

Bacterial Biodiversity of Bay of Bengal at Orissa Coast: The Unseen Gifts and Threats.

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Bacterial biodiversity of the coastal waters of Bay of Bengal was studied for the first time in the state of Orissa. The entire 460 km. long coastline which starts from Chandaneswar in the North to Bahuda in the South were divided into four study sites such as Chandipur, Paradip, Puri & Gopalpur. From all the four study sites a total number of 36 bacterial strains were isolated. Presence of coliforms in the water samples of Chandipur and Puri coast indicates faecal contamination of these water bodies which could be due to heavy tourist activity. These 36 bacterial strains were categorized into 9 different genera as per their biochemical characterisation. Gram reaction of these isolates indicates 83% of the total isolates belong to Gram –ve type. Most of these bacterial isolates were found to be potential producers of various types of enzymes such as amylase, lipase, pectinase, cellulase etc. Presence of a large numbers of pathogenic *Vibrio* sp. in the coastal water indicates the probability of infection to human beings as well as other aquatic animals. The enzymatic activity and antibiogram pattern of these marine bacterial isolates were studied in details for industrial application as well as for human safety.

Keywords: Marine bacteria, Antibiogram, Enzyme activity.

Microorganisms shape the biosphere of the earth by their spectacular geo-chemical activities. Microbes form an integral part of ecosystem and are represented by groups such as eubacteria, archaea, fungi, unicellular algae, and protozoa etc., which are divided among three domains of life. The later three are eucaryotes-single cells or simple colonies of cells whose nuclei are wrapped in membranes. Microbes have proven to be a rich source of novel compounds with diverse distribution and biological activities (Davis 1999; Demain 1998 and 1999; Imada and Hotta 1992; Bernen *et al.* 1997).

The marine environment is proving to be a valuable source of novel bioactive compounds with antibacterial, antiviral, anticancer and antifouling properties (Colwell and Hill, 1992). Research in varied aspects of marine biotechnology has thrown up a number of technologies. Nowadays, marine microbes are of

varied commercial applications and used in the production of biopolymers, food and food industries, the production of enzymes, pharmaceutical industries, as biological response modifiers and environmental biotechnology (Vandamme 1989). Presently microbiologists are focusing to churn the ocean or seawater to explore and exploit the marine microbial diversity. The curiosity of the researchers is to see the unseen that is present in the vast, variable and versatile ocean as well as to exploit the natural resources for the well being of the humanity. A lot of work has already been initiated in this direction in various part of the Globe including Asia. The 460 km long coast of Orissa has not yet been studied, in terms of its microbial wealth. This part in the east coast of the Bay of Bengal consists of the largest brackish water lake of Asia i.e. the Chilka, vast golden sand beaches of Puri and Konark, unique sea beaches of Chandipur, the only artificial port of India i.e. Paradip port, dense mangrove forests of Bhitarkanika where thousands of Olive Riddley turtle came every year

to hatch their young ones and also declared as the Ramsar site of international importance. So far microbial ecology and diversity of these coastal areas have not been explored. Therefore it renewed our interest, to explore this virgin long coastal belt to unravel its microbial wealth, for possible commercial applications. Such studies could be a pre-requisite for tapping the biotechnological potential and/or other industrial application of these microbes.

Washed by the salty waters of the Bay of Bengal on the eastern coast, Orissa is situated from 17° 49' to 22° 34' North latitude and from 81° 29' to 87° 29' East longitude. Depending on the geographical location the entire coast was divided into four locations, e.g. Chandipur, Paradip, Puri and Gopalpur-on-sea and were chosen as the study areas.

MATERIALS AND METHODS

Collection of water samples

Random collections of water samples were carried out aseptically from the above study sites during the pre monsoon seasons, i.e. (April-May 2004) following the methods of ZoBell (1946). From each site three water samples (100 ml each) were collected aseptically covering a distance of five km. All the three samples were then mixed together in a sterile container and were transported to the laboratory immediately for further processing and analysis.

Microbiological analysis

The water samples collected from four different sites of the coastal waters of Orissa state were processed in the laboratory of P.G. Department of Microbiology, Orissa University of Agriculture and Technology (OUAT) to determine the total aerobic heterotrophic bacterial load, number of

coliforms, isolation of different strains of bacteria, making axenic culture of the strains, studying their morphological and physiological parameters, assigning strain no, identification through a series of biochemical characteristics and also other features required for their characterization following standard microbiological techniques of Collins and lyne (1970) and Hansen *et al.* (1991).

Enumeration of aerobic heterotrophic bacteria

Enumeration of total aerobic heterotrophic bacterial load in the samples was estimated by 10-fold serial dilution technique followed by spread plating. Diluents were prepared with sterilized phosphate buffer having pH 7.2 to avoid osmotic shock to the bacteria. From each dilution tube 100µl of samples were used for spread plating on presterilised ZoBell Marine Agar (ZMA) plates. Three replicates of each dilution were used for spread plating to minimize the error. All the plates were incubated at 30°C ± 2°C for 24-48 hrs. Plates having viable colony count (30-300 colonies per plate) were selected for the enumeration of the water samples.

Estimation of total coliforms

Total coliform counts of the water samples were estimated using the Most Probable Number (MPN) method of Mackie and Mc Cartany (1996). The MPN of total coliforms present per 100 ml of each water sample was determined with the help of a standard chart (i.e. modified Mackie and McCartney 5-tube method) based on the number of tests that had turned positive.

Pure culture and maintenance of the bacterial isolates

Selected bacterial colonies showing different morphological features were picked up from the ZMA plates and were restreaked several times on presterilized ZMA plates to obtain pure culture

Table 1. Total coliforms & aerobic plate count

Sampling sites	MPN-index (coliforms/100ml. water sample)	Colony forming units/ml
Chandipur	20	7.2 X 10 ⁴
Paradip	0	6.5X10 ⁴
Puri	35	3.5X10 ⁵
Gopalpur	0	3.9X10 ⁵

of the isolates. The pure cultures of the isolates were preserved by restreaking on ZMA slants at 4°C for future use.

Identification of the bacterial isolates

The isolated bacterial strains from the coastal waters of Orissa were identified following various morphological and bio-chemical methods. Identification was done on the basis of their colony characteristics on different media, Gram reaction, different biochemical tests, various sugar utilisation tests, halophilic nature, pH tolerance capacity, growth at different temperatures and enzymatic activities shown by the isolates.

Test for enzymatic activity of the marine bacterial isolates

All the isolates were screened on pseudo-selective media for production of various industrially important exocellular enzymes like amylase, cellulase, pectinase, alginase, gelatinase, caseinase and lipase following standard microbiological methods of Collins and lyne 1970.

Antibiotic sensitivity of the marine bacterial isolates

Eight pathogenic strains of bacteria isolated from the coastal waters of Bay of Bengal were revived in ZMB broth after overnight incubation at 30°C. Then by following the disc diffusion methods of Bauer *et al.* (1966) the test organisms were spreaded over the surface of pre-sterilized Muller Hinton agar plates with the help of a sterilised cotton swab. Precaution was taken while sweeping the plates with broth culture of the test organisms to make a uniform lawn culture of the isolate. Selective antibiotic discs were put uniformly throughout the MHA plates. The antibiotic discs were selected depending on their use and mode

of action. The plates were incubated at 30°C for 24 hrs. The zone of inhibition was measured with the help of a scale and was categorised as sensitive, moderately sensitive and resistant.

RESULTS AND DISCUSSION

Total bacterial load and coliform count of the samples

The total bacterial load as well as total coliforms present in all the four samples is given in table 1. The average bacterial load was found to be 10^4 colony forming units/ml of water. The coliforms counts were 20 and 35 per 100 ml of water sample in Chandipur and Puri respectively. Whereas no coliform count was observed in the other two study sites like Paradip and Gopalpur-on-Sea. The presence of coliforms and higher bacterial load in Chandipur and Puri could be attributable to higher tourist load indicating more anthropogenic activities, nearer to the township, due to dumping of garbage and discharge of sewage to the seawater.

Isolation of marine bacteria

A total number of 36 different types of colonies were picked up from the basal medium i.e. ZoBell Marine Agar (ZMA). They were restreaked several times to check for their purity and were kept in slants as pure culture.

Identification of marine bacterial isolates

All the 36 strains of bacteria were put into a number of biochemical tests for their identification following the methods of Hansen *et al.* (1991) and Oliver (1982). The growth of bacterial colonies on different mediums and bio-chemical characteristics including oxidation &

Table 2. Distribution of Gram +ve and Gram -ve bacteria at four different sites in the Orissa coast

Sampling sites	Gram+ rods	Gram+ve cocci	Gram-ve rods	Gram-ve cocci
Chandipur	Nil	1	6	Nil
Paradip	1	1	7	Nil
Puri	1	1	9	Nil
Gopalpur	Nil	1	8	Nil
Total Nos.	2	4	30	Nil

fermentation test of the sugars and Gram's reaction of the 36 isolates were studied. All the test results were matched with Bergy's Manual and with the help of software, PIBwin (version 1.9.2.) devised by Dr. T. Bryant, University of Southampton. Basing on the results all these 33 identified strains belonged to 9 genera and 18 different species. Approximately 10% (3) of the isolates could not be identified with the above process.

Distribution of marine bacterial isolates

Regarding the distribution of bacterial types at various collection sites, it was observed that coastal waters at Puri, Paradip, Gopalpur, and Chandipur contain 11, 9, 9 & 7 nos. of bacteria respectively. This result indicate that at Puri because of its tourist location and shore activities more number of bacteria were isolated which could be due to contamination from anthropogenic sources thereby increasing the population of bacteria. Whereas at Paradip, Gopalpur and Chandipur less number of bacterial load indicate less external contamination of the sample. The distribution of bacteria as per Gram reaction indicates the dominance of Gram -ve bacteria. 83% of the total isolates were found to be of Gram -ve type where as only 17% belongs to Gram +ve type (Table 2).

These results corroborated the observations of Rheinheimer (1985) and Schut *et al.* (1997).

The generic composition indicates 44% (*Vibrio* sp.), 8% (*Pseudomonas* and *Aeromonas* sp.), 5.6% (*Photobacterium*, *Micrococcus*, *Enterococcus*, and *Bacillus* sp.) and 3% *Enterobacter* sp. Similar results were obtained by Simidu *et al.* (1962) from the coastal waters of Kamagawa Bay in Japan.

Antibiotic sensitivity pattern of the isolates

As 49% of the isolates belonged to the genera *Vibrio*, reported to be common pathogens to marine animals and also are the disease causing organism to humans, the antibiotic sensitivity pattern of these organisms was studied. From the sensitivity test it was observed that all the 8 strains of the bacteria were sensitive to most of the commonly used drugs such as Ciprofloxacin, Norfloxacin, Amikacin and Cephataxim. *Vibrio fluvialis* was the only organism that showed 100% sensitive to all the drugs, whereas *Vibrio diazotrophicus* showed maximum resistance to four of the drugs. But the over all results indicated that these potential pathogens were showing the trend of resistance, which is a major concern regarding the use of antibiotics for the treatment of diseases that might be caused/ causing due to these marine microbes in the coastal region of Orissa (Table 3).

Enzymatic activity of the isolates

The detailed enzymatic activities of all the 33 bacterial strains isolated from coastal waters of

Table 3. Sensitive and resistance pattern of selected bacterial isolates from coastal waters of Orissa

Species	Strain no	Sensitive	Moderately sensitive	Resistant
<i>V. parahaemolyticus</i>	SOC-1	Ce, Cf, Ak, G, Am	A, T, S, Nx	Co
<i>Vibrio cholerae</i>	SOP-18	Cf, Nx, Ce, T, Ak	A, Am, Co, G	S
<i>Vibrio alginolyticus</i>	SOD-8	A, Am, Cf, Nx, Co, Ce, G, Ak -		T, S
<i>Vibrio vulnificus</i>	SOG-31	A, Am, Cf, Nx, G, Ce, Ak, S -		Co, T
<i>Vibrio fluvialis</i>	SOP-21	Cf, Nx, Co, Ce, Am, T, Ak,	A, G, S	-
<i>Vibrio nereis</i>	SOD-10	Cf, Nx, Co, Ce, Ak	A, G, S	Am, T
<i>Vibrio diazotrophicus</i>	SOC-5	Cf, Nx, Ce, Ak	Am, G	A, Co, T, S
<i>P. fluorescence</i>	SOD-11	Ce, Nx, Cf, Ak	Am, A, G, T	Co, S

Antibiotics used:

Ce: Cefotaxim, Cf: Ciprofloxacin, Ak: Amikacin, G: Gentamycin, Am: Amoxycillin, A: Ampicillin, T: Tetracycline, S: Streptomycin, Nx: Norfloxacin, Co: Trimoxazole

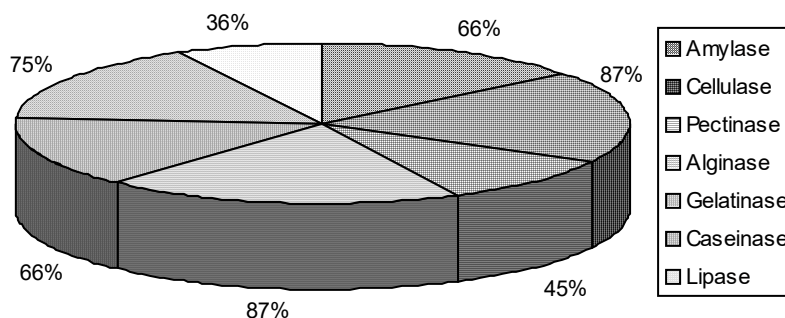


Fig. 1. Percentage of bacterial isolates showing enzyme activity.

Orissa were given in Fig. 1. Analysis of the data on the enzymatic activity of all the 33 bacterial strains showed that 66%, 87%, 45%, 87%, 60%, 75% and 36% of the isolates were found to be potential producers of amylase, cellulase, pectinase, alginase, gelatinase, caseinase and lipase respectively. It was also observed that most of these bacteria were capable of producing more than one type of enzymes.

CONCLUSION

The above study gives an insight into the types of marine bacteria present in the coastal water sample as well as their potential use as a biotechnological tool for production of enzymes and other bioactive substances. Side by side the presence of a large numbers of pathogenic marine *Vibrio* sp. is of great concern because these organisms are not only act as a public health hazard but also they are an economic hazard, because due to *Vibrio* infection a sizeable amount of shellfish, shrimp, etc. were killed in the initial stages of their growth. (Lowrie *et al.* 2000).

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