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Research Article

BIOREMEDIATION OF SEA FOOD WASTE IN SOLID STATE FERMENTATION ALONG WITH PRODUCTION OF BIOACTIVE AGENTS

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ABSTRACT

Sea food processing generates large volumes of waste products such as skin, head, tails, shells, scales, backbones, etc. Pollution due to conventional methods of sea food waste disposal causes negative implications on environment, aquatic life, and human health. Moreover, these waste products can be used for the production of high-value products which are still untapped due to inappropriate management. *Paenibacillus* sp. AD, known to act on chitinolytic and proteinaceous waste was explored for its potential to degrade various types of sea food waste in solid state fermentation. Effective degradation of sea food waste, generated from variety of sources such as fish scales, crab shells, prawn shells and mixture of such wastes was observed. 30 to 40 percent degradation in terms of decrease in the mass was achieved. Along with the degradation, chitinolytic and proteolytic enzymes were produced which can have various biotechnological applications. Apart from this, value added products such as chitin oligosaccharides and peptides of various degree of polymerization were also produced which can be used for various therapeutic purposes. Results indicated that *Paenibacillus* sp. AD can be used for the development of a process for the infield degradation of sea food waste.

Keywords: Chitin, Chitin-oligosaccharides, Chitinase, Protease, Biodegradation, Crab shells, Prawn shells, fish scales.

1. INTRODUCTION

Every year huge amount of waste is generated from sea food industry. Approximately, 50-70% of the raw material goes as waste during the processing of sea food [1]. Conventional methods such as ocean dumping, incineration, land-filling etc. are generally used for its disposal. However, all these methods lead to heavy environmental pollution. An eco-friendly and most suitable alternative to reduce this pollution load is bioremediation [2]. Bioremediation involves the usage of organisms usually microorganisms to degrade the waste into harmless forms. For the bioremediation of sea food waste, major limitation is the biodegradation of recalcitrant polysaccharide, chitin, which is a major component of the waste [3]. Apart from chitin, proteinaceous components are also present in varying proportion depending on the type of waste [4]. Microbial enzymes are known to play role in number of industrial and environmental processes [5-8]. Microorganism producing chitinolytic and proteolytic enzymes can be explored and exploited for the bioremediation of sea food waste [9]. Apart from reducing the pollution load, it also leads to the production of products which can have applications in various fields.

Although there are numerous studies which have reported the microorganism producing chitinolytic and proteolytic enzymes [10, 11] but very few studies are available which have shown their application in the bioremediation of sea food waste [12, 13]. *Paenibacillus* sp. AD has been reported to degrade shrimp shells along with the production of chitinase(s) and protease(s) in submerged conditions [14]. However to carry out bioremediation at large scale, it is required that organism should be able to degrade the waste under solid state conditions. Moreover it should have the capacity to degrade waste generated from variety of sea food sources. Therefore, in the present study, potential of *Paenibacillus* sp. AD to degrade sea food waste generated from variety of sources such as fish scales, crab shells, prawn shells and mixture of such wastes have been evaluated. Value added products such as enzymes, chitin-oligosaccharides and peptides produced by the biodegradation of sea food wastes have also been analyzed.

2. MATERIAL AND METHODS

2.1. Microorganisms and chemicals

Paenibacillus sp. AD MTCC No. 12619 (isolated earlier in our laboratory) was used in the present study [14]. Chitin oligosaccharide standards were purchased from Qingdao BZ Oligo Biotech Co., Ltd. China. Media components were procured from Hi Media. All other chemicals used in the study were of analytical grade.

2.2. Substrates for solid state fermentation

Sea food waste such as fish scales, crab shells, prawn shells were collected from sea food waste dumping sites of south India. Wastes were transported to the laboratory under frozen condition and processed thereafter by thawing followed by washing and then drying in oven at 50°C. After drying, all the wastes were stored at room temperature in air tight plastic containers until further use.

2.3. Colloidal chitin preparation

Colloidal chitin was prepared according to the modified method as described in Faramarzi *et al*. [15]. In brief, chitin flakes were crushed and 5g of chitin powder was added to 60ml of concentrated HCl and incubated for 2-3h at 28-30°C with constant stirring. 200ml of ice-cold distilled water was added and kept for overnight incubation at 4°C. Precipitates obtained were washed repeatedly with distilled water until its pH became 7.0.

2.4. Inoculum preparation

Paenibacillus sp. AD was grown in MMHL agar [14] supplemented with 0.5% (w/v) of colloidal chitin as substrate. Organism grown in nutrient broth at 37°C, 150 rpm min^{-1} for 24h was used as inoculum.

2.5. Biodegradation of sea food waste under solid state fermentation (SSF)

Three grams of sea food waste after autoclaving in 250ml flask was mixed with MMHL moistening solution and inoculum keeping the moisture ratio to 1:4 (substrate:moisture). SSF was carried out at 37°C, 150rpm per min. After incubation, fermented solid was mixed with 20ml of autoclaved distilled water and mixed thoroughly on a rotary shaker at150 $rpmmin^{-1}$ for 1h. Entire content was then squeezed through a muslin cloth. Remaining waste was dried, weighed and degradation was calculated [13]. For the removal of any remains left of unhydrolyzed material, squeezed hydrolysate was centrifuged at 10000 rpm for 15 minutes, supernatants obtained were used for analysis of enzymes, chitin oligosaccharides and peptides.

2.6. Enzymes assays

2.6.1. Chitinase assay

Chitinase activity was determined by using 1.0% colloidal chitin as a substrate in phosphate buffer of 0.1M (pH 7.0) by Reesieg method [16]. 500µl of colloidal chitin was pre-incubated at 45°C for 2min. 500µl of appropriately diluted enzyme in phosphate buffer was added in colloidal chitin and incubated at 45°C for 5min. The tubes were placed in ice cold water bath to end the reaction. After incubation, all the tubes were centrifuged at 5000rpm for 10 minutes. 0.5ml of supernatant was taken and 0.1ml of potassium tetraborate buffer (0.8M, pH 9.0) was added to each tube and incubated at 100°C for 3min. The tubes were cooled under tap water and 3ml of freshly prepared p-Dimethylaminobenzaldehyde (DMAB) was added. Tubes were incubated at 37°C for 20 minutes and OD_{585nm} was taken. Chitinase activity was expressed in International Units (IU). One unit of enzyme activity was defined as micromoles of N-acetylglucosamine (GlcNac) produced by one ml of enzyme in one minute in standard assay protocol. Total enzyme activity was calculated by multiplying with the volume of enzyme extract and it was converted to enzyme yield per gram of substrate.

2.6.2. Protease assay

Protease activity was determined by casienolytic method in which casein was used as a substrate [17]. Enzyme assay was carried out under the standard conditions of 37°C, pH 7.2 for 5min and the reactions were stopped with 0.5ml of 5.0% TCA. In control, all the reagents were added similar to test but only 5% TCA was added prior to enzyme. After that all the tubes were centrifuged at 10,000rpm for 10min at 4°C. The supernatant obtained after centrifugation was used to determine the tyrosine produced by Lowry method [18]. Protease activity was expressed in International Units (IU). One IU of protease activity was defined as the amount of enzyme required to release one micromole of tyrosine per minute under the standard assay conditions. Total enzyme activity was calculated by multiplying with the volume of enzyme extract and it was converted to enzyme yield per gram of substrate.

2.7. Analysis of value added products

For the detection of chitin oligosaccharides (COS) and peptides, two volume of chilled ethanol were added to the supernatants obtained and kept at -20°C for 2h for the precipitation of molecules of higher molar mass. Centrifugation was done at 10000rpm for 15min.

Ethanol was evaporated from the supernatants at 50°C and the concentrated samples were used to detect COS and peptides by thin layer chromatography (TLC) with respect to degree of polymerization.

2.7.1. Detection of chitin oligosaccharides by thin layer chromatography

Samples were applied on TLC plate as spots of 2.0µl. N-Butanol: Ethanol: water (2:1:1) was used as mobile phase and COS were detected by spraying 2.0% (w/v) aniline-diphenylamine reagent prepared in acetone: ortho-phosphoric acid (8:1) followed by heating the plate at 100°C for 5min [14]. A mixture of N-acetyl glucosamine (GlcNAc), N-acetyl chitobiose $(GlcNAc)_2$, N-acetyl chitotriose $(GlcNAc)_3$ and N-acetyl chitotetroses $(GlcNAc)_4$ were used as standards.

2.7.2. Detection of peptides by thin layer chromatography

Samples were applied on TLC plate as spots of 2.0µl. N-Butanol: Ethanol: water (2:1:1) was used as mobile phase and peptides were detected by spraying 0.1% ninhydrin mixed in ethanol followed by heating at 100°C for 5min.

3. RESULTS AND DISCUSSION

3.1. Evaluation of different types of sea food waste degradation in solid state fermentation (SSF)

Major components of sea food waste are chitin and proteins [10, 11], therefore, two important enzymes required for its degradation are chitinase(s) and protease(s). *Paenibacillus* sp. AD, a marine bacterium, has been reported to produce extracellular chitinase and protease [14]. The organism has been reported to degrade shrimp shell waste in submerged fermentation in a very effective manner [14]. For in-field application, potential of *Paenibacillus* sp. AD to degrade wide variety of sea food waste generated from different sources was evaluated in solid state fermentation.

3.1.1. Biodegradation of fish scales

A huge amount of fish scales are generated as waste which are mainly composed of chitin and proteins [10]. Fish scales were inoculated with *Paenibacillus* sp. AD in solid state fermentation at 37°C. After 4 days of incubation, significant degradation was achieved (approximately 40% decrease in the mass of the waste) (fig.1A; table 1). A number of reports have shown the degradation of fish scales in submerged fermentation using various organisms [18-20]. Meruvu, 2018 is the only report of degradation of fish scales by SSF in which production of chitinase(s) have been standardized, however, decrease in the mass of the waste was not evaluated [21].

3.1.2. Biodegradation of crab shells

Crab is one of the important crustacean foods. The main waste of this sea food is the shells [22]. Chitin is the main structural compounds of crab shells in the cuticle layer [23]. Generally this waste is used for the extraction of chitin using treatment with strong acids and bases [24] which causes lots of pollution [25]. As *Paenibacillus* sp. AD is a potent sources of chitinase therefore degradation of crab shells were evaluated under solid state fermentation. About 30% degradation was achieved in 10 days at 37°C (fig.1B; table 1). The longer degradation time than the fish scales might be due to the higher content of chitin in crab shells than fish scales [26].

3.1.3. Biodegradation of prawn shells

Prawns are also consumed as sea food in many parts of the world and its shells are generated as waste [27]. Prawn waste can also be used as substrate for SSF. *Paenibacillus* sp. AD effectively degraded the prawn shells, 30% degradation was achieved in 4-6 days (fig.1C; table 1). Suresh and Chandrasekaran used prawn shells as substrate for the production of chitinase [28].

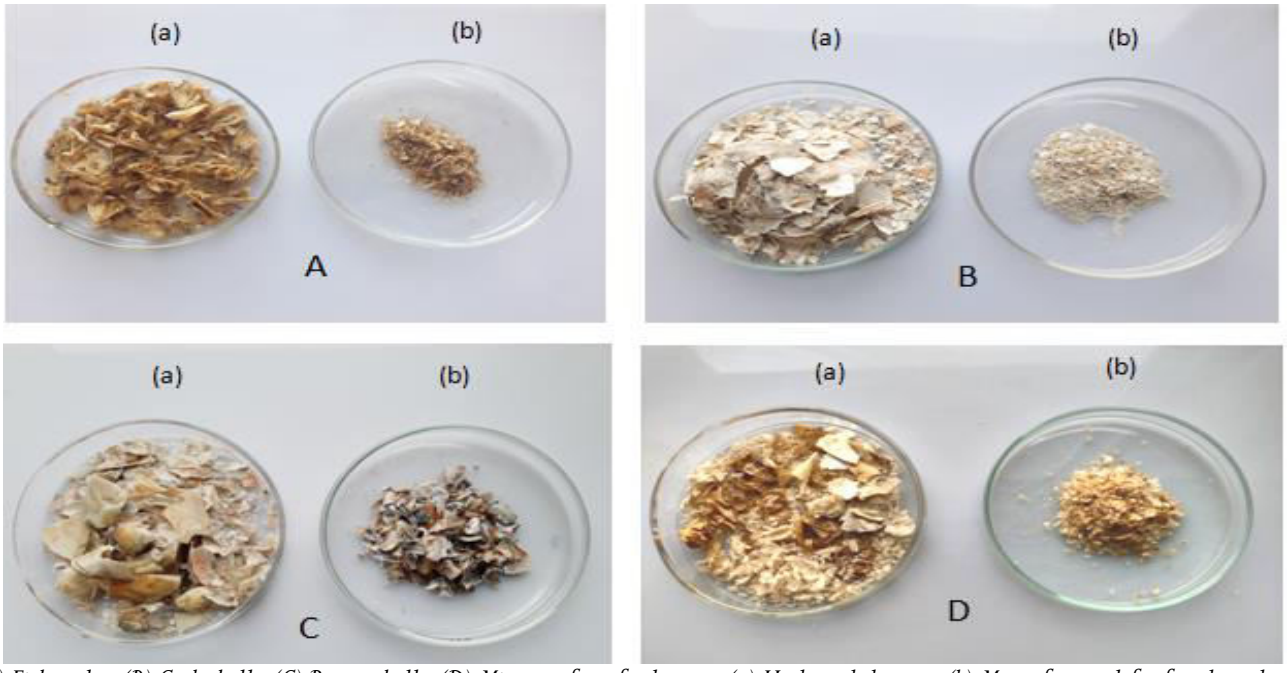
3.1.4. Biodegradation of mixture of sea food waste

Results indicated that *Paenibacillus* sp. AD can degrade waste from wide variety of sources in SSF such as fish scales, prawn shells and crab shells. Besides this, shrimp shells waste were also degraded by the organism (data not shown). Generally wastes from different types of sea foods are present together at dumping sites [29]. For the infield application of any process, it should be capable to degrade various types of waste in a mixture. Basic composition of all these wastes is similar with some variation in the proportion of different components [1]. As *Paenibacillus* sp. AD was able to degrade wide variety of sources, therefore, its capacity of degraded waste from all these sources in mixture was evaluated. Organism was effectively able to degrade waste in mixture also. A degradation of about 35% was achieved in 4-6 days (fig.1D; table 1).

3.2. Enzymes produced with biodegradation of sea food waste

As chitin and proteins are the major components of sea food waste [3, 4] therefore, hydrolysate of the degraded waste was analyzed for presence of enzymes such as

chitinase(s) and protease(s) both of these enzymes were produced with different yields using different types of waste (table 2). Protease yield in fish waste degradation was higher as in fish scales proteinaceous components are more than the other type of wastes [4]. Protease(s) and chitinase(s) have application in number of industrial processes [11, 17, 30].



(A) Fish scales; (B) Crab shells; (C) Prawn shells; (D) Mixture of sea food waste, (a) Undegraded waste; (b) Mass of waste left after degradation

Fig. 1: Biodegradation of various types of sea food waste in solid state fermentation using *Paenibacillus* sp. AD

Table 1: Degradation of sea food waste in terms of percent decrease in the mass

Sea food waste	Percent decrease in the mass
Fish scales	40
Crab shells	30
Prawns shells	30
Mixture of sea food waste	35

Table 2: Enzyme production with the biodegradation of various types of sea food waste

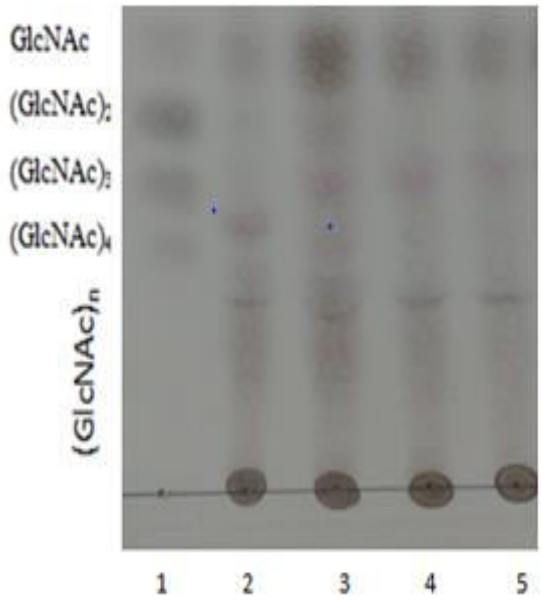
Type of sea food waste	Enzyme yield IUg^{-1}	
	Protease	Chitinase
Fish scales	10	5.2
Crab shells	5.37	8.3
Prawns shells	4.22	7.2
Mixture of sea food waste	6.8	7.5

3.3. Analysis of value added products formed during biodegradation of sea food waste

The added advantage with the biodegradation of sea food waste is that it produces number of products which can have various applications [9]. Two important value added products produced are chitin oligosaccharides and peptides [14]. The hydrolysate produced by the degradation of various types of sea food waste using *Paenibacillus* sp. AD was analyzed for the presence of these products.

3.3.1. Analysis of chitin oligosaccharides

The hydrolysate produced due to degradation of waste was analyzed by TLC using specific procedures for the analysis of oligosacharrides. A mixture of chitin oligosaccharides of varied degree of polymerization were presented in the hydrolysate. Along with N-acetyl glucosamine (GlcNAc), COS of various degree of polymerization such as N-acetyl chitobiose, $(GlcNAc)_2$; N-acetyl chitotriose, $(GlcNAc)_3$; N-acetyl chitotetraoses $(GlcNAc)_4$ and oligosaccharides of higher degree of polymerization were detected (Fig.2). Prebiotics and probiotics play a significant role in improving the gut microflora [31]. Like other types of oligosaccharides [32] chitin oligosaccharides are known to have prebiotic and number of other useful properties [14].

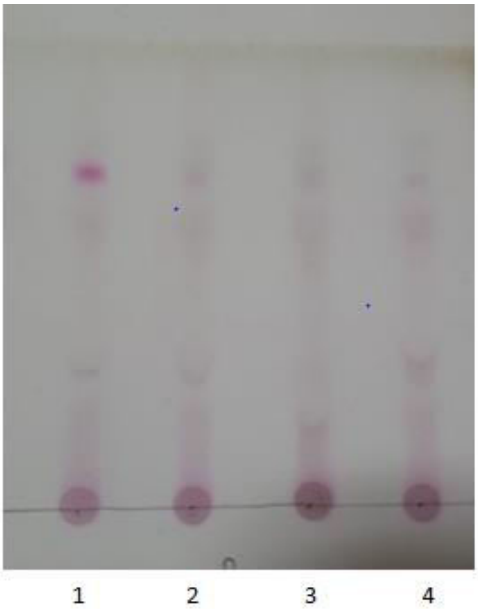


(1) Standard Chitin oligosaccharides (2) Fish scales (3) Crab shells (4) Prawn shells (5) Mixture of sea food waste

Fig. 2: Analysis of chitin oligosaccharides by TLC

3.3.2. Analysis of peptides

The hydrolysate produced after biodegradation of waste was also analyzed for proteins/peptides. Results showed the presence of peptides of various sizes in the hydrolysate (fig.3). These peptides can be explored for their application as therapeutic agents [7].



(1) Fish scales (2) Crab shells (3) Prawn shells (4) Mixture of sea food waste

Fig. 3: Analysis of peptides by TLC

4. CONCLUSION

Biodegradation of sea food waste is important to reduce the pollution load generated by the conventional methods of disposal. *Paenibacillus* sp. AD was able to degrade the waste generated from wide variety of sources in solid state fermentation individually and in mixture. Degradation of 30-40 percent was achieved depending on the type of waste. Along with degradation, a number of value added products such as enzymes, chitin oligosaccharides, peptides were also formed which can be used for various applications.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

1. Toliba, AO, Rabie MA, El-Araby GM. *Zagazig J Agric Res*, 2014; **41(5):** 1067-1076.
2. Aanand, S, Divya, M, Deepakon T, Padmavathi P, Manimekalai D. *Int J Agric Res*, 2017; **3(7):**1-4.
3. Das S, Roy D, Sen R. *Adv Food Nutr Res*, 2016; **78**:27-46.
4. Ghaly AE, Ramakrishnan VV, Brooks MS, Budge SM, Dave D. *J Microb Biochem Technol*, 2013; **5(4):**107-129.
5. Chauhan PS, N Gupta. 2017, *Crit Rev Biotechnol*; **37 (2):** 190-201
6. Chauhan PS, Soni SK, Sharma P, Saini A, Gupta N. *Int J Pharm Bio Sci*, 2014; **5(1):**237-251.
7. Chauhan PS, George N, Sondhi S, Gupta N. *Int J Pharma Bio Sci*, 2014; **5 (1):**176-192.
8. Fantahun W, Virk AP, Naveen G, Prince S. *J Microbiol Biotechnol Res*, 2013; **3(4):**32-41.
9. Rathore AS, Gupta RD. Enzyme Res, 2015; **2**: 1-8.
10. Gupta N, Kumar A, Laksh, Angural S, Rana M, *Eur J Pharmaceut Med Res*, 2017; **4 (9):**474-480.
11. Kumar A, Laksh, Angural S, Kumar D, Gupta N, Puri N. *Int J Eng Sci Res*, 2017; **5 (10):**28-47.
12. Farag AM, Abd-Elnabey HM, Ibrahim HA, El-Shenawy M. *Egypt J Aquat Res*, 2016; **42(2):**185-192.
13. Sridevi S, Reddy DSR. *Indo Am J Pharm Res*, 2016; 4634-4638.
14. Kumar A, Kumar D, George N, Sharma P, Gupta N. *Int. J. Biol. Macromol*, 2018; **109**:263-272.
15. Faramarzi MA, Fazeli M, Yazdi MT, Adrangi S, Al-Ahmadi KJ, Tasharrofi N, Mohseni FA. *Biotechnology*, 2009; **8(1):**93-99.

16. Reissig JL, Strominger JL, Leloir LF. *J Biol Chem*, 1955; **217(2):**959-966.
17. David A, Chauhan PS, Kumar A, Angural S, Kumar D, Puri N, Gupta N. *Int. J. Biol. Macromol.*, 2018; **108:**1176-1184.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. *J Biol Chem*, 1951; **193(1):**265-275.
19. Ajayi AA, Onibokun EA, Adedeji OM, George FOA. *Int J Adv Sci Eng Technol*, 2015; **3(4):**88-95.
20. Ghanem KM, Al-Garni SM, Al-Makishah NH. *Afr J Biotechnol*, 2010; **9(32):**5135-5146.
21. Meruvu H. *New Mater Compd Appl*, 2018; **2(3):**261-273.
22. Dliyauddin M, Ardyati T, Suharjono. *Biodiversitas*, 2020; **21(1):**211-218.
23. Chen PY, Lin AYM, McKittrick J, Meyers MA. *Acta Biomater*, 2008; **4(3):**587-596.
24. Tolaimate A, Desbrieres J, Rhazi M, Alagui A. *Polymer*, 2003; **44(26):**7939-7952.
25. Younes I, Rinaudo M. *Mar Drugs*, 2015; **13(3):**1133-1174.
26. B. Vinusha, Ch. Vijaya. *Int J Res Appl Sci Eng Technol*, 2019; **7(4):**2217-2220.
27. Tarafdar A, Biswas G. *Int J Theor Appl Res Mech Eng*, 2013; **2(3):**17-24.
28. Suresh PV, Chandrasekaran M. *World J Microbiol Biotechnol*, 1998; **14(5):**655-660.
29. Sasidharan A, Baiju KK, Mathew S. *Int J Environ Waste Manag*, 2013; **12(4):**422-441.
30. George N, Sondhi S, Soni SK, Gupta N. *Indian J Microbiol*, 2014; **54 (2):**139-142.
31. Sharma N, Angural S, Rana M, Puri N, Kondepudi KK, Gupta N. *Trends Food Sci Technol,* 2020; **96:**1-12.
32. Rana M, Kumar A, Kumar D, Mazumder K, Gupta N. *J Adv Sci Res,* 2020; **11(6):**1-6.