J. Mar. Biol. Ass. India, 48 (2) : 233 - 236, July - December 2006

Note

Chitinolytic activity of marine bacteria isolated from marine sediments in the continental slope of Bay of Bengal

Surajit Das, P. S. Lyla and S. Ajmal Khan*

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai- 608 502, Tamilnadu
**E-mail: s_ajmalkhan@rediffmail.com*

Abstract

Chitin, a polymer of N-acetylglucosamine (GlcNAc), is one of the most abundant organic substances in the marine environment and the chitinolytic bacteria help to recycle chitin. The present study was undertaken to investigate the chitinolytic activity of marine bacteria isolated from the sediments collected from the continental slope of Bay of Bengal. Total heterotrophic bacterial counts ranged from 0.42 to 37.38×10^4 $CFUg^{-1}$ dry sediment weight. Only 66.5% isolates showed the *in situ* chitin degradation. Totally, 11 genera of marine bacteria were identified of which 7 were chitinoclasts. The chitinoclasts percentage was maximum in *Vibrio* (17%) followed by *Pseudomonas* (13%), *Micrococcus* (12%), *Flavobacterium* (10%), *Bacillus* (6%), *Alteromonas* (5%), *Cytophaga* (4%) and unidentified (1%). The percentage occurrence of bacterial genera *vis-à-vis* chitinoclasts showed *Flavobacterium* as the most efficient and *Bacillus* as less efficient chitin degrading bacteria.

Degradation of complex carbohydrates such as chitin, present in filamentous fungi and in the exoskeleton of arthropods and cellulose, hemicelluloses and lignin- structural polymers in plants is quite essential. Without microbial conversion, these polymers would accumulate huge amounts of carbon from the biosphere and blocking a multitude of biological processes that allow micro- and macro organisms to thrive. In the absence of these microbial degradative processes, life on earth would soon falter.

Chitin is the second most abundant biopolymer in nature and its decomposition is accomplished by microbial action of chitin degrading bacteria which are collectively called as 'chitinoclast'. Bacteria are responsible for large scale degradation and rapid recycling of chitin produced in the marine environment. Hence, there is no accumulation of chitin in the environments.

Marine sediment contains less chitin which can only be explained if chitin is weathered at the same rate as it is produced. Chitin is decomposed by exoenzymes 'chitinase' (EC 3.2.1.14) present in bacteria, resulting in the production of material like N-acetyl glucosamine. This together with chitobiose or chitotriose is further broken down by chitobiase to produce the glucosamine or glucose. Since this decomposition process releases mineralized nutrients for primary producers, the activities of chitin decomposing bacteria, therefore, directly or indirectly affect the productivity of organisms at various trophic levels in the sea. Till date there is no report on the distribution and activities of the chitinoclasts from the continental slope of the Bay of Bengal. In this backdrop, the present study was undertaken to test this hypothesis that the deep sea sediment off Bay of Bengal contains less chitin due to the chitin mineralizing bacterial activities.

Materials and methods

The sediment samples were collected by Smith McIntyre Grab ($0.2m^2$ coverage area) during the scientific cruise (No. 225) of FORV *Sagar Sampada* in April-May, 2004 between latitude 10°36'N & 20°01'N and longitude 79°59'E & 87°30'E, covering 33 stations over 11 transects in the continental slope of Bay of Bengal (Fig. 1). On each transect three stations, each at the depth of ca. 200, 500 and 1000m, were sampled.

The central portions of sediment subsamples from 33 stations were collected aseptically from the grab. Immediately after collection of samples, surface plating for cultivable fraction of aerobic bacteria was carried out on board onto Zobell's Marine Agar 2216e medium (HiMedia, India) in duplicate after suitable serial dilutions. After incubating for 3 days at 27°C the number of Colony Forming Units (CFU) were counted and expressed as $CFUg^{-1}$ dry sediment weight.

The representative colonies were picked up and isolated by successive streaking and the strains were stored in Zobell's marine agar slants at 4°C. The bacterial isolates were then screened for chitinolytic activity. Chitinolytic

bacteria may be detected by either the production of clearing zones on agar containing chitin or hydrolysis of a fluorogenic substrate analogue of chitin (Cottrell *et al.*, 1999). In the present study, the former method which is more efficient was used. The colloidal chitin precipitate was prepared as proposed by Lingappa and Lockwood (1961) and modified by Booth (1971). Five grams of purified chitin was taken in beaker and 140ml of 50% (v/v) H_2SO_4 was added with continuous stirring in an ice bath for 60 min. The insoluble fraction was filtered off by passing through glass wool and the clear solution was poured into 2 l deionised distilled water in order to precipitate the chitin. The suspension was kept overnight at 4°C, the supernatant decanted out and the sedimented residue was resuspended in deionised distilled water, allowed to settle and decanted. This process was repeated until the *p*H of the liquid became neutral. The colloidal chitin was collected and supplemented in 1000ml mineral medium (K_2HPO_4: 1g, $MnSO_4$, $7H_2O$: 0.5g, NaCl: 0.5g, $CaCl_2$: 0.1g, Fe $(NH_4)_2$ SO_4. $6H_2O$: 5mg, NH_4Cl: 1g, Agar: 15g, *p*H: 7.6, 50% sea water: 1000 ml). The isolated strains were streaked onto the plate and incubated at dark for 2-3 weeks period. Colonies which exhibited clearing zones around them indicating chitin utilization were counted as chitinoclastic bacteria.

Standard bacteriological procedures were carried out to identify the isolates up to generic level following the scheme given by Baumann *et al.* (1972), Buchanan and Gibbons (1974) and Sneath (1986).

Results

Total heterotrophic bacterial (THB) counts ranged from 0.42 to 37.38×10^4 $CFUg^{-1}$ dry sediment weight. Totally 541 isolates obtained from all the stations were screened for the chitinolytic activity. Among all the isolates tested, only 360 isolates (67%) showed the chitinolytic property.

As many as 11 bacterial genera were identified, viz. *Pseudomonas* (19%), *Bacillus* (18%), *Vibrio* (18%), *Micrococcus* (9%), *Corynebacterium* (6%), *Arthrobacter* (5%), *Alcaligenes* (5%), *Cytophaga* (5%), *Flavobacterium* (5%), *Alteromonas* (5%), *Acinetobacter* (4%) and unidentified (1%). But the chitinolytic activity was recorded only in 7 genera (Table 1). Maximum isolates belonged to *Vibrio* (17%) followed by *Pseudomonas* (13%), *Micrococcus* (12%), *Flavobacterium* (10%), *Bacillus* (6%), *Alteromonas* (5%) and *Cytophaga* (4%). However, the percentage occurrence of bacterial genera *vis-à-vis* chitinoclasts (Fig. 2) showed *Flavobacterium* as the most efficient and *Bacillus* as the least efficient chitin degrading bacteria.

Table 1. *Percentage occurrence of bacterial genera and the chitinoclasts*

Bacterial genera	% occurrence	% of chitinoclasts
Pseudomonas	19	13
Vibrio	18	17
Flavobacterium	5	10
Alcaligenes	5	-
Cytophaga	5	4
Alteromonas	5	5
Acinetobacter	4	-
Micrococcus	9	12
Bacillus	18	6
Arthrobacter	5	-
Corynebacterium	6	-
Unidentified	1	-

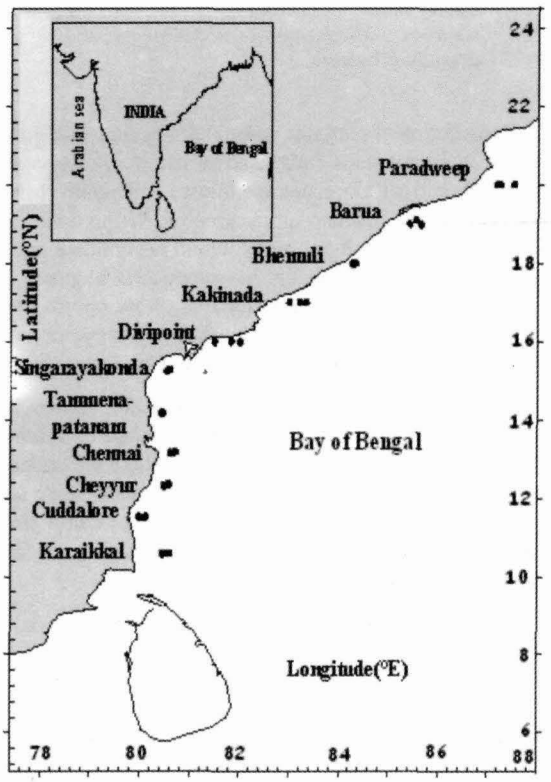

Fig. 1. Study area- continental slope of Bay of Bengal

Discussion

The marine realm rich in chitinous material is an excellent source of chitinoclastic bacteria. The production of large amount of chitinous material has a definite impact

on the associated microbial flora. Microbial flora active in decomposition of chitin has also been enumerated by plate count methods (Zobell and Rittenberg, 1938) and by most probable number (MPN) (Colwell, 1979). Subsequently many kinds of chitin degrading microorganisms have been isolated from marine sand, mud and water (Hock, 1941).

In the present study, 67% bacterial isolates were chitinoclasts and the same level of chitin decomposers was observed in the bottom mud (Okutani and Okaichi, 1971). The higher level of chitinoclasts in the present study may be attributed to the deep sea environment where larger accumulation of chitin occurs. Thus, 200m had more abundance of chitinoclasts than 500 and 1000m because of the temperature difference as maximum chitinase (*in situ*) was reported at 25°C (Zhiying *et al.*, 1999).

Totally 11 genera were identified and the gram negative genera (61%) contributed more than gram positive (39%). It had already been reported that marine environment favors gram negative genera than the gram positive (Nair, 1979). Among the identified genera, *Pseudomonas* was found to be abundant (19%), followed by *Bacillus* (18%) and *Vibrio* (18%). In the marine environment *Pseudomonas* and *Vibrio* are the native and dominant bacterial flora than other genera (Baumann *et al.*, 1972).

The size and qualitative composition of microbial flora largely depends on the interaction of various factors prevalent in the marine environment. Several microbial groups develop and multiply quickly under favourable conditions. Some forms are better adapted to the conditions and have better metabolic capabilities. Such forms thrive well and always dominate. In chitinoclastic group, though members belonging to various genera are present, only some strains always dominate and it is important to study the generic composition of this group in the marine environment. In the present investigation, stations at three depths (ca. 200, 500 and 1000) were found to have

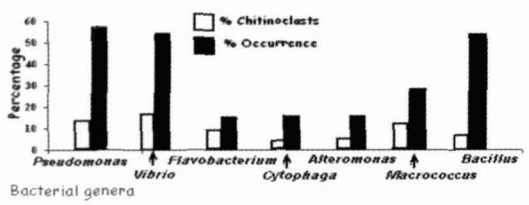

Fig. 2. Total bacterial genera (cumulative % occurrence) *vis-à-vis* chitinoclasts.

chitinolytic bacteria and the overall range observed, was in general, of the same magnitude as reported by Zobell and Rittenberg (1938).

Among the chitinolytic genera, *Vibrio* (17%) was found to be more abundant in the present study followed by *Pseudomonas* (13%) and *Micrococcus* (12%). But, in terms of activity *Flavobacterium* showed more efficiency than the other genera. However, *Vibrio,* a good chitinoclast was dominant in the present study as reported earlier by Svitil *et al.* (1997). *Vibrio* plays a major role in the degradation of chitin in the marine environment, because of their flagellation (both polar and lateral) which can adhere firmly to chitinous particles. Normally, attachment to surface provides enhanced survival and multiplication of *Vibrio* in the marine environment (Cowell, 1979). *Vibrio* can also grow faster than other marine bacteria which enabled it as a good chitinoclast along with other genera. The present study has brought to light the chitinolytic activity of bacteria in the continental slope of Bay of Bengal.

Acknowledgements

The authors are thankful to Prof. T. Balasubramanian, Director, Centre of Advanced Study in Marine Biology for the encouragement, the authorities of Annamalai University for the facilities and the Centre for Marine Living Resources and Ecology, Ministry of Ocean Development, Government of India for the financial support.

References

Baumann, L., P. Baumann, M. Mandel and R.D. Allen. 1972. *J. Bacteriol.,* 110: 402-429.

Booth, T. 1971. *Can. J. Bot.,* 49: 939-949.

Buchanan, R.E. and N.E. Gibbons. (Eds.) 1974. *Bergey's manual of determinative Bacteriology.* 8th Edition. The Williams and Wilkins, Baltimore, 1246 pp.

Colwell, R.R. 1979. Enumeration of specific populations by the most probable number (MPN) method. *In*: Costerton, J.W. and R.R. Colwell (Eds.), American Society for testing and Materials *Native Aquatic bacteria: Enumeration activity and Ecology*, p. 56-61.

Cottrell, M.T., J.A. Moore and D. Kirchman. 1999. *Appl. Environ. Microbiol.,* 65 (6): 2553-2557.

Hock, C.W. 1941. *J. Mar. Research.,* 4 (2): 99-106.

Lingappa, Y. and J.L. Lockwood. 1961. *Nature,* 189: 158-159.

Nair, S. 1979. *Indian J. Mar. Sci.,* 8 (4): 227-231.

Okutani, K. and T. Okaichi. 1971. *Tech. Bull. Fac. Agri.,* Kagawa University, 23 (51): 137-148.

Sneath, P.H.A. 1986. *Bergey's manual of systematic bacteriology*. Vol. 2, The Williams and Wilkins, Baltimore, 1599 pp.

Svitil, A.L., S.M. Ni Chadhain, J.A. Moore and D.L. Kirchman. 1997. *Appl. Environ. Microbiol.*, 63 (2): 408-413.

Zhiying, Li., H. Baoqin, J. Zhiliang and D. Jixun. 1999. *J. Ocean. Univ. Qingdao,* 29 (4): 643-648.

Zobell, C.E. and S.C. Rittenberg. 1938. *J. Bacteriol.*, 35: 275-287.

Received: 27 July 2006
Accepted: 25 October 2006