

Marine origin *Bacillus* sp. : A potential collagenase source for fishery waste utilization

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Fisheries contributes immensely to global food and nutritional security. Substantial part of the fish produced were lost as fish waste discards and various other losses. Fish head, bones, gut, scales and skin are the major discard materials and anticipated to generate 32 million tonnes of waste from the processing operations (Kristinsson and Rasco, 2000). In this diction, utilization of fish waste is an important step. Among the discards collagen is a rich source found in skin, scales, and bones of over 7% of the total body weight of fish. Collagen is a major structural protein of animal origin which constitutes about 30% of total protein which is an insoluble fibrous protein. Among the enzymes highly marketed commercially, proteases takes the maximum share (Garcia-Carren˜o *et al.*, 1994; Arvanitoyannis and Kassaveti, 2008). Collagen hydrolysate prepared enzymatically using collagenase enzyme from microbial sources are comparatively better than thermo-chemically produced with strong alkali and high temperature (Rochima *et al.*, 2016).

In this context, a study was undertaken to screen seawater for isolating bacteria with collagenase activity/collagen degrading capability. Fifteen morphologically distinct bacteria were isolated from the seawater samples and identified both biochemically and molecularly using 16s rDNA sequencing analysis. All the 15 bacteria were characterized for the exo-enzyme activities such as lipase, protease, amylase and chitinase actives. Furthermore, the bacteria were tested for the collagenase activity with Azocoll Assay.

The study revealed that among the 15 bacterial strains isolated, 13 of them belonged to *Bacillus* sp. and two belonged to *Staphylococcus* sp. (Fig.1). Seven isolates were able to produce



Fig.1. Morphological characteristics of bacteria isolated from collagen medium

collagenase activity at 24 h of the growth and all the them belonged to *Bacillus* sp. (*Bacillus amyloliquefaciens*, *B. Velezensis* and *B. subtilis*) by Azocoll Assay. The sequencing study revealed the species *albeit*, there exist a variation in the biochemical characteristics which emphasize the potential novel species among the *Bacillus subtilis* group complex exempting *Bacillus velezensis*. The study also concludes the potential use of this enzymes in fish processing industry to convert waste to wealth.

Several attempts were made to isolate collagenase producing organism. However, all the attempts were confined to terrestrial ecosystem or food resulting in isolation of a few pathogenic organisms *viz.*, *Clostridium perfringens* and *C. histolytica*, *Bacillus subtilis* FS-2 (Nagano, 1999), *Bacillus subtilis* CN (Tran and Nagano, 2002), *Bacillus subtilis* AS1.398, (Rui *et al.*, 2009), *Bacillus pumilus* Co-J (Wu *et al.*, 2010), *Bacillus cereus* (Liu *et al.*, 2010) and *Streptomyces*

sp. Strain 3B (Petrova, 2006) and pathogenic *Vibrios*. The study reveals the potential use of this enzymes in the fish processing industry to convert waste to wealth and the possibility of new strain or species among the Subtilis group.

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Immobilization and sulphur oxidation capability of sulphur oxidizing bacteria

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Sulphur oxidizing bacteria (SOB) are one group of beneficial bacteria capable of utilizing toxic form of sulphur produced by a group of bacteria called sulphur reducing bacteria (SRB) in natural environment during anoxic conditions (Friedrich *et al.*, 2001). The autotrophic SOB plays an important role in sulphur cycle in maintaining the levels of hydrogen sulphide. The count

of these bacteria in water and soil are usually very low and depend on the availability of the sulphur compounds in oxidized state. Natural polymers such as chitin, alginate, cellulose and chitosan are commonly used as carrier materials for immobilization of microorganisms where the bacterial cells are trapped. However, there is no such study on the immobilization of SOB. The present study is carried out to immobilize