bacterial fraction was carried out by pour plate method. For qualitative estimation of bacteria, distinct colonies were selected from each plate and isolated in pure culture on Marine Agar (Himedia, India) and incubated at 30°C ± 0.2°C for 24 to 48 hrs. For taxonomic identification, purified bacterial samples were subjected to morphological and biochemical characterization such as Gram stain, catalase test (3% H₂O₂), oxidase test, indole [9], oxidative/fermentative acid production from glucose, citrate utilization, nitrate reduction, Methyl red (MR)-Voges Proskauer (VP), degradation of urea, followed by growth on TCBS (thiosulphate citrate bile salts sucrose), growth at different salt (NaCl) concentrations and different temperature, were performed according to the standard methods [10].

Molecular characterization

For molecular identification, pure cultures of the phenotypic isolate in log phase in Luria-Bertani (LB) broth were used for pure genomic DNA extraction using bacterial genomic DNA extraction kit (Sigma, USA). For identification of bacterial isolates (1-7) 16S rRNA gene was amplified in a thermal cycler (Eppendorf, Germany) using the combination of universal primers 27F (5' AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' GGTTACCTGTTACGACTT 3'), while specific primers were used for confirming the *Vibrio harveyi* (VAHF1) 5'-ATCATGAATAAAAC TATTACGTTACT-3' and (VAHR1) 5'-AAAGGATGGTTTGACAAT-3' following Fukui and Sawabe [11] and *Vibrio algenolyticus* VA-F (5' -CGAGTACAGTCACTTGAAAGCC- 3') and VA-R (5' -CACAACAGAACTCGGTTACC- 3') following Abdel-Aziz et al. [12]. The amplified PCR products were confirmed by running the amplification product in 1% agarose gel in 1X TAE. The amplified products (1500 bp) were purified individually using the nucleic acid purification kit (Axygen Biosciences, USA) and sanger sequenced. Bacterial identity was deduced by searching Genbank using BLAST algorithm. The partial 16S rDNA sequences derived in this study have been deposited in GenBank.

RESULTS AND DISCUSSION

Marine substrates generally provide a nutrient-rich environment where bacteria grow in complex interactive biofilm communities [13]. Total of 9 isolates were selected for present study for biochemical and molecular characterization. These included *Bacillus firmus*, *Bacillus cereus* (MG782844), *Bacillus subtilis* (MG782866), *Oceanomonas smirnovii* (MG800693), *Ferrimonas balearica* (KP406159), *Acinetobacter sps* (KP718120), *Bacillus aryabhatai* (KP718121), *Vibrio harveyi* and *Vibrio algenolyticus*. Phenotypic characterization of bacterial isolates showed creamy yellow, dull white, pale yellow colour with most of the isolates having round to irregular shaped colony (Table 1). All the isolates except *Acinetobacter sp* were motile. Gram-positive bacteria comprise a relatively large proportion in the marine sediments and are likely to play important microbiological roles in the marine environment mainly the biogeochemical processes. In the present study, the selected isolates comprised of 40% gram positive and 60% were gram negative. Among the gram positive group, the genus *Bacillus* is one of the well-known genera and members of these genera have strong adaptability to diverse conditions and several species belonging to the genus are capable of producing highly resistant spores [14]. During the present study, 4 isolates were found to be gram positive which includes *Bacillus cereus*, *Bacillus subtilis*, *Bacillus aryabhatai* and *Bacillus firmus*. Miranda et al. [15] reported that, *Bacillus cereus* and *Bacillus subtilis* were the most frequently (78%) occurring strains from marine sediments. *B. cereus* causes two distinct types of food poisoning i.e. diarrhoeal and emetic (vomiting), causing two types of illness [16] and also fatal meningitis [17]. This organism is also responsible for spoilage of different food products especially of plant origin, but also isolated from the meat, egg and dairy products [18]. Isolates of *Bacillus subtilis* from the marine sediments has many industrial application. *B. subtilis* were used to increase the rotifer production and control bacterial growth in the cultures [14]. Study also shown that *B. subtilis* can be used in laboratory based bioremediation experiments along with other species of *Bacillus* viz *B. megaterium* and *B. polymyxa* [19]. Ray et al. [20] isolated *Bacillus aryabhatai* the plant growth promoting bacteria from the wetlands of east Kolkata and reported that the isolate can survive in extreme condition. Shvajji et al. [21] initially isolated this

bacterium from cryotubes used to collect air samples from the Earth's stratosphere at an altitude between 27 and 41 km. Lee et al. [22]; Pailan et al. [23] and Yan et al. [24] isolated this bacterium from rhizosphere soil from South Korea, India, and Tibet. During the present study this bacterium was isolated from the sediment collected from marine cage farm but the source of this bacterium in sediment is unclear. *Bacillus firmus*, lead resistant bacteria used for its ability in biosorption of lead was isolated from the cage farm sediments. Alireza et al. [25] isolated to *B. firmus* from the surface sediment of Imam Khomeini port.

Acinetobacter is a gram-negative, coccobacilli, nonspore-forming, and non-motile bacteria with milky white colored colonies. Members of the genus *Acinetobacter* are ubiquitous in nature and can be isolated from soil, water samples and sewage with appropriate enrichment techniques [26]. During present study this bacterium was isolated from the marine cage farm sediment and presence of *Acinetobacter* in the sediment indicates the competitive degradation of organic matter [27].

Among the gram negative bacteria, vibrios are the most commonly occurring group in the marine environment either in the free form in the water and sediment or associated with the diverse marine animals. *Vibrio alginolyticus* and *Vibrio harveyii* are most frequently isolated from the coastal sediments and water and are considered as opportunistic pathogens for aquatic animals such as fishes, shellfishes and crustaceans causing serious economic damage in the culture by causing the disease [28]. During the present study, *V. alginolyticus* and *V. harveyii* were isolated from marine farm sediment from Polem. Simillar reports were made by Liu et al. [29] in the sea cage farm of Xiangshan Bay, China. *Ferrimonas balearica* a new facultative iron reducing bacteria isolated from the sediment during the present study. Similar reports on isolation of this bacteria were made by Rossello' -Mora et al. [30] from marine sediment at the Mediterranean coast of Mallorca (Spain). The genus *Oceanimonas* are the group of marine Proteobacteria, which are motile- gram negative rods comprising of three known species *O. baumannii*, *O. doudoroffii* and *O. smirnovii*. Among this *O. smirnovii*. is a novel bacterium isolated from the marine sediments [31].

Table 1: Biochemical characteristics of the bacterial isolates

	<i>Bacillus firmus</i>	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Bacillus aryabhatai</i>	<i>Acinetobacter sps</i>	<i>Ferrimonas balearica</i>	<i>Oceanomonas smirnovii</i>	<i>Vibrio harveyi</i>	<i>Vibrio algenolyticus</i>
Morphology	Creamy yellow	Opaque	Dull white	Creamy	Pale yellow	Pale yellow	Light creamy	Pale yellow	White
Shape	Round	Irregular	Irregular	undulated	Smooth	Round	round	Round	Round
Gram Stain	Rods +ve	Rods +ve	Rods +ve	Rods +ve	-ve Bacilli	Rods -ve	Rods -ve	Rods -ve	-ve
Motility	+	+	+	+	-	+	+	+	+
Spore formation	+	+	+	+	-	-	-	-	-
Indole	-	-	+	-	-	-	+	+	+
MR	-	-	-	-	-	-	-	+	+
VP	-	+	+	+	-	-	-	-	-
Citrate	-	+	+	-	+	-	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	+	-	+	+	-	+	+	+	+
H₂S production	-	-	-	-	ND	+	-	-	-
Urease	-	-	-	+	-	+	-	ND	-
Nitrate	+	+	+	+	-	+	+	+	+
Glucose	+	+	+	+	+	-	+	-	+
Sucrose	+	+	+	-	-	-	-	-	+
Gas production	-	-	-	-	-	-	-	-	-
Lactose	-	-	+	+	+	ND	-	-	-
Starch	+	+	+	+	-	-	-	+	+
TCBS	-	-	-	-	-	Green	ND	Green	Yellow
Growth at									
5^o C	-	+	-	-	-	-	-	-	-
10^o C	-	+	+	+	+	+	+	-	-
40^o C	+	+	+	+	+	+	+	-	-
50^o C	-	-	+	-	-	-	-	-	-
NaCl growth (%)	2-6%	2-6%	2-8%	2-6%	0-6%	0-8%	0-8%	2-8%	2-6%
Gen Bank Acce. No	NS	MG782844	MG782866	KP718121	KP718120	KP406159	MG800693	NS	NS

VP, Voges Proscauer; MR, Methyl red; ND, not done; (+), growth; (-), no growth; NS (not submitted)

CONCLUSION

Identification of microbial fauna from the sea sediment is one the difficult area of biodiversity research which helps in understanding the role of these organisms in community assembly and ecological processes. The heterotrophic bacterial diversity in marine cage sediments is little known, which can have considered as bio indicator for assessing the health of farm environment.

ACKNOWLEDGEMENTS

Authors are thankful to Director ICAR-Central Marine Fisheries Research Institute (CMFRI), Kochi, India and Scientist-in-Charge, Karwar Research Centre of CMFRI for providing facilities to carry out this work. Thanks are due to the staff members of Fisheries Department, Directorate of fisheries, Goa.

REFERENCES

- Deming J.W. Baross J.A. The early diagenesis of organic matter: bacterial activity. In: Engel M.H. and Macko S. (eds), *Topics in Geobiology*. Plenum Press, New York, 119-144, (1993).
- Danovaro R. Benthic microbial loop and meiofaunal response to oil induced disturbance in coastal sediments: A review. *Inter J Environ Poll.* 13(1-6), DOI: 10.1504/IJEP.2000.002327, (2000).
- Whitman W.B, Coleman D.C. Wiebe W.J. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America.* 95: 6578-6583, (1998).
- La Rosa T, Mirto S, Mazzola A, Danovaro R. Differential responses of benthic microbes and meiofauna to fish-farm disturbance in coastal sediments. *Environ Pol.* 112: 427-434, (2001)
- Stabili L, Cavallo R.A. Biodiversity of culturable heterotrophic bacteria in the southern Adriatic sea, Italian coastal waters. *Sci Mar.* 68 (1): 31-41, (2014).
- Gontang E.A, Fenical W, Jensen P.R. Phylogenetic diversity of Gram-positive bacteria cultured from marine sediments. *Appl Environ Microbiol.* 73:3272-82, (2007).
- Wilson G.S, Raftos D.A, Corrigan S.L, Nair S.V. Diversity and antimicrobial activities of surface-attached marine bacteria from Sydney Harbour, Australia. *Microbiol Res.* DOI 200910.1016/j.micres.2009.05.007, (2009).
- Nithya C, Pandian S.K. Isolation of heterotrophic bacteria from Palk Bay sediments showing heavy metal tolerance and antibiotic production. *Microbiol Res.* 165(7): 578-593, (2010).
- Kovacs N. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nat Lon.* (178): 703, (1956).
- Hugh R, Leifson E. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J Bacteriol.* 66: 24-26 (1953).
- Fukui Y, Sawabe T. Improved one-step colony PCR detection of *Vibrio harveyi*. *Microbes Environ.* 22: 1-10, (2007).
- Abdel-Aziz M, Eldin Eissa A, Hanna M, Abou Okada M. Identifying some pathogenic *Vibrio/Photobacterium* species during mass mortalities of cultured Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) from some Egyptian coastal provinces. *Inter J Vet Sci Med.* 1: 87-95 (2013).
- Egan S, Thomas T, Kjelleberg S. Unlocking the diversity and biotechnological potential of marine surface associated microbial communities. *Curr Opin Microbiol.* 11: 219-25, (2008).
- Murillo I, Villamil L. *Bacillus cereus* and *Bacillus subtilis* used as probiotics in rotifer (*Brachionus plicatilis*) cultures. *J Aquacult Res Develop.* DOI: 10.4172/2155-9546.S1-007, (2011).
- Miranda C.A, Martins O.B, Clementino M.M. Species-level identification of *Bacillus* strains isolates from marine sediments by conventional biochemical, 16S rRNA gene sequencing and inter-tRNA gene sequence lengths analysis. *Antonie van Leeuwenhoek*, 93: 297-304, (2008).
- Granum P.E, Lund T. *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiol Lett.* 157:223-228, (1997).
- Evreux F, Delaporte B, Leret N, Buffet-Janvresse C, Morel A. A case of fatal neonatal *Bacillus cereus* meningitis. *Arch Pediatr.* 14: 365-368, (2007).
- Kramer J.M, Gilbert R.J. *Bacillus cereus* and other *Bacillus* species. In: Doyle MP, editor. Foodborne bacterial pathogens. New York: Marcel Dekker. 21-77, (1989).
- Tariq A.L, Sudha S, Reyaz A.L. Isolation and screening of *Bacillus* species from sediments and application in bioremediation. *Int J Curr Microbiol and Appl Sci.* 5(6): 916-924, (2016).
- Ray S, Datta R, Bhadra P, Chaudhuri B, Mitra A.K. From space to earth: *Bacillus aryabhatai* found in the Indian sub-continent. *Biosci Discov.* 3(1):138-145 (2012).
- Shivaji S, Chaturvedi P, Begum Z, Pindi P.K, Manorama R, Padmanaban D.A, Shouche Y.S, Pawar S, Vaishampayan P, Dutt C.B, Datta G.N, Manchanda R.K, Rao U.R, Bhargava P.M, Narlikar J.V. *Janibacter hoylei* sp nov., *Bacillus isronensis* sp nov and *Bacillus aryabhatai* sp nov., isolated from cryotubes used for

- collecting air from the upper atmosphere. *Int J Syst Evol Microbiol*. 59:2977-2986 (2009).
22. Lee S, Ka J.O, Song H.G. Growth Promotion of *Xanthium italicum* by Application of Rhizobacterial isolates of *Bacillus aryabhatai* in Microcosm Soil. *J Microbiol* . 50(1):45-49, (2012).
 23. Pailan S, Gupta D, Apte S, Krishnamurthi S, Saha P. Degradation of organophosphate insecticide by a novel *Bacillus aryabhatai* strain SanPS1, isolated from soil of agricultural field in Burdwan, West Bengal, India. *Int Biodeterior Biodegradation*. 103:191-195, (2015).
 24. Yan Y, Zhang L, Yu M.Y, Wang J, Tang H, Yang Z.W. The genome of *Bacillus aryabhatai* T61 reveals its adaptation to Tibetan Plateau environment. *Genes and Genom*. 38(3):293-301, (2016) .
 25. Alireza S, Razieh L, Negin S, Hajar A. Isolation and identification of *Bacillus firmus* from the marine sediments of Imam Khomeini port and study its ability in biosorption of lead. *J Oceanogra*. 5 (17): 11-12, (2014).
 26. Towner K.J. Biology of *Acinetobacter* spp. In: Bergogne Berezin E, Joly-Guillou ML, Towner KJ (eds) *Acinetobacter: Microbiology, Epidemiology, Infections, Management*. Boca Raton, CRC Press. 13-36, (1996).
 27. Sekiguchi H, Koshikawa H, Hiroki M, Murakami S, Xu K, Watanabe M, Nakahara T, Zhu M, Uchiyama H. Bacterial distribution and phylogenetic diversity in the Changjiang estuary before the construction of the Three Gorges Dam. *Microb Ecol* . 43: 82-91, (2002).
 28. Chen M.X, Li H.Y, Li G, Zheng T.L. Distribution of *Vibrio alginolyticus*-like species in Shenzhen coastal waters, China. *Braz J Microbiol* 42: 884-896, (2011).
 29. Liu L, Ge M, Zheng X, Tao Z, Zhou S, Wang G. Investigation of *Vibrio alginolyticus*, *V. harveyi*, and *V. parahaemolyticus* in large yellow croaker, *Pseudosciaena crocea* (Richardson) reared in Xiangshan Bay, China. *Aquacul Rep*. 3: 220-224, (2016).
 30. Rossello' -Mora R.A, Ludwig W, Kampfer P, Amann R, Schleifer K.H. *Ferrimonas balearica* gen. nov., spec. nov., a new marine facultative Fe (III)-reducing bacterium. *Syst Appl Microbiol*. 18: 196-202, (1995).
 31. Ivanova E.P, Onyshchenko O.M, Christen R, Zhukova N.V, Lysenko A.M, Shevchenko L.S, Buljang V, Hambly B, Kiprianova, E.A. *Oceanimonas smirnovii* sp. nov., a novel organism isolated from the Black Sea. *Syst Appl Microbiol*. 28: 131-136, (2005).

Received:04.05.18, Accepted: 07.06.18, Published:01.07.2018

***Corresponding Author:**

J L Rathod*

Email: jagannathrathod9@gmail.com