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SCREENING OF MARINE VIBRIO SP ISOLATED FROM THE BAY OF BENGAL, INDIA FOR CHITINASE ENZYME PRODUCTION

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ABSTRACT

A total of 12 water, sediment and plankton samples were collected from different sampling stations of open sea off the Bay of Bengal coast of Chennai, Tamilnadu, India and 160 isolates of marine Vibrio sp were isolated using Thiosulphate Citrate Bile Sucrose (TCBS) agar medium. The entire isolates were screened for the production of chitinase enzyme on to colloidal chitin agar plates. One of these strains with high ability to produce chitinase was selected and identified as *Vibrio alginolyticus* JN863235 by morphological and biochemical properties along with 16S rDNA partial gene sequence analysis. The production of chitinase by *Vibrio alginolyticus* JN863235 was optimized using different substrate concentrations, pH, and temperature and incubation period. The maximum chitinase production was observed with 0.6% colloidal chitin at pH 6.5 at temperature 30°C after 3 days incubation. Whereas the chitinase production on the media containing colloidal chitin and its combination with glucose, yeast extract, K2HPO4 showed that the highest activity 19.31, 19.28, 17.11 U/ml respectively was achieved on day 3.

KEY WORDS: **marine bacterium, *Vibrio alginolyticus*, medium optimization, chitinase**

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INTRODUCTION

Marine microorganisms are increasingly becoming an important source in the search for industrially important molecules. Today both academic and industrial interests in marine microorganisms are on the rise, because unique and biologically active metabolites have been reported from marine organisms (Imada 2004; Zhang *et al*., 2005). Marine environment contains a wide range of distinct microorganisms that are not present in the terrestrial environment. Most of the organic matter in marine ecosystems consist of compounds of a high molecular weight and polymeric structure, mainly proteins, starch, lipids, pectin, cellulose, chitin, nucleic acids, or lignin (Arnosti *et al*., 1998; Poremba *et al*., 1995; Unanue *et al*., 1999).For heterotrophic bacteria, those high molecular weight biopolymers constitute an important source of carbon, nitrogen, and energy used for biosynthesis or respiration (Brown *et al*., 1996; Patel *et al*., 2000). As polymeric molecules are too large to be directly incorporated into bacterial cells (Hoppe *et al*., 2002), they have to be decomposed by extracellular enzymes into simple compounds (Unanue *et al*., 1999) that can easily diffuse into the periplasmic space (Mallet *et al*., 1999). Many heterotrophic bacteria are known to carry genetic and metabolic potentials to synthesise and control extracellular enzymes, which can degrade and modify large variety of natural polymers in water basins (Mudryk *et al*., 2004). For this reason, according to Boetius *et al*.,1995, Jackson *et al*.,1995, Mallet and Debroas (Mallet *et al*., 1999), enzyme assays can provide powerful tools for studying organic matter degradation and nutrient cycling in aquatic ecosystems.Marine microorganisms have recently emerged as a rich source for the isolation of industrial enzymes (Chandrasekaran, 1997). Marine bacterial enzymes have several advantages for industrial utilisation (Ventosa *et al*., 1995). The optimum activity of marine bacterial enzymes usually occurs at high salinity, making these enzymes utilisable in many harsh industrial processes, where the concentrated salt solutions used would otherwise inhibit many enzymatic transformations. In addition, most marine bacterial enzymes are considerably thermo tolerant, remaining stable at room temperature over long periods.Chitin which is the second most abundant biopolymer on the planet is an insoluble linear polymer of β-1, 4 linked N-acetyl glucosamine (Shahidi and Abuzaytoun, 2005). It is widely distributed in nature as a structural component of crustaceans, fungi, protozoa and insects (Flach *et al*., 1992). Chitinase (EC 3.2.1.14) are glycosyl hydrolases which catalyse the degradation of chitin. These enzyme have a wide range of biotechnological applications such as preparation of pharmaceutically important chitoligosaccharides and N-acetyl-D-glucosamine (Kuk *et al*., 2005), isolation of protoplast from fungi and yeast (Dahiya *et al*., 2006), control of pathogenic fungi (Mathivanan *et al*., 1998) and treatment of chitinase waste (Wang and Hwang, 2001). The enzyme found in numerous bacteria, fungi, insects, plants and animals are involved in natural protection mechanism. More notables among the chitin degrading prokaryotes are gliding bacteria, *Pseudomonas, Vibrio, Enterobacter*, *Actinomycetes*, *Bacillus, Aeromonas, Serratia* and *Clostridia* (Jami al ahmadi *et al* .,2008).Microbial production of chitinase has captured the worldwide attention of both industrial and scientific environments, not only because of its wide spectrum of applications but also for the lacuna of an effective production method (Jami al ahmadi *et al*., 2008). Although 10^{15} metric tons of chitin is produced annually in the aquatic biosphere alone, there is no substantial accumulation of chitin in ocean sediments (Anil Kumar Sing, 2010; Purwani *et al*., 2004). This is because, a bioconversion process is naturally driven by chitinolytic marine bacteria especially *Vibrio species* (Suresh *et al*., 1998). These bacteria utilize chitinous material very efficiently by converting them into organic compounds that then can be used as carbon and nitrogen sources. However such application requires chitinase to be produced in large quantities which in turn optimization of nutritive and physical parameter like pH and temperature for its production by selected isolate (Anil Kumar Sing, 2010).

Generally, chitinase produced from microorganisms is inducible in nature. Extracellular chitinase production is reported to be influenced by media components such as carbon sources, nitrogen sources, and agricultural residues such as rice bran, wheat bran, etc, (Dahiya *et al.,* 2005). Several statistical and non statistical methods are available for optimization of medium constituents (Montgomery, 2002). Before statistical optimization of medium for production of desired product from a new source bacterium it is essential to screen a large number of possible medium constituents. Component replacing is the most commonly used method for screening number of carbon; nitrogen and phosphorous sources (Jatinder *et al*, 2006). This approach can generate information on medium constituents for desired product from organism under study and can also identify new compounds affecting its production. Relatively, the Bay of Bengal, an arm of the Indian Ocean has rarely been explored for microbial diversity and microbial metabolites. Hence, there is an immense possibility to identify new marine Vibrio sp in the Bay of Bengal to discover a novel chitinase enzyme. Accordingly, the present study was aimed to investigate the diversity marine Vibrio sp in the Bay of Bengal with the ultimate objective of discovering novel chitinase enzyme.

MATERIALS AND METHODS

Study area and sampling

The study area covered the Bay of Bengal coast of Chennai, Tamilnadu from different sites of the open sea off Chennai (lat. $9^0$25' and $10^0$10'; long. $76^0$13' and $76^0$30'). A total of 12 water, sediment and plankton samples were collected from different sampling stations as given in Table 1. Water samples were collected in sterile wide mouthed bottles from a depth of 10 to 15 M. Sediment samples were collected using Peterson's grab. From the central portion of the collected sediment samples, about 100 g portions were transferred aseptically into a fresh polythene bag and both water and sediment samples were transported to the laboratory in ice box for analysis. Zooplanktons were collected using a Bongo net of 200μm sieve size. Net was operated horizontally at a constant speed of 20 min. A portion of the plankton samples were transferred aseptically into bottles containing sterile sea water for bacteriological analysis. Other portion of the sample was preserved in 10% neutralised formalin for the identification of major component species.

Physico-chemical analysis of water sample

Temperature, pH, salinity and dissolved oxygen of water samples were noted. Temperature was determined *in situ* with a mercury thermometer. The sample for the estimation of dissolved oxygen was fixed and brought to the laboratory for the analysis following Winkler's method (APHA, 1980). pH and salinity were determined in the laboratory by using digital pH meter (Digison Electronics, Chennai) and Mohr's argentometric titration method (Strickland and Parsons, 1972) respectively.

Isolation of total halophilic bacteria and marine Vibrio

Sea water, sediment and plankton were serially diluted 10 to 1,000 fold in sterile seawater and spread on Trypticase Soy Salt Agar (TSSA) supplemented with 3% sodium chloride and Thiosulphate Citrate Bile Sucrose (TCBS) agar medium for isolation of total Halophilic bacteria and total Vibrio, respectively. The plates were incubated for 48h at room temperature (28 ± 2^0C). Plates with colonies 30 to 300 were counted and multiplied with dilution factor to obtain the Colony Forming Units (CFU) per ml of the sample.

Identification of the isolates

From each TCBS plate a representative population of vibrios were selected randomly ranging from 20-30 colonies. A total of 500 presumptive isolates were further classified into species level as per the scheme of Alsina and Blanch (1994). Out of this 500, 160 representative cultures were selected and the identification tests were done as per the methodology described in Table 2. The identifying characters were cross checked with Bergey's Manual of Determinative Bacteriology (Baumann *et al.,* 1984).

Primary screening of marine Vibrio sp for extracellular chitinase enzyme production

The entire isolated marine Vibrio sp was screened for the production of chitinase enzyme on to colloidal chitin agar plates (Colloidal chitin 12.0 g, (NH4)2SO4 2.0 g, KH2PO4 0.7 g, Na2HPO47H2O 0.2 g, FeSO47H2O 1.0 mg, MnSO45H2O 1.0 mg, agar 15 g, dis.H2O 500ml, aged sea water 500 ml, pH 7.0) and incubated at room temperature/ two weeks. After incubation, a clear zone formating bacteria were selected as chitinase producer (chitin utilizer). Among 17 *Vibrio* sp the isolate *Vibrio alginolyticus* JN863235 was selected for further study of chitinase due to its formation of the largest clear zone on the chitin agar plate.

Taxonomic studies

Isolation of genomic DNA, PCR amplification and sequencing of PCR product for analysis of 16S rRNA were conducted according to Lee *et al*., 2007. A similarity search for the nucleotide sequence of 16S r RNA of the test isolate was carried out using a Blast search at NCBI (http://www.ncbi.nlm.nih.gov).

Nucleotide sequence accession number

The partial 16S rRNA sequence of the isolate was deposited in the GenBank database under accession number JN863235.

Preparation of colloidal chitin

Colloidal chitin was prepared by the method of Rodriguez-kabana *et al* (1983), by partial hydrolysis of chitin (Sigma Chemical Co, USA) with 10 N HCL for 2h at room temperature. The colloidal chitin was washed several times with large volumes of distilled water to adjust the pH to 7.0.

Chitinase assay

Chitinase activity was measured by the release of N-acetyl-D-glucosamine equivalents from colloidal chitin by following the method of Reissig *et al* (1955). The reaction mixture consisted of 1 ml of enzyme preparation and 1 ml of 0.1% (w/v) colloidal chitin in sodium acetate buffer (0.05 M, pH 5.2) and incubated at 37ºC for 2 h. The reaction mixture was centrifuged at 3000 rpm for 3 minutes. 0.1 ml of potassium tetra borate buffer (0.08M, pH 9.2) was added to 0.5 ml of the supernatant and boiled for 3 minutes at 100ºC and cooled. To this 3 ml of diluted dimethyl aminobenzaldehyde reagent was added, incubated at 37ºC for 20 minutes for colour development. A heat killed enzyme following the same procedure was kept as control, and read at 585 nm in Milton Roy 601 Spectrophotometer.

Effect of substrate concentrations on chitinase production

The production medium was prepared with different concentrations of chitin like 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. Fifty ml of the medium were inoculated with 2ml suspension and incubated at 120 rpm at 37˚C temperature.

Screening of essential medium components

Thirty different possible media constituents including 5 different concentration of colloidal chitin source ,5 carbon source,5 nitrogen source,5 phosphorous source, 5 incubation temperature and 5 initial pH of the medium were screened for 5 days for their effect on chitinase production by *Vibrio alginolyticus* JN863235. Effect of different concentration of colloidal chitin was checked with different carbon source by replacing 2% of different test sugars (with chitin) in the basal medium. Nitrogen and phosphorous sources were screened by replacing the corresponding source in the basal medium.

Enzyme production

Cultivation of the isolate for continuous production was carried out in 500 ml production medium in 2 L Erlenmeyer flasks and incubated under 140 rpm in a shaker at 30°C for 6 days. After cultivation, the cells were removed by centrifugation at 5000 rpm for 20 minutes at 4˚C. The clear supernatant was used as crude enzyme extract and measured by absorbance at 585 nm.

RESULTS AND DISCUSSION

Ecology and distribution of Vibrio species in marine environment

Marine environment is the biggest reservoir of chemical and biological diversity. Therefore, research focus on marine environment has been gaining importance in recent years. However, still it has not been fully explored and there is tremendous potential to identify novel organisms with various biological properties. In line with this view, the present research has been initiated to identify novel *Vibrio* sp from Indian marine environment, because its rich microbial diversity has been studied only to a limited extent. Totally 36 different marine samples were collected from various locations of the Bay of Bengal, India (Table.1).

Table 1
Locations of the sampling stations for the collection of water, sediment and plankton samples

Station No.	Location
1	9^0 58.7'N 76^0 10.8'E
2	10^0 0.1'N 76^0 0.9'E
3	9^0 58.7'N 76^0 0.09'E
4	10^0 0.01'N 76^0 0.09'E
5	9^0 58.1'N 76^0 11.2'E
6	10^0 0.5'N 76^0 0.6'E
7	9^0 58.1'N 76^0 0.3'E
8	9^0 58.5'N 76^0 10.7'E
9	10^0 0.2'N 76^0 0.7'E
10	N off Chennai 340^0E-W
11	2.5 km N off I station 340^0E-W
12	2.5 km S off II station 340^0E-W

Among them, a total of 26 *Vibrio* sp were isolated from marine sea water, sediments and plankton. Physico-chemical parameters recorded from the sea water samples collected off Chennai coast are presented in the Table 2.

Table 2
Physico-chemical parameters recorded for water samples collected from various stations off Chennai coast

Station No.	Depth (m).	Temperature (^{0}C)	p^H	Salinity (ppt)	Dissolved Oxygen (ppm)
1	10	28	7.4	30.4	8.4
2	10	27	7.7	35	6.8
3	10	27	7.5	32.1	8.8
4	11	26	7.9	36.74	8.7
5	11	27	7.6	31.01	6.2
6	10	26.5	8.1	34.6	6.4
7	10	27	8.1	34.1	7.8
8	10	27.5	7.9	34.5	8.2
9	10	27.5	7.6	33.1	8.2
10	14	28	8	35.2	7.5
11	14	28	8.1	35.2	5.4
12	14	29	8	33	7.6

Temperature, pH, salinity and dissolved oxygen were in the range of 26-29^{0}C, 7.4-8.1, 30.40- 36.74 ppt and 5.4-8.8 mg l^{-1} respectively. The values recorded in the present study were in accordance with previous data (Balakrishnan and Shynamma, 1976; Pradeep, 1986). Sreeja and Ravindran (1999) also reported similar results from Mangalore coast. *Vibrio* population is not significantly (P<0.05) related to the tested physical parameters like temperature, salinity, pH and dissolved oxygen. Total halophilic bacterial count (THC) varied from 7.0 x 10^3 to 6.4 x 10^5 cfu ml^{-1} and total *Vibrio* count (TVC) from 6.0 x 10^2 to 8.2 x 10^3 cfu ml^{-1} (Table 3). Density of *Vibrio* in the coastal water of Korea was reported to be 0.2 x 10^1 to 9.0 x 10^3 ml^{-1} (Jung and Shin, 1996). Sreeja and Ravindran (1999) reported be 0.2 x 10^1 to 9.0 x 10^3 ml^{-1} in coastal water and 0.8 x 10^1 to 3.0 x 10^1 ml^{-1} in open water off Mangalore coast of India. (Table 3).

Table 3

Bacteriological parameters recorded for water samples collected from various stations off Chennai coast Occurrence of Vibrios in the sediment samples collected from various stations off Chennai coast. Occurrence of Vibrios in plankton samples collected from various locations off Chennai coast

Station No.	Total Bacterial Count	Total *Vibrio* Count	Percentage of vibrios to total bacteria	Total halophilic bacteria	Total *Vibrio*	Percentage of *Vibrio* to total halophilic bacteria	Total Halophilic bacteria	Total *Vibrio*	Percentage of *Vibrio* to Total Halophilic bacteria
	x $10^4 g^{-1}$	x $10^2 g^{-1}$		(x $10^7 g^{-1}$)	(x $10^6 g^{-1}$)		x $10^8 g^{-1}$	x $10^7 g^{-1}$	
1	9.4	48	5.1	18	46	25.5	2.5	4.7	18.7
2	64	82	12.8	12	9.2	7.67	1.4	1.8	12.86
3	0.7	13	18.57	46	31	6.47	5.7	18.4	32.34
4	0.96	6	6.3	52	72	7.74	1.5	3.9	25.83
5	1.8	6	3.33	62	64	10.32	5	5.4	10.8
6	3.4	13	3.82	10	18.3	18.3	3.6	9.4	26.14
7	0.81	9	11.11	81	73	9.01	2	8	40
8	1.6	8.2	5.13	9.2	8.2	8.91	5.5	18.1	32.91
9	1.3	8	6.15	32	37	11.6	11	9.2	8.36
10	1.5	9.8	6.53	43	22	5.12	4.8	11	22.92
11	12	56	4.66	4.1	0.98	23.9	2.4	1.9	7.92
12	2.4	12	5	5.2	2.4	4.62	7.3	18.5	25.34

The percentage of *Vibrio* to the total flora isolated from the sea water varied from 3.33 to 18.57 with a mean value of 6.9%. In 1989, Alavandi, 1989 has reported 5% value from the same area. However, the percentage of Vibrios is on a lower side when compared to the earlier report that 22% of the bacterial flora of Cochin backwaters was constituted by *Vibrio* (Chandrika and Nair, 1994). *Vibrio* as a dominant flora with incidence 35% preponderance in sea water and sediment collected from Madras coastal waters was also established (Prabhu *et al*., 1991).Sediment samples collected from twelve stations off Chennai area were analysed for THC and TVC and the average values were tabulated in Table 4. In sediment sample, as in sea water, *Vibrio* load is significantly related to total halophilic bacteria ($r=0.71$; $P<0.05$). Percentage of *Vibrios* to total bacteria varied from 4.2 to 25.5% with a mean value of 11.59. THC was also high ranging from9.2 x 10^7 to 8.1 x 10^8 cfu g^{-1}. This corroborates with the earlier report of 22% of the total flora from sediment of Cochin area (Chandrika and Nair, 1994). Similarly, *Vibrio* comprising 35% of total flora was reported from water and sediment of Madras coast (Prabhu *et al*., 1991). Generally sediment provide better micro environment than water and thus rich flora can flourish. It was reported earlier that the flora of sediment was 3 times (Williams and LaRock, 1985) and 10 times (Pagnocca *et al*., 1991) higher than the water. This high value might be due to the comparatively higher nutritional status, availability of substrate for attachment or the positive interactive effect of organisms present in the sediment. Preferential chitinoclastic activity of *Vibrio* gave selective and advantage over other bacteria to flourish in the chitin rich sediments (Ivanova *et al*., 1993; Montgomery and Kirchman, 1994).Zooplankton harboured heavy load of *Vibrio* with a highest concentration of 1.8 x 10^8 cfu g^{-1}. Heterotrophic bacterial count of the plankton were also found to be high, with value ranging from 1.4x10^8 to 1.1x10^9 cfu g^{-1} (Table 5). *Vibrio* constituted 7.92 to 40.0% (mean value is 24.18) of the total bacteria attached to zooplanktons. Colwell (1994) also established association of *Vibrio* with plankton. Zooplankton blooms might provide favourable microcosm, supplementing substrate for attachment, growth factors and other nourishment as their exocrine. Chitin degrading ability of Vibrios was extensively studied by Ivanova *et al.,* (1993). *Vibrios* were found to attach on the zooplankton due to its chitinoclastic nature (Montgomery and Kirchman, 1994). The relative frequency of *Vibrio* species in different marine samples collected off Chennai was tabulated in table 6.

Table 6
Relative frequency of Vibrio species in different marine samples collected off Chennai

	Vibrio spp.	Sea water	Sediment	Plankton
	Number of samples	12	12	12
1	*V. alginolyticus*	9	8	7
2	*V. campbellii*	8	7	7
3	*V. carchariae*	1	--	--
4	*V. cholerae*	--	2	--
5	*V. cincinnatiensis*	3	--	2
6	*V. costicola*	--	--	--
7	*V. damsela*	--	--	--
8	*V. fluvialis*	--	3	--
9	*V. furnissii*	--	2	--
10	*V. harveyi*	4	8	6
11	*V. hollisae*	--	--	2
12	*V. logei*	7	--	--
13	*V. marinus*	--	1	4
14	*V. mediterranei*	4	3	8
15	*V. metschnikovii*	--	--	--
16	*V. mimicus*	6	9	--
17	*V. natriegens*	--	--	--
18	*V. orientalis*	8	9	--
19	*V.parahaemolyticus*	7	11	10
20	*V. pelagius I*	--	2	--
21	*V. pelagius II*	4	6	--
22	*V.. proteolyticus*	--	--	--
23	*V. splendidus I*	--	--	--
24	*V. splendidus II*	--	--	7
25	*V. vulnificus*	1	9	7
26	*V. vulnificus B2*	--	--	--

Primary screening of marine Vibrio sp for extracellular chitinase enzyme production

With the growing awareness on environmental protection, the use of enzymes, gained considerable attention in many industrial processes. In recent years, the microbial enzymes have been replacing chemical catalysts in manufacturing chemicals, textiles, pharmaceuticals, paper, food and agricultural chemicals. Enzyme-based industrial bioprocess now directly competes with established chemical-based process within the processed foods, pharmaceutical and allied fermentation industries. The entire isolated marine Vibrio sp was screened for the production of chitinase enzyme on to colloidal chitin agar plates at different temperature results were tabulated in table 7.

Table 7
Chitinase enzyme production at different temperatures by various Vibrio species isolated during the present study

Number of strains producing chitinase enzyme at the temperatures						
Vibrio species	No of strains tested	6 ± 1^0C	10^0C	28 ± 1^0C	37^0C	42^0C
V. alginolyticus	10	0	0	10	10	2
V. campbellii	10	0	0	4	6	1
V. carchariae	5	0	0	5	5	0
V. cincinnatiensis	10	0	0	4	4	0
V. damsela	10	0	0	0	0	0
V. fluvialis	10	0	0	6	6	2
V. furnissii	10	0	0	0	0	0
V. harveyi	10	0	0	8	7	0
V. hollisae	5	0	0	5	3	0
V. logei	10	0	0	8	6	0
V. metschnikovii	10	0	0	8	8	0
V. mimicus	10	0	0	8	6	0
V. orientalis	10	0	0	6	6	0
V. parahaemolyticus	10	0	0	8	8	0
V. pelagius II	10	0	0	8	7	0
V. splendius II	10	0	0	4	4	0
V. vulnificus	10	0	0	4	6	0
Total strains	160	0	0	96	94	5

A total of 160 isolates of *Vibrio* comprising 17 prevalent species were tested for the production of chitinase enzymes at temperatures ranging from 6±2^{0}C to 42^{0}C. Maximum number of strains produced chitinase when the plates were incubated at 37^{0}C and at the room temperature. Of the 17 species, 15 were capable of producing chitinase. Among the isolate *Vibrio alginolyticus* JN863235 was selected for further study of chitinase due to its formation of the largest clear zone on the chitin agar plate. The isolate was identified as *Vibrio alginolyticus* JN863235 based on the morphological, physiological and biochemical characteristics and 16 S rRNA sequence analysis.

Screening of essential medium components

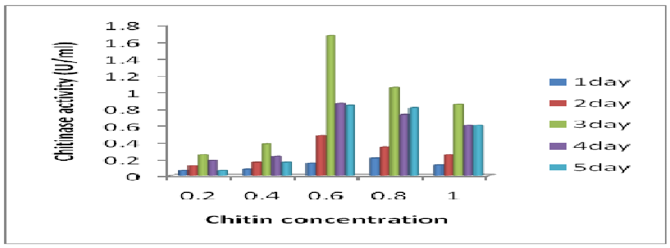

Figure1
Production of extracellular chitinase by Vibrio alginolyticus JN863235on different concentration of colloidal chitin

Various chitin sources were tested for chitinase production. 0.6% of the colloidal chitin was proven to be the best carbon source for chitinase production (fig.1) at pH 6.5 at temperature 30°C after 3 days incubation (fig 2).

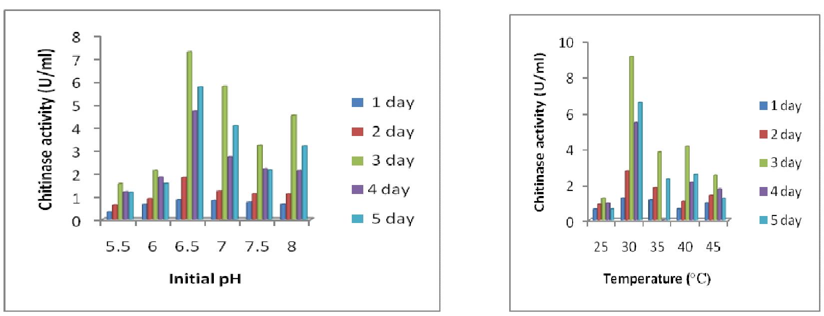

Figure 2
Production of extracellular chitinase by Vibrio alginolyticus JN863235on different pH and temperature (°C)

A part from chitin we have checked other carbon source. Glucose also supported chitinase production followed by maltose to some extent while in the presence of fructose and dextrose, chitinase production was very less (fig.3) appreciable chitinase activity was observed. Andronopoulou and Vorgias (2004) previously reported colloidal chitin as the best source for chitinase production by *Thermococcus chitonophagus*. Glucose was found to repress chitinase production in all the strains of *streptomyces* .NK1057, NK528 and NK951 (Nawani and Kapadnis, 2004), addition of glucose contributed to the obtaining of maximum chitinase activity in *Vibrio alginolyticus* H-8 (kazoo Ohishi *et al.*, 1996).Nitrogen source are very important for the microbial growth and to maximize the final reaction product next to carbon. Nitrogen sources the medium supplemented with beef extract, peptone, casein, yeast extract and gelatin at 0.1% along with glucose as a carbon source to the production medium.

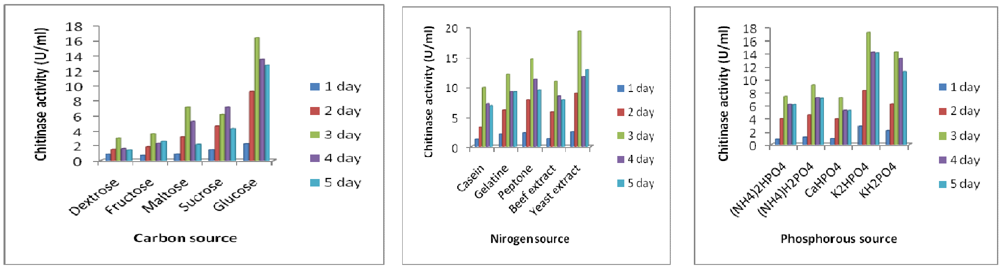

Figure 3
Effect of different carbon, nitrogen and phosphorus source on chitinase production

Among the various nitrogen sources involved in reaction the results were shown Yeast extract has the significant increasing order than other sources added to the medium followed by peptone. Gelatin also supported the chitinase production to some extent while in the presence of beef extract and casein, chitinase production was less(fig.3).Addition of yeast extract to the medium reported to enhance chitinase enzyme production in *serratia marcescens* (Kannan Natarajan and Ramachandramurty) and A*spergillus cameus.* Addition of yeast extract has been reported to increase chitinase activity in *Alcaligenes xylosoxydans* and *Paenibacillus Sabina* strain JD2 (Vaidya *et al*., 2001; Patel et al, 2007). Gohal *et al*. (2006) had reported significant influence of urea, peptone and yeast extract on chitinase production by *Pantoea dispersa.* K2HPO4 was identified as the best phosphorus source for chitinase production by *Vibrio alginolyticus* JN863235 (fig.3). Similar results were observed in *Paenibacillus* sp.D1 (Singh, 2010), Nawani and Kapadnis (2004) reported increase in concentration of K2HPO4 exhibited a little effect on chitinase production in all the strains of *Streptomyces* sp NK951, NK1057 and NK5289.

CONCLUSION

Marine vibrios are metabolically active more vigorously in the marine environment, which leads to the production of chitinase enzymes. With respect to present results and comparison with our best knowledge about other chitinase producers, this isolate has capability for the production of novel chitinase. This microorganism may be useful for treatment of chitinous waste and also for the production of different product of hydrolyzed chitin for various applications. It would be necessary to study the purification, characterization of the enzyme in future.

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