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Deproteination of Shrimp Shell Wastes using Immobilized Marine Associated Pseudomonad Amet1776

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Chitin is a naturally abundant amino polysaccharide found in the shell of crustaceans, insects etc. It has become of great interest because of their biological, industrial and biomedical applications. Shrimp by product has become available in abundance in India in the last few years. The conventional demineralization, deproteination and decoloration method of extraction of chitin from crustacean waste is costly and causes environmental problems. In this study bioconversion of chitinous material has been proposed as a waste treatment alternative to the disposal of shellfish wastes. A total of 79 bacteria were isolated from different marine samples samples collected in Kanathur Chennai. Among 79, 13 have produced fluorescent pigments in Kings B Agar Medium. Primary screening for enzyme production revealed that 22 strains were having the ability to show chitinase activity and 54, 41 and 35 strains exhibited proteolytic activity when skimmed milk, gelatin and casein were used as substrates respectively. From the primary screening, 8 strains were shortlisted based on their ability to produce fluorescent pigments and strong chitinase and protease activity. Selected 8 strains alone subjected to secondary screening where quantification of chitinase and protease, chitinase and protease activity in high salinity and shelf life during immobilized state was analyzed and found that threestrains namely AMET1756, AMET 1767 and AMET1776are the most suitable for deproteination of shrimp shell wastes. However, all the eight bacterial strains were immobilized with sodium alginate and tested for their effect on deproteination of shrimp shell powder (SSP). It has been observed that a protein removal of 44.20% was observed after 7 days of incubation for AMET 1776 and 40.80 % using AMET 1767. Both the strains have retained their deproteinization potential even after reuse of the same beads (22.70% and 23.20%) respectively. Among these strains, AMET1776 was selected and identified as *Pseudomonas* sp. Thus, the study increases the scope of using these two efficient marine associated fluorescent pseudomonads for the deproteination of shrimp shell wastes.

Key words: Chitin, shrimp shell waste, bioconversion, immobilization, *Pseudomonads.*

Chitin is a high molecular weight polysaccharide, composed primarily of N-acetylglucosamine (NAG), units linked by β-1,4glycoside bonds[35].It is the second most naturally occurring polymer derived fromthe exoskeleton of crustaceans, insects and fungi (Halder *et al*., 2012; Kandra *et al*., 2012).Chitin and its product has a wide application in the field of waste water treatment (No &Hur, 1998; Lora & Brennan, 2009), cosmetics (Felse& Panda, 1999),

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health care (Suresh &Chandrasekaran, 1998; Franca *et al*., 2008), agriculture (Ait *et al*., 2004; Wang *et al*., 2005), biotechnology (Gentili *et al*., 2006; Mati-Baouchea *et al*., 2014) and food (Yen *et al*., 2009; Van den Broek *et al*., 2014).Chitin degradation and recycling helps in maintaining global carbon and nitrogen circulation(Hoang *et al*., 2011).

About 10^{11} tones of chitin is produced annually in the aquatic biosphere alone (Wang *et al*., 1998). 100 billion tones of chitin are produced every year by crustaceans, molluscs, fungi and insects. Chitin is also a major source of surface pollution in coastal areas because of the crustacean shell wastes (Madhavan, 1992; Zhai& Hawkins, 2002; Gimeno *et al*., 2007). Global production of crustacean shells has been estimated to be 1.9 million metric tons (Peberdy, 1999). Only 65% of the shrimp is edible,and the remainder (cephalothorax and exoskeleton) is discarded as inedible waste. These potential renewable resources are however, discarded through ocean dumping, incineration and land filling with not only complete disregard to economical costs but also leading to environmental pollution and resource depletion (Rattanakit *et al*., 2002).

Shrimp by-products have been identified as an animal protein source of great potential; also, as an important source of chitin (Shahidi and Synowiecki, 1991). Bioconversion of chitinous materials has been proposed as a waste treatment alternative to the disposal of shellfish wastes. The enzymes proteases and chitinases have very essential role in the biological treatment of chitinous shell wastes (Manni *et al*., 2009). Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity and their susceptibility to genetic manipulation. Microbial proteases account for approximately 40% of the total worldwide enzyme sales (Godfray and West, 1996) and are preferred to plant and animal proteases since they possess almost all the characteristics desired for biotechnological applications (Rao*et al.*, 1998). Proteases can be used for the deproteinization of crustacean shells for the production of chitin. Several workers have used bacterial protease for the removal of proteinous substances from the chitinous shell wastes (Oh *et al*., 2008, Pacheco *et al*., 2010, Liua *et al*., 2014). Chitinase also play an important role in Immobilization of whole cells for the production of extracellular enzymes. It offers many advantages such as the ability to separate cell mass from the bulk liquid for possible reuse, facilitation of continuous operation over a prolonged period and enhanced reactor productivity (Zhang *et al*., 1989; Galazzo and Bailey, 1990). In this scenario, the present work has been initiated with the following objectives. (1) Isolation of chitinolytic marine bacteria from shell wastes. (2) Selection and identification of efficient bacteria with both chitinoylytic and proteolytic potentials. (3) Immobilization of selected efficient bacteria and evaluation of their role in deproteinization of chitinous shell wastes.

MATERIALS AND METHODS

Materials

Bovine Serum Albumin, L-Tyrosine and N acetyl glucosamine were purchased from Sigma Chemicals Co. USA; Shrimp shell wastes were obtained from local restaurants and fish market (Kanathur, Chennai, India). All other biochemicals were of AR grade and obtained from SD Fine chemicals (Mumbai, India) or Himedia (Mumbai, India) unless stated otherwise.

Chitin and colloidal chitin preparation

Shrimp shell waste collected (during 2010) from a local restaurant and fish market, was washed with warm tap water to remove adhering materials and remaining muscle particles. The shells were then dried and ground to coarse powder with a grinding mill. The extraction of chitin from shell waste was performed following the methods of No &Meyers (1995). The chitin obtained was then converted into colloidal chitin using the method of Skujins*et al.* (1965).

Isolation of bacteria from shrimp shell wastes

A total of 12 marine associated samples such as crustacean shell wastes, intertidal sediments and coastal water were collected from three different places within the Kanathur Beach, Chennai, India (12°50’53.72"N, 80°14’55.36"E). Isolation of bacteria were carried out in Kings B Agar medium (Peptone 20 g; Glycerol 20 g; K_2HPO_4 1.5 g; $MgSo_4$ 1.5 g; Agar 18 g; Distilled water 1000 ml; pH 7.2) and sub-culturing was performed in Nutrient Agar (Peptone 5 g; Yeast extract 3 g; NaCl 5 g; Agar 18 g; Distilled water 1000 ml; pH 7.0)

slants at 4°C**.**

Screening of marine associated bacteria for chitinase and protease activity

For screening of chitinase producing bacteria, 0.5% colloidal chitin agar (CCA) medium consisting of (g/l) colloidal chitin, 5.0; yeast extract, 0.5; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.3 and KH_2PO_4, 1.36 pH 7.0 was used.Test bacterial strains were spot inoculated on the plates and incubated at room temperature (35±2°C) for 3 days. After incubation, plates were flooded with 0.3% congo red solution for 15 mins in a shaker. And then the plates were washes in running tapwater and destained using 1N NaCl. Chitinase activity was observed as a zone of clearance around bacterial growth amidst reddish background. Protease activity of all the bacteria was performed qualitatively on three different substrates (gelatin, skimmed milk and casein). Half strength nutrient agar medium supplemented with different substrates for protease at a final concentration of 0.5% was prepared and plated. Test bacterial strains were spot inoculated on the plates and incubated at room temperature for 3 days. After incubation, plates were flooded with saturated ammonium sulphate solution prepared in 1N HCl. Protease activity was observed as a zone of lysis around bacterial growth.

Quantification of protease and chitinase

Test bacterial strains were grown for 3 days in nutrient broth medium supplemented and cell free supernatants were obtained through centrifugation (1000o rpm 10 mins at 4°C). Protease assays was done as described by Wang *et al.* (2006) where one unit of protease activity was defined as the amount of enzyme required to release 1 ¼mol of tyrosine per min at reaction conditions (Wang *et al*. 2008). Protein content in the culture filtrates were measured by Bradford method using Bovine Serum Albumin as standard (Bradford, 1974). Test bacterial strains grown for 5 days in a liquid medium containing (g/l) chitin 5.0; yeast extract, 0.5; $(NH_4)_2SO_4$, 1.0; $gSO_4 \cdot 7H_2O$, 0.3 and KH_2PO_4, 1.36 and cell free culture filtrates were analyzed for chitinase enzyme as described by Kim *et al*. (2003) where one unit of enzyme activity was defined asthe amount of enzyme that catalyzed the release of 1 µmol of N-acetyl glucosamine per ml in 60 min.

Effect of Salinity of Enzyme Activity

Nutrient Broth medium containing 0%, 3%, 6% and 9% of NaCl was prepared and 100 µl inoculums of selected strains were added to 5 ml of liquid medium in 20 ml test tube and incubated for 48 hr at 30 ± 2 °C on a rotary shaker (180 rpm). Subsequently the culture filtrate was collected by centrifugation at 10000 rpm for 10 min. Both chitinase and protease activity was tested using qualitative plate assay and zone of clearance was considered to grade the bacteria.

Immobilization of bacterial cells

2g Alginate was dissolved in 100 ml of boiling water and autoclaved at 121°C for 15 min. Cells were harvested during the mid-logarithmic growth phase by centrifugation (10000 rpm, 10 min), resuspended in 0.2% sterilized alginate solution. This alginate/cell mixture (with stirring) was extruded drop by drop into a cold, sterile 0.2 M $CaCl_2$ solution through a sterile 5 ml pipette in a laminar air flow chamber. Gel beads of approximately 5 mm diameter were obtained. The beads were hardened by re-suspending into a fresh $CaCl_2$ solution for 24 h at 4°C with gentle agitation. Finally these beads were washed with sterile distilled water to remove excess calcium ions and un-entrapped cells.

Effect of immobilized bacteria on deproteination of shrimp shell powder (SSP)

Ten immobilized bacterial cell beads were transferred to 30 ml of medium containing (g/l) shrimp shell powder 30.0; yeast extract, 0.5; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.3 and KH_2PO_4, 1.36. The culture was grown with agitation (180 rpm) and at 1, 3, 5 and 7 days respective samples were taken for protease and chitinase activity as described previously.The supernatant after determined period (1, 3, 5 and 7 days) were centrifuged at 10,000 rpm for 10 min. The precipitate was dried and analyzed for protein content using Bradford method to find the degree of deproteination. For determining 0% deproteination, 10 mg of the shrimp shell powder was mixed with 10 ml of distilled water, it was mixed well and 1 ml of the solution was used for the measurement of the protein content. 100% deproteination was calculated by complete deproteination of the shrimp powder using 1N NaOH (w/v) for 2 h at 65°C solid: solvent (1:10, w/v).

Reuse of the immobilized beads for the deproteination of SSP

After completion of first batch of

experiments as mentioned above, the immobilized beads containing cells were first washed in sterile distilled water and then transferred to 30 ml of fresh medium with shrimp shell powder in Erlenmeyer flask and incubated further for 7 days in rotary shaker with agitation (180 rpm) at 35°C. Deproteination efficiency was calculated as described above.

Biochemical test of the efficient bacterium

The efficient strain AMET1776 was tested for selected biochemical and staining techniques as described by Cappuccino and Sherman (2004) and the results were interpreted with the key provided in the Bergy's Manual of Determinative Bacteriology (Holt *et al*., 2004).

RESULTS

Chitin and colloidal chitin preparation

22.8 g of chitin was prepared from 80g of the shrimp shell powder. The efficiency of chitin preparation was found to be 28.5%. 15 g of chitin powder was then converted into the colloidal chitin from which 6.2 g of colloidal chitin was recovered at the end of the process.

Isolation and primary screening of marine associated bacteria for chitinase and protease activity

A total of 79 bacteria were isolated from the samples collected from Kanathur Beach, Chennai, India. Out of 79 isolates thirteen were found to have produced the yellow green or blue green fluorescent pigments in King's B agar medium. From the 79 bacterial isolates, 22 bacteria were found to exhibit chitinolytic activity on CCA (Fig. 1.). Among the 22 chitinolytic bacteria, AMET1742 showed the maximum chitinolytic activity with the Zone of lysis (ZOL) measuring 2.6 cm. While screening for protease activity a total of 54, 41 and 35 isolates exhibited proteolytic zones in skimmed milk, gelatin and casein amended media.

Measurement of Enzyme Activity

Based on the primary screening for both chitinase and protease enzyme activities, eight bacterial isolates such as AMET1034, AMET1039, AMET1054, AMET1056, AMET1067, AMET1069, AMET1076 and AMET1077 were selected for further studies. These eight bacteria alone were subjected to quantitative measurement of both the enzymes. Among the eight, AMET1769 and AMET 1777 showed the maximum chitinase specific activity of 0.35 U/mg (Table 1) and showed the maximum specific protease activity of 7.23 U/mg protein.

Effect of salinity on Chitinase and protease activity

Since all the bacteria were isolated from samples collected in saline environment, the effect of salinity on the enzyme production was also studied. All the isolates showed chitinase activity at 0% of NaCl. The chitinase activity of the isolates decreases with the increasing concentration of NaCl. AMET1734, AMET1756, AMET1767, AMET1776 and AMET1777 lost their chitinase activity at 3% NaCl concentration onwards. The chitinase activity of AMET1739 and AMET 1754 were lost only after 6 % of NaCl concentration (Table 2) which indicates their potential in enzyme production even in saline environment. Similarly, strains AMET1734, AMET1756, AMET1776 and AMET1777 showed widest range of protease activity up to 12% of NaCl concentrations while strains AMET 1739 & AMET1754 showed no

Table 1. Quantification of chitinase and protease activity of the eight selected bacterial strains

Strain	Chitinase activity		Protease activity	
No.	Total activity (U/ml)	Specific activity (U/mg protein)	Total activity (U/ml)	Specific activity (U/mg protein)
AMET 1734	0.059	0.164	0.277	4.629
AMET 1739	0.08	0.307	0.163	1.561
AMET 1754	0.045	0.105	0.4	4.888
AMET 1756	0.074	0.246	0.2	2.469
AMET 1767	0.08	0.297	0.363	5.05
AMET 1769	0.084	0.35	0.227	3.156
AMET 1776	0.08	0.297	0.81	7.052
AMET 1777	0.084	0.35	0.144	7.238

protease activity above 6% of NaCl. The protease activity of AMET1767 & AMET1769 was lost above 9% of NaCl concentration.

Effect of immobilized bacteria on deproteination of shrimp shell powder (SSP)

The immobilization of all the eight selected bacteria was done and found that the beads are of good quality, uniform in size and having desirable hardness. Deproteination of SSP was studied by incubating the SSP with the immobilized beads of the selected eight isolates for a period of seven days. The deproteination efficiency (%) of different treatments with immobilized cells in fresh condition up to 7 days was represented in Table 3. The strain AMET1776 has produced higher deproteination efficiency

Table 2. Protease and Chitinase activity of the eight selected strains at different salinity (plate assay)

Strain No	Protease activity (diameter in cm)					Chitinase activity (diameter in cm)				
	0% NaCl	3% NaCl	6% NaCl	9% NaCl	12% NaCl	0% NaCl	3% NaCl	6% NaCl	9% NaCl	12% NaCl
AMET 1734	1.4	1.6	1.1	1.1	1	0.7	0	0	0	0
AMET 1739	1.3	0.8	0	0	0	1.2	1.4	1.2	0	0
AMET 1754	1	0.8	0	0	0	1.4	1.3	1.4	0	0
AMET 1756	1.2	0.9	0.8	0.8	0.8	0.7	0	0	0	0
AMET 1767	1.4	0.6	0.5	0	0	1.6	0	0	0	0
AMET 1769	1	0.7	0.5	0	0	1.3	1.4	0	0	0
AMET 1776	1.5	0.9	0.9	0.7	0.7	1.4	0	0	0	0
AMET 1777	1.4	1.1	0.9	0.7	0.7	1.5	0	0	0	0

Table 3. Comparative study of deproteination using fresh and used immobilized bacteria

Strain No.	Deproteination efficiency (%) of Immobilized beads (Fresh)				Deproteination efficiency (%) of Immobilized beads (Used)			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
AMET 1734	13	18.90	29.20	38.20	8.20	11.20	18.20	22.70
AMET 1739	15.80	21.50	28.90	31.60	9.60	14.10	14.70	19.60
AMET 1754	11.50	18.50	26.10	30.40	7.20	10.20	15.60	19.20
AMET 1756	14.20	20.40	30.00	38.90	8.90	14.60	18.80	26.10
AMET 1767	15.80	21.10	31.50	40.80	11.20	15.30	19.20	23.20
AMET 1769	13.90	19.20	30.80	37.60	7.40	10.60	14.30	19.80

Table 4. Protease activity of fresh and used immobilized bacterial cells during the deproteinization of SSP

S. No	Isolate code	Protease activity of fresh beads		Protease activity of used beads	
		Total Activity (U/mL)	Specific Activity (U/mg protein)	Total Activity (U/mL)	Specific Activity (U/mg protein)
1.	AMET 1734	0.239	3.87	0.224	3.17
2.	AMET 1739	0.217	4.24	0.172	3.41
3.	AMET 1754	0.257	5.26	0.215	3.54
4.	AMET 1756	0.214	2.16	0.202	2.08
5.	AMET 1767	0.224	3.22	0.210	3.25
6.	AMET 1769	0.247	4.07	0.221	3.54
7.	AMET 1776	0.247	4.27	0.226	3.27
8.	AMET 1777	0.214	3.08	0.297	3.19

TA- Total Activity; SA- Specific Activity

Table 5. Chitinase activity of fresh and used immobilized bacterial cells during the deproteinization of SSP

S. No	Isolate code	Chitinase activity of fresh beads		Chitinase activity of used beads	
		Total Activity (U/mL)	Specific Activity (U/mg protein)	Total Activity (U/mL)	Specific Activity (U/mg protein)
1.	AMET 1734	0.034	0.326	0.031	0.307
2.	AMET 1739	0.054	0.229	0.052	0.257
3.	AMET 1754	0.066	0.211	0.034	0.214
4.	AMET 1756	0.036	0.227	0.034	0.164
5.	AMET 1767	0.058	0.225	0.44	0.222
6.	AMET 1769	0.042	0.204	0.031	0.162
7.	AMET 1776	0.092	0.217	0.057	0.227
8.	AMET 1777	0.058	0.217	0.037	0.222

TA- Total Activity; SA- Specific Activity

(44%) up to 7 days after incubation. Similarly, the deproteination efficiency of the immobilized beads for the second use was also tested and results are compared with fresh beads. The re-used cells of strain AMET 1756 has produced higher deproteination efficiency (24%) followed by the straiun AMET1776 which produced 22.70% after 7 days (Table 3). However, in general all the strains have retained at least 60% of their deproteination efficiency when re-used.

Quantification of protease and chitinase during on deproteination of shrimp shell powder (SSP) by fresh and used immobilized cells

The protease activity of fresh and used immobilized bacterial cells during the deproteinization of SSP was studied up to 7 days and results are represented in table 4. The strain AMET 1754 has produced a maximum of 5.26 U/mg protease specific activities when used for the first time and the same has produced 3.54 U/mg protease specific activities. The fresh beads of strain AMET1776 has produced specific protease activity of 4.27 U/mg and the also has produced specific protease activity of 3.27 U/mg when re-used. Similarly, the chitinase activity of fresh and used immobilized bacterial cells during the deproteinization of SSP was studied for 7 days and results are represented in table 5. The strain AMET 1734 has produced 0.326 U/mg highest chitinase specific activities when used for the first time and the same has produced 0.307 U/mg protease specific activities. The fresh beads of strain AMET1776 has produced specific chitinase activity of 0.217 U/mg and the also has produced specific protease activity of 0.227 U/mg when re-used.

Selection and identification of efficient bacterium

Based on the quantitative activity of chitinase and protease, the efficiency of protein removal a strain designated as AMET1776 was

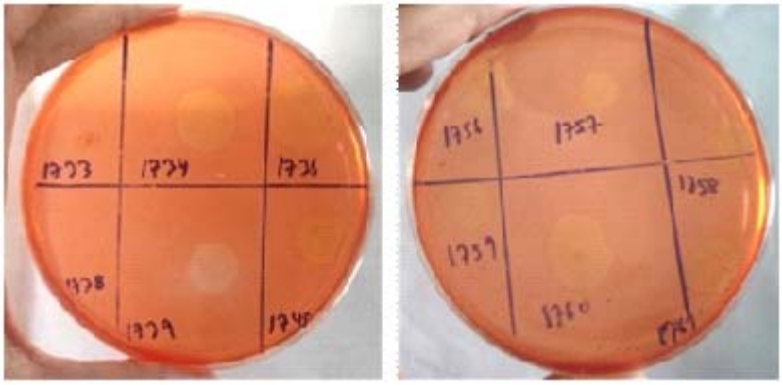

Fig. 1. Screening of marine associated bacteria for chitinase production

found to be the most suitable for the deproteination of SSP. Since the best performing strains in different parameters were not consistent in all the characteristics, though not the best in all tests, AMET1776 was selected as a potential strain and subjected to identification using biochemical tests. AMET1776 is a gram negative, non spore forming, rod shaped bacterium and tested positive for catalase activity. It exhibited positive result for gelatin liquefaction, casein and skimmed milk hydrolysis. AMET1776 is indole negative, citrate positive and MRVP negative. It grows well in Cetrimide agar and produces yellow green fluorescent pigments in King's B Agar Medium. The results have convincingly proved that both the strain AMET1776 is *Pseudomonas* sp.

DISCUSSIONS

Shrimp aquaculture in India has grown to a great extent in the last few years due to excessive global demand. Asian countries accounts for 80% of the shrimp production, with India being the pioneer in the field (Chakrabarti, 2002; Kandra *et al*., 2012). Out of 85 species of shrimp in Indian waters, 55 species are either commercially important or have high local/international demands (Ramyadevi *et al*., 2012). Shrimp industry in India generates a large volume of waste material. In India, 1,25,000-1,50,000 tones of shrimp wastes are discarded per year by the shrimp processing industry alone (Sachindra *et al*., 2006). The conversion of these waste material into value-added forms like chitin, chitosan etc. will not only be economic value but also be environmental friendly.

Crustacean shells mainly contain about 20~30% chitin on a dry basis (Acousta*et al.*, 1995). About 1.2 x 10^5 metric tons of chitin is annually accessible from shellfish waste on worldwide basis (Knorr, 1991). Methods of production of chitin vary considerably; however, they all invariably involve deproteinization and demineralization of the crustacean shells.The better economic use can be the conversion of chitin to chitosan which includes N-deacetylation (Williams and Tverezovskaya, 2013). Chemical treatments by acids and bases are used in most research on deproteination of SSP. Some drastic treatments with highly concentrated acids may result in modification such as depolymerization and deacetylation of the native chitin (No and Meyers, 1995). Hence in this study, optimum demineralization was achieved by constant stirring of the dried ground shrimp shell with 1N HCl for 30 min at ambient temperature and a solid to solvent ratio of 1:15 (w/v) (No *et al*., 1989). We have obtained an appreciable amount (22.8 g) of chitin from 80 g of the shrimp shell powder with an efficiency of 28.5%. Similar trend was reported by Nair and Madhavan, 20.5% chitin in body shell of crab *Scylla serrata*. Thirunavukkarasu *et al*. (2011) have reported relatively lower chitin yield 10.62% in mantis shrimp, *Oratosquilla quinquedentata*. Shrimp Shell mixed with crab shell powder medium has also been used in many studies (Oh *et al*., 2000; Wang *et al*., 1995; Wang & Chang, 1997; Chang *et al*., 2003; Wang &Yeh, 2006).

There were few studies on the use of proteolytic enzymes for the deproteination of crustacean wastes. Broussignac, 1968 demonstrated that use of papain, trypsin or pepsin produced chitin with as little deacetylation as possible. Tuna proteins, papain and few bacterial proteins have also been used for the deproteination step. Bustos and Muchael (2004) have compared the effects of microbial and enzymatic deproteination and found a maximum value of 82% deproteination was achieved with *P. Maltophila a*fter seven days of incubation. When purified microbial protease was used no more than 64% deproteination was achieved under the same conditions. Microorganisms seem to best alternative to harsh chemical treatment for deproteination of Shrimp shell waste.

Pseudomonads are group of bacteria which produces extracellular proteases and chitinase (Aji *et al*., 2006). Among Pseudomonads, *Pseudomonas* sp. has been reported to be an excellent producer of both protease as well as chitinase. Fluorescent pseudomonads (FPs) are one of the important environmental microbial communities which were reported to number of beneficial properties. There are very few reports available in the past about the chitin degrading fluorescent pseudomonads (Wang *et al*., 1995, 1997, 2008). Ajit *et al*. (2006) have studied the extracellular chitinase activity in fluoroscent pseudomonads and found that it has antifungal activity against *Fusarium oxysporum*. In the present study, 16.5%

of the isolates were FPs. Among the six FPs which showed chtinolytic activity, AMET 1776 showed maximum chitinolytic activity (Table 1). Chitin from shrimp shell is reported to be an excellent material for production of chitinases (Chang *et al*., 2010; Hoang *et al*., 2011). Study also suggests colloidal chitin as an inducer of chitinase (Zarei *et al*., 2011).

Numerous investigators have studied the protease activity of *Pseudomans* in deproteination of shrimp shell waste (Oh *et al*., 2000; Ghorbel-Bellaaj *et al*., 2011). Triki-Ellouz *et al*. (2003) have demonstrated very high activity of protease (7,800 U/ml) activity of *Pseudomonas aeruginosa* MN7in media prepared with fish raw material. Previous reports have much convincingly proved that sodium alginate is relatively non toxic, efficient and cost effective immobilizer of either whole cells or enzyme (Yu-Si *et al*.,1999). Immobilization increases the shelf-life, facilitates reuse and improves the enzyme activities. Hence, the selected efficient bacteria were immobilized on sodium alginate beads. In our studies, AMET 1776 and AMET 1777 had showed 44.2% and 40.8% of protein removal after 7 days of incubation. These immobilized beads were also reused. After seven days of incubation, deproteination capability of both the marine bacteria (AMET 1767 and AMET 1776) was found to be above 20% which shows their efficiency in deproteination of SSP in used form. As FPs are one of the most studied bacterial communities, further optimization of genetic improvement studies can be done without much difficulty. In this scenario, the present finding assumes much significance.

CONCLUSION

The present study proves that marine associated fluorescent pseudomonads with the ability to produce both chitinase and protease have immense potential to be used for the deproteination of shrimp shell wastes.

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