ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Isolation and Characterization of Marine Micro-Flora from Coast of Paradip and Screening for Potent Enzymes.

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ABSTRACT

Sea water samples were collected from 3 different sites of Paradip and analyzed for their micro-flora using standard microbiological protocols. The average pH of collected samples was 7.55. About 18 bacterial and 9 fungal isolates were considered for further study, belonging to genera *Vibrio* spp., *Pseudomonas* sp., *Bacillus* sp., *Aeromonas* sp., *Photobacterium* sp., and fungi belong to *Penicillium* spp. and *Aspergillus* sp. Among the above *Vibrio* spp. and *Penicillium* spp. were predominant. About 94.4% bacterial isolates were moderately halophilic and *Pseudomonas fluorescence* was found to be moderately halophilic by tolerating 15% (w/v) NaCl and 77.8% bacterial isolates could tolerate 5 - 12 pH, which confirmed them as alkali- and halo- tolerant bacteria. All the bacterial isolates had capacity to degrade cellulose and could produce alginase but only *Bacillus* sp. had capability to utilize phosphate. In case of fungi *Aspergillus niger* showed positive for degradation of cellulose, pectin and tributyrin substrates. This study not only provides proper insight about the microbiological analysis of the samples but also production of extracellular enzymes by potent bacterial isolates. These bacteria can be further exploited for biotechnological purposes.

Keywords: Alkali-tolerant, extracellular enzymes, halo-tolerant, marine microbes

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ISSN: 0975-8585

INTRODUCTION

Research interest in microbial diversity over the past years has increased markedly because of its significance in various ecological and biotechnological processes such as industrial, pharmaceuticals and agricultural aspects. Microbes have played an essential role in making the Earth a suitable place for more complex life forms including human beings. Marine ecosystems are dominated by various microbes which have significant economic impacts. Although about 70% Earth's surfaces are covered by water, however the marine micro-flora yet remains an unknown and unexploited resource.

In rapidly emerging field of marine biotechnology, much of its success relies on investigation, characterization and maintenance of marine biodiversity. For this purpose, bio-prospecting and bio-harvesting are now popularly used by many researchers. Marine microbes have immense applications towards various field viz. productions of bio-polymers, bio-surfactants, enzymes used in food and pharmaceutical industries, and also regarded as a source for single cell protein (SCP) and single cell oils (SCO) etc. Enzymes produced by marine microorganisms are important in biotechnological aspects due to their high salt tolerance and thermo-stability. Most of the marine bacteria are well known for their association with the wide variety of functions like bio-surfactant production [21], biodegradation and bioremediation of hydrocarbons [22], oil biodegradation [24], bioremediation of diesel-contaminated soils [8], agar degradation [34], degradation of plastic debris [6], and anti-biofilm activity [12] etc.

Marine microbes usually require sodium and potassium ions for their growth and to maintain osmotic balance of their cytoplasm [15]. They have facultative psychrophilicity [2], higher tolerance to pressure than their terrestrial counterparts [35], capacity to survive in high salt concentrations of seawater and they are mostly Gram negative rods [4], and motile spore formers [5]. Marine bacteria used β-aminoglutaric acid or β-glutamate as osmolytes which are present in higher amounts in marine sediments [29].

The 460 km long coast of Odisha has not yet been explored microbiologically. The coastal water of Odisha has been found to be clean except at the river mouths which showed lower values of dissolved oxygen due to oxidation of heavier organic matters carried by the rivers and the near shore waters contain relatively higher bacterial pollution than the offshore (Coastal Ocean Monitoring and Prediction Systems (COMAPS) programme, Government of India, 2000-01). Paradip is a Port Town in the district of Jagatsinghpur in coastal region. Although Paradip port (20.2654 °N, 86.6763 °E) is the only artificially created port in the eastern India and has remained a popular tourist spot, its microbial diversity has not yet been studied. Located on the shores of Bay of Bengal, is a port township with a number of industries and all the sewage and effluent are discharged into the sea including those from the ships. The estuary of the river Mahanadi is also present here and prawn hatcheries are present at some distance from the township. Considering the importance of the port township, the present work was undertaken to explore the microbial diversity of sea water.

MATERIALS AND METHODS

Sample collection and analysis

Water samples were collected aseptically in sterile sampling bottles from three sites of Paradip Port, Odisha, India, i.e. dock of Paradip, beach and estuary near Nehru Bungalow. After that the pH of samples were analysed by digital pH meter 335 (Systronics, India) [19].

Microbiological analysis

Microbiological analysis was done using various tests for the isolation of bacteria and fungi. Bacteriological analysis was conducted using various techniques i.e. total plate count (TPC) using zobell marine agar (ZMA) plate method, total coli-form test using MPN method, and faecal enterococci determination using ethyl violet azide broth [1]. For mycological analysis, total plate count (TPC) using 10-fold serial dilution method and spread plate technique was used in czapek dox agar plate prepared with sea water and distilled water (1:1) [1].

Identification and characterization of isolates

Identification of bacteria were done on the basis of their colony characteristics on different media, Gram reaction, different biochemical tests, sugar utilization tests, and other physiological tests like pH, temperature and salt tolerance tests. Different media used for investigation were nutrient agar (with sea water), nutrient agar (without sea water), macConkey Agar (MA), eosin methylene blue agar (EMBA), Salmonella-Shigella agar (SSA), thiosulphate citrate bile salts sucrose agar (TCBSA), Pseudomonas isolation agar (PIA), Photobacterium agar (PBA), thioglycollate agar (TGA) and sea water agar (SWA). All the physiological tests were done using ZMA at respective conditions. Thermal death point (TDP) of bacteria was calculated using Zobell marine broth (ZMB). For this, bacteria were pre-incubated in ZMB for 24 h. Then 100 µL of this was transferred to 10 mL of Normal saline solution (NSS) and incubated at different temperatures from 40 - 90°C for 10 min. After that a loop-full of samples were inoculated onto ZMA plate and incubated at 37°C for 24 - 48 h. Viability test of bacteria was done using ZMB with optimum NaCl observed from salt tolerance test. Viability was tested by sub-culturing them to ZMA plates at interval of 7 - 8 days for 46 days. Identification of fungal isolates was done by using slide culture method [27] and on the basis of micro-morphological study using lacto phenol cotton blue staining.

Enzymatic screening of isolates

The isolated bacterial and fungal strains were spot inoculated on pseudo selective agar medium having respective substrates for screening of enzymes produced at 37°C for 24 - 48 h in zobell marine agar and at 28°C for 48 - 72 h in czapek Dox agar respectively. After incubation the plates were assayed by observing zones of clearing around the colonies and were measured in mm. as the difference between the total diameter and the fungal colony. The zone of clearances was observed for cellulase and lipase by following the general protocols. Iodine cubes were used to test the production of amylase, 15% acidic $HgCl_2$ for caseinase and hexadecyltrimethly-ammonium bromide (1%) was used for pectinase test. In case of alginase, sodium alginate-ZMA plates were prepared using N/5 zobell marine broth, 1% (w/v) sodium alginate and 1.5% agar. The zone of clearance was observed as the protocol followed in case of cellulase.

Statistical analysis

The data recorded during the course of investigation was subjected to significance test using T- test and Pearson Correlation coefficient. Statistical significance was set at $P < 0.05$. Results were denoted as mean ± S.D. (standard deviation) of triplicate experiments.

RESULTS

About 15 water samples from 3 different sites were studied. From pH analysis of all water samples it was found that the pH of the Paradip is near to neutral or slightly alkaline in nature which ranges from 7.38 - 7.63 (Table 1). Table 1 also represents the total plate count of bacteria and fungi, coli-forms and faecal enterococci determination. Bacterial and fungal load was found to be more in case of water samples from dock and near Nehru Bungalow respectively. Statistical analyses of these parameters were also determined.

Table 1: pH and microbial loads of water samples from different sites of Paradip

Water samples		pH[a]	Total plate count (TCP) in CFU/ml		Total Coli-forms (MPN/100 mL)	Presence of faecal Enterococci
			Bacterial load[a]	Fungal load[a]		
1	Dock	7.63	4.3×10^4	2.0×10^1	-	+
2	Beach	7.63	3.2×10^4	1.5×10^1	-	+
3	Near Nehru Bungalow	7.38	1.21×10^5	3.5×10^1	-	+

+: Positive; - : Negative

[a] Results were average of 5 independent samples from each sites

ISSN: 0975-8585

Table 2: Biochemical characterization and probabilistic identification of bacterial isolates

Isolates	Gram reaction	Cell morphology	TSI						MMT			Nitrate reduction	Indole	Urease	Citrate	MR	VP	Catalase	Oxidase	IDENTIFICATION
			Glucose	Sucrose	Lactose	Acid	Gas	H_2S	Mannitol	Motility	Gas									
PPB1	-	Short rods, comma shaped	+	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+	-	*Vibrio alginolyticus*
PPB2	-	-do-	+	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+	+	-do-
PPB3	-	-do-	+	-	-	+	-	-	+	+	-	-	-	-	+	-	-	+	+	-do-
PPB4	-	Minute rods	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	Unidentified
PPB5	-	Short fine rods	+	+	+	+	-	-	+	+	-	+	+	-	+	-	-	+	+	*Vibrio alginolyticus*
PPB6	-	Rods of varied sizes	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	Unidentified
PPB7	-	Short rods	+	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	+	*Vibrio vulnificus*
PPB8	-	Small, bacilli	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	+	-	*Pseudomonas fluorescence*
PPB9	-	Small, bacilli	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-do-
PPB10	-	Short rods	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	+	Unidentified
PPB11	+	Big bacilli	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	+	-	*Bacillus* sp.
PPB12	-	Cocco bacilli	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	Unidentified
PPB13	-	Short rods, comma shaped	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-	+	+	*Vibrio alginolyticus*
PPB14	-	Short, curved rods	+	+	+	+	-	-	+	+	-	+	+	-	+	+	-	+	+	*Vibrio nereis*
PPB15	-	Very short fine rods	+	+	+	+	-	-	+	-	-	+	-	-	+	+	-	+	+	*Aeromonas schubertii*
PPB16	-	-do-	+	+	+	+	-	-	+	-	-	+	-	-	-	+	-	+	+	-do-
PPB17	-	-do-	+	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	+	-do-
PPB18	-	Minute rods	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	*Photobacterium angustum*

+ : Positive; - : Negative; TSI: Triple sugar iron test; MMT: Mannitol motility test

ISSN: 0975-8585

Table 3: Sugar utilization tests of alkaline protease producing isolates

Isolates		Xy	Ar	Ga	Mo	De	Fr	Ma	Ce	La	Mb	Su	Rf	Tre	Sa	In	Du	Sb	Is	Ad
PPB1	O	-	-	-	+	+	+	+	-	+	-	+	-	+	-	-	+	-	-	+
	F	-	-	+	+	+	+	+	+	-	+	+	-	+	+	-	-	-	-	-
PPB4	O	-	-	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-
	F	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-
PPB5	O	-	-	+	+	+	+	+	-	-	-	+	-	+	-	-	-	-	-	-
	F	-	-	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-
PPB6	O	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-
	F	-	-	+	-	+	+	+	+	-	+	+	-	+	+	-	-	-	-	-
PPB7	O	-	-	+	+	+	+	+	-	+	-	+	-	+	-	-	-	-	-	-
	F	-	-	+	+	+	+	+	+	-	-	+	-	+	+	-	-	-	-	-
PPB8	O	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PPB10	O	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	-	-
	F	-	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-
PPB11	O	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-
	F	-	-	+	-	-	+	+	+	-	+	-	-	+	-	+	-	-	-	-
PPB12	O	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	F	-	-	-	+	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-
PPB13	O	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-
	F	-	-	-	+	+	+	+	+	-	-	+	-	+	+	+	-	-	-	-
PPB14	O	-	-	+	+	+	+	+	+	-	-	+	-	+	-	+	-	-	-	-
	F	-	-	-	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-
PPB15	O	-	-	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-
	F	-	-	-	+	+	+	+	-	-	-	+	-	+	+	+	-	-	-	-
PPB18	O	-	-	+	+	+	+	-	+	-	-	+	-	+	-	-	-	-	+	-
	F	-	-	-	+	+	+	+	-	-	-	+	-	+	+	+	-	-	-	-

O: Oxidative mode; F: Fermentative mode; Xy: Xylose; Ar: Arabinose; Ga: Galactose; Mo: Mannose; De: Dextrose; Fr: Fructose; Ma: Maltose; Ce: Cellobiose; La: Lactose; Mb: Melibiose; Su: Sucrose; Rf: Raffinose; Tre: Trehalose; Sa: Salicin; In: Inulin; Du: Dulcitol; Sb: Sorbitol; Is: Inositol; Ad: Adonitol; (+): Positive; (-): Negative

ISSN: 0975-8585

Table 4: Effect of NaCl concentrations on growth of bacterial isolates

Isolates	NaCl concentration % (w/v)							
	0	2.5	5.0	7.5	10.0	12.5	15.0	20.0
PPB1	+++	+++	+++	++	++	+	-	-
PPB4	+++	+++	+++	++	+	-	-	-
PPB5	+++	+++	+++	+++	++	-	-	-
PPB6	+++	++	+	+	-	-	-	-
PPB7	+++	+++	+++	++	+	-	-	-
PPB8	+++	+++	+++	++	++	+	+	-
PPB10	+++	+++	++	++	-	-	-	-
PPB11	+++	++	+	+	-	-	-	-
PPB12	+++	++	-	-	-	-	-	-
PPB13	+++	+++	+++	++	+	-	-	-
PPB14	+++	+++	+++	++	-	-	-	-
PPB15	+++	+++	++	-	-	-	-	-
PPB18	+++	+++	++	-	-	-	-	-

+++: Luxuriant growth; ++: Moderate growth; +: Slow growth; - : No growth

Table 5: Effect of pH on growth of bacterial isolates

Isolates	pH tolerance									
	3	4	5	6	7	8	9	10	11	12
PPB1	-	-	++	+++	+++	++	++	++	++	++
PPB4	-	-	++	+++	+++	++	++	++	++	-
PPB5	-	+	++	+++	+++	++	++	++	++	++
PPB6	-	-	-	++	++	++	++	+	+	+
PPB7	-	-	++	+++	+++	++	++	++	++	-
PPB8	-	+	++	+++	+++	++	++	++	++	+
PPB10	-	-	-	+	+	+	+	+	+	-
PPB12	-	-	-	+	++	++	++	-	-	-
PPB13	-	-	++	++	+++	+++	+++	++	++	++
PPB14	-	-	++	++	+++	+++	+++	++	++	++
PPB15	-	-	+	++	+++	++	++	++	++	++
PPB18	-	-	++	++	+++	+++	+++	+++	+++	+++

+++: Luxuriant growth; ++: Moderate growth; +: Slow growth; - : No growth

Table 6: Effect of temperatures on growth of bacterial isolates

Isolates	Temperatures in °C							
	4	10	15	20	25	30	35	40
PPB1	+++	+++	+++	+++	+++	+++	+++	+++
PPB4	+++	+++	+++	+++	+++	+++	+++	+++
PPB5	+++	+++	+++	+++	+++	+++	+++	+++
PPB6	+	+	++	++	++	+++	+++	+++
PPB7	+++	+++	+++	+++	+++	+++	+++	+++
PPB8	++	++	+++	+++	+++	+++	+++	+++
PPB10	+++	+++	+++	+++	+++	+++	+++	+++
PPB11	++	++	+++	+++	+++	+++	+++	+++
PPB12	+	+	++	++	++	+++	+++	+++
PPB13	+++	+++	+++	+++	+++	+++	+++	+++
PPB14	++	++	+++	+++	+++	+++	+++	+++
PPB15	+	+	++	++	++	+++	+++	+++
PPB18	+	+	++	++	++	+++	+++	+++

+++: Luxuriant growth; ++: Moderate growth; +: Slow growth; - : No growth

ISSN: 0975-8585

Table 7: Enzymatic screening of bacterial isolates

Isolates	Amylase	Cellulase	Pectinase	Alginase	Gelatinase	Caseinase	Lipase	Phosphatase
PPB1	+	+	w+	+	+	-	+	-
PPB4	+	+	+	+	+	-	-	-
PPB5	+	+	w+	+	+	-	-	-
PPB6	+	+	-	+	-	-	-	-
PPB7	-	+	-	+	+	-	-	-
PPB8	-	+	w+	w+	-	-	-	-
PPB10	-	+	w+	w+	+	-	-	-
PPB11	+	+	+	w+	-	+	-	-
PPB12	-	+	w+	w+	+	-	-	-
PPB13	+	+	w+	w+	+	+	-	-
PPB14	-	+	w+	w+	-	+	+	-
PPB15	+	+	w+	w+	+	+	-	-
PPB18	+	+	w+	w+	+	+	-	-

+: Positive; - : Negative; w+: Weakly positive

A total of 19 bacterial isolates and 9 fungal isolates were taken for further studies and identified on the basis of their colony morphology in various growth media, Gram reaction, biochemical tests, sugar utilization tests and other physiological tests and the fungi were identified on the basis of their macro- and micro- morphological studies.

Gram staining revealed that except one isolate, all other isolates were Gram negative (Table 2). About 38.89% were belonged to *Vibrio* spp., 16.67% to *Aeromonas* spp., 11.11% *Pseudomonas* spp. and 5.55% *Bacillus* sp. (PPB11) and *Photobacterium* sp. (PPB18). From sugar utilization tests it was found that majority of the isolates were positive for dextrose, fructose, maltose, and trehalose. About 7.69% isolates were positive for xylose and arabinose; 15.38% for raffinose and adonitol; 23.07 % for sorbitol and inositol; 53.83% for inuline and 69.21% for galactose (Table 3). Only one isolate (PPB8) could tolerate 15% NaCl incorporated in ZMB (Table 4). Most of the isolates have pH optima 7 - 9 but few isolates can able to tolerate pH 12 (Table 5). All the isolates can grow at different ranges of temperatures from 4 - 40°C (Table 6). Thermal death point test revealed that all isolates can grow at 40°C (100%) and one of them can grow well at 90°C (7.69%). Besides about 46.15% isolates were active at temperature of 50°C and 15.39% isolates at 60°C. While studying the viability pattern of the bacterial isolates it was observed that all the strains remain viable even after 45 days in ZMB with optimum concentration of NaCl.

Among the 9 fungal isolates, 6 were identified as *Penicillium* spp. (PPF1, PPF3, PPF5, PPF7, PPF8 and PPF9) and 2 were as *Aspergillus* spp. (PPF2 and PPF6) on the basis of their macro- and micro- morphological studies.

The ability of both bacterial and fungal isolates to produce extracellular enzymes was investigated using different pseudo selective agar medium respectively. All the bacterial isolates were positive for cellulase production and some are for amylase whereas few for lipase and caseinase and only one isolate can produce phosphatase enzyme (Table 7). In case of fungal isolates only 3 (PPF4, PPF5 and PPF9) out of 9 were positive for amylase where as only few for others like PPF2 for both lipase and cellulase and PPF3 for caseinase. Besides, no one can produce phosphate solubilising enzymes.

DISCUSSION

As the pH of the medium plays a vital role in the growth, development and distribution of microorganisms, during the present investigation, near neutral pH was favourable for the isolated marine bacteria and fungi. The average pH of the surface sea water is generally 8.5 though deviations of varying magnitude may be seen [26]. The bacterial load and fungal load varied at varied sampling sites which may be due to pH of the samples. As in the other two samples except near Nehru Bungalow, the pH of the water is near to alkaline which is not suitable for fungi to grow and vice versa is correct for the bacterial load. Statistical

analysis revealed that there is a negative but significant correlation among the pH and fungal load (r = -0.97) which corroborates with the findings of [19]. It denotes lower the pH more is fungal load, which is true for most fungi as they love to grow nearer acidic pH than basic. While there is a positive and significant correlation among pH and bacterial load (r = 0.94). This was similar with the findings of [16] where bacterial load increases with increase in pH. There is a negative and significant correlation among the fungal load and bacterial load (r = -0.82). T- test revealed that there is a significant difference among the population means of all the parameters at $P < 0.05$.

All the water samples were negative for total coli-forms but were positive for the presence of faecal enterococci during the present investigation. Absence of coli-forms indicates a number of possibilities such as the presence of a high amount of coli-phages or chemical pollution of the water etc, while the presence of faecal enterococci in the samples indicated biological pollution as they are generally used as the pollution indicators in marine and brackish water environments [28].

A total of 18 bacterial isolates and 9 fungal isolates were isolated from coastal water at Paradip. Bacterial isolates were identified on the basis of Bergey's manual [10] and PIBWin software [3]. Fungal isolates were identified with the help of a Fungal Atlas from Mycology Online devised by Dr. David [7], University of Adelaide, Australia. About 17 isolates (94.4%) of bacteria were found to be Gram negative. These results corroborated with the other researchers [26, 31, 36].

Out of 18 isolates, 7 (38.89%) were identified as different species of Vibrio such as *V. alginolyticus, V. vulnificus* and *V. nereis*. According to the Aquarium Frontier Feature, marine *Vibrio* spp. are the most common bacteria found in sea water and are known to cause bio-corrosion [23]. Some of them were also considered to pose health risks to both human and animals. Since a long time, *Vibrio alginolyticus* has been known as a potential cause of sea food poisoning while halophilic marine vibrios such as *V. vulnificus* are reported to cause gastroenteritis, sepsis, cellulitis leading to necrotizing soft tissue infection after exposure to sea water or consumption of raw sea food [11].

Gram negative rods other than *Vibrio* found during the present study were identified as *Pseudomonas fluorescence*, *Aeromonas schubertii* and *Photobacterium angustum* while one was Gram positive rod, *Bacillus* sp. Fungal isolates were identified as *Aspergillus* spp. and *Penicillium* spp. Four bacterial isolates (22.22%) and one fungal isolate obtained during the study remained unidentified. Most of the marine microbes were difficult to be identified by conventional methods, no matter which determinative keys are used [30].

About 12 isolates (67%) found during the current investigation were determined as facultative anaerobes. The facultative anaerobic bacteria grow better in the presence of oxygen [26]. Three of the unidentified bacteria and one fungus were noticed as growing slowly and faintly. Zobell reported that many of the marine bacteria generally grow more slowly than their terrestrial counterparts [37].

According to the classification of halophiles [14], 94.4% of the bacterial isolates were categorized as moderately halophilic and 5.6% were slightly halophilic. Only one isolate, *Pseudomonas fluorescence*, was found to be halophilic (up to 17% NaCl). Eight isolates (44.4%) were found to have an obligate requirement for Na^+ in the medium for growth. These isolates were identified as truly obligate marine microbes [36]. The halophilic microbes are well sought after in the field of marine biotechnology.

About 70% isolates can tolerate pH range 5 - 12. Most of the marine bacteria can grow at wider range of pH [26]. Most of these isolates due to their alkalitolerant nature have great importance in treatment of various industrial effluents. All the bacterial isolates can grew over a wide ranges of temperatures (4 - 40°C), though having growth optima at 37°C. Similar results were obtained by [33]. TDP of the spore former *Bacillus* spp. was found to be above 90°C while that of 8 isolates (47%) were determined to be at 40 - 50°C and of the others at 50 - 60°C.

In the present study, about 13 isolates were selected on the basis of biochemical and halophilic nature for the enzymatic characterization. About 61.5% isolates were found to be potential producers of amylase, 100% for cellulase, 84.6% for pectinase, 100% for alginase, 69.2% for gelatinase, 30.8% for caseinase, 15.4% for lipase, and phosphate solubilization was seen in case of only 7.7% isolates. The isolate PPB11 was identified as *Bacillus* sp. which has capability of degrading various substrates like starch, cellulose, pectin, and

casein. The similar results were also reported [16, 17, 25, 32]. Out of 9 fungal isolates, 33% were identified as potential amylase producers, 11.1% for cellulase, 22.2% for pectinase, 11.1% for caseinase, and lipase and none of them were able to degrade phosphate. The present finding of getting *Aspergillus* sp. as good producers of cellulase and lipase is corroborated with the findings of [18, 19, 20]. It was also seen in the present study that *Penicillium* spp. were good producers of amylase, pectinase and caseinase which was also in accordance with [18, 19]. The enzymatic study of these isolates gave a broad outlook regarding their utilization of various substrates.

CONCLUSION

The present investigation study regarding microbiological loads of coastal water at Paradip provides certain valuable information which leads to the following conclusions. Presence of number and variety of bacteria and fungi in the coastal water at Paradip indicated a rich microbial diversity at this area of Bay of Bengal. Further studies should be done over a longer period in different seasons to provide information regarding seasonal variations of various native as well as exotic species. Presence of a high number of *Vibrio* spp., pollution indicator organisms like faecal enterococci, a high number of bacterial isolates having the ability to degrade starch, cellulose, pectin, alginate, gelatine, casein, tributyrin and a high number of alkalitolerant bacteria in the sea water indicated microbial diversity of the coastal water at Paradip. As it is a port township, the above findings is an insight about the probable causes of pollution due to anthropogenic factors as well as transportation and industrial developments. Conservation of all isolates has been done so as to serve as reference standards for future studies. Selected isolates having potentiality to produce a number of extracellular enzymes can be employed in biodegradation, biotechnological and industrial sectors for which further analyses are required.

ACKNOWLEDGEMENTS

The authors would like to thank the Head, Department of Microbiology, C.B.S.H., Orissa University of Agriculture and Technology, Bhubaneswar, for the laboratory support.

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