International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com

Research Article

Isolation and screening of protease producing marine Actinomycetes from Chennai coastal region

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Abstract

Actinomycetes are an important group of microorganisms that has wide industrial application in the production of antibiotics and enzymes. In this study 109 isolates of Actinomycetes were isolated from marine soil samples collected from the coastal area of Chennai, India. The isolates were identified by morphological studies. Form the total 109 isolates, 40 showed proteolytic activity which was indicated by growth and clear zone formation on casein-skim milk agar media plate. Among the 40 protease producing strains the enzyme activity was highest in 8 strains namely, MB22, MB41, MB16, MB47, BN3, BN16, NK17, NK15. Potential strains were further used for protease production and for the purpose of screening for fibrinolytic enzymes.

Keywords: Actinomycetes, Protease, Isolation, Screening, fibrinolytic.

Introduction

Protease are among the most important class of industrial enzymes, about 80% of enzymes produced annually are simple hydrolytic enzymes, in which 60% are protease. Different bacteria, fungi, and actinomycetes are the major source of microbial protease enzyme. Protease are also isolated from marine algae (Swapnil and Jeyanthi Rebecca, 2014; Sharmila *et al*., 2012). Protease have been partially purified from marine waste like fish scales and crab and prawn shells (Jeyanthi Rebecca *et al*., 2012a; Jeyanthi Rebecca *et al*., 2012b) They have extensive industrial applications in the production and processing of detergent, food, pharmaceuticals, leather, textiles etc. (Gupta *et al*., 2002). In spite of considering actinomycetes to be among the most important producers of antibiotics (Kavitha *et al*., 2010), the present knowledge concerning protease of actinomycetes to be the most important group of secondary metabolites are widely exploited (Limkhada *et al*., 2010).

Microbial enzymes represent 60% of the world market of industrial enzymes. They find commercial application in toothpaste as anti-plaque and anti-tartar, in cosmetics and for the recovery of silver from used x-ray films (Ishikawa *et al*., 1993) as well as in bioremediation process (Anwar and Saleemudin, 1998; Gupta and Roy, 2002; Oskouie *et al*., 2008)
Trypsin and chymotrypsin acts as anti-inflammatory and antioxidant agents on burn wounds (Bitange *et al*., 2008). Actinomycetes particularly *Streptomyces* are known to secrete multiple proteases in culture medium (Siva Kumar, 2001). This study aimed to isolate actinomycetes from marine soils in Chennai coastal region and screen it for their potential to produce protease.

Materials and Methods

Sample collection

The marine soils were collected from Chennai coastal region namely from Marina beach, Elliots beach and Neelankarai beach. The samples were collected using alcohol rinsed person grab and were transferred to new zip lock bags using sterile spatula (Dhevendaran *et al*., 2002) the samples were transported to the laboratory for the isolation of actinomycetes.

Isolation of actinomycetes enrichment

One gram of soil sample was serially diluted in sterile distilled water. One ml of each aliquot was spread on starch casein agar (SCA) medium and used for the isolation of actinomycetes (Radhakrishnan *et al*., 2011). The antibiotics such as nalidixic acid and nystatin were added in to the medium, in order to inhibit bacteria and fungi, respectively. The plates were incubated at 28˚C for 6 to 7 days (Savitri *et al*., 2003). The isolated agar plates were observed for the presence of actinomycete colonies from 3rd day onwards. Single separated colonies were selected and the subculture was maintained in SCA slants at 4˚C until further use.

Identification of marine actinomycetes by cover slip method

The isolated strains were confirmed as actinomycetes by studying their morphology under microscope. The SCA was poured on sterile slides and allowed for solidifying. Then the organisms were streaked on it and incubated at 37˚C for 48 hrs. After incubation the cover slip was carefully removed with respect to its orientation and placed upwards on a slide. About 2 drops of methylene blue dye was added and was allowed to stand for a minute then the slide was covered with cover slip and the morphology was observed under microscope (Prazeres *et al*., 2006).

Microscopic Examination

The following microscopic observations were recorded using cover slip culture.

- Presence or absence of substrate mycelium
- Fragmentation of substrate mycelium
- Presence of sclerotia or sporangia
- Spore chain morphology

The generic level identification was carried out by using Bergey's Manual of Determinative Bacteriology 8th edition (Holt 1994).

Protease Activity

The isolated actinomycete colonies were plated onto skim-milk-agar plates containing; Casein 5.0 g/l, yeast extract 2.5 g/l, dextrose 1.0 g/l, skim milk 28.0 g/l, agar 20 g/l, and pH 7.0. Plates were incubated for 5 days at 28˚C. A clear zone on skim-milk hydrolysis gave an indication of protease producing strains. Different colonies from the plates were purified through repeated streaking on fresh agar plates (Panuwan *et al*., 2002).

The enzyme activity was visualized as clear zones around the wells due to hydrolysis of substrates in the presence of indicator solution, and the diameter of the photolytic zone was measured. The strains with maximum protease activity were selected and the subcultures were maintained in SCA slants at 4˚C for further investigation.

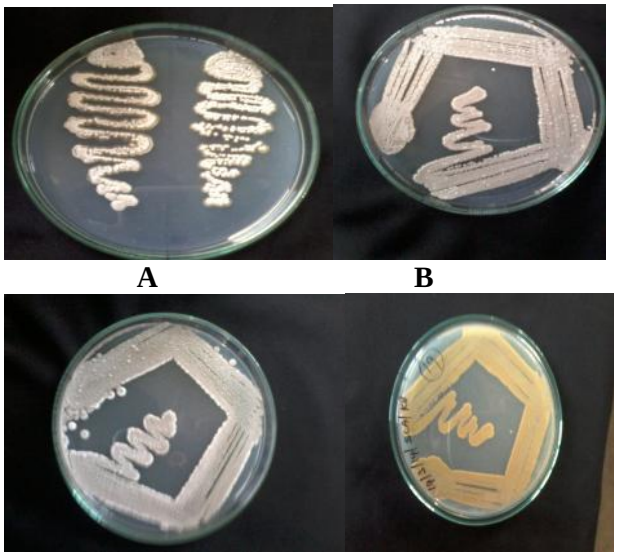
Result and Discussion

In this study, from the soil samples collected, 109 isolates of actinomycetes were isolated (Table 1)

Table 1: Collection of Soil Samples for isolation of Actinomycetes

S. No	No of Isolates	Symbol	Collection area
1	57	MB1-MB57	Marina Beach
2	40	BN1-BN40	Besant nagar
3	17	NK1-NK17	Neelankarai

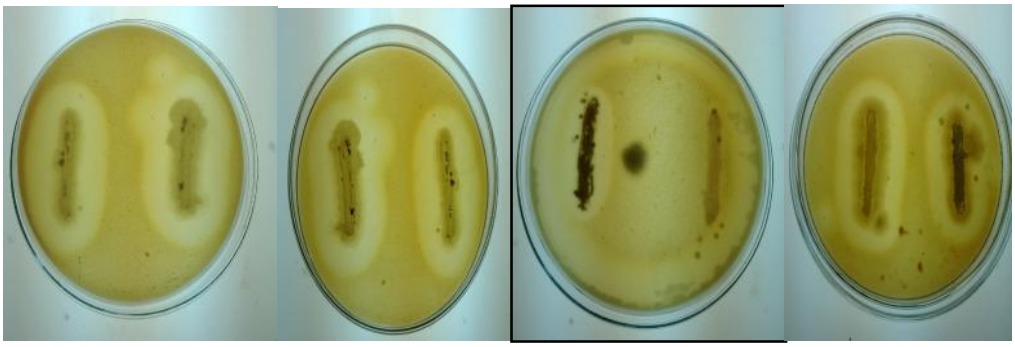
The colony color of actinomycete strains isolated was categorized into light whitish, greyish, light dark grey brownish (Figure1). These isolates were identified according to their morphological test. The actinomycetes isolates were cultured on starch casein agar media for five or six days.



A B C D

Figure 1, Colony colour of isolated actinomycetes, A) light whitish, B) greyish, C) light dark grey and D) brownish

It was then tested for their ability to produce protease enzyme using skim milk agar plate method. A clear zone was observed and protease activity of actinomycetes was high in casein. The appearance of clear zone around the colonies surrounded by white color background with skim milk agar plate (Figure 2) indicated the presence of protease activity.



MB22 & MB41 MB16 & MB47 BN3 & BN16 NK17 & NK15

Figure 2: Protease activity (Clear zone)

Protease is considered as an industrially important enzyme that showed a wider range of application in pharmaceutical, leather, laundry, food, waste processing as well as in textile industries. In our search for protease enzyme activity, out of 109 actinomycete strains isolated only 40 strains showed zone of clearance for protease activity as they were screened using skim milk agar plate method. It was observed previously that use of casein as the substrate under the standard assay conditions gave the highest activity of pH8.0. (Yanga *et al*., 2000). Several investigators used different screening plate media in their search for alkaline protease (Aftab *et al*., 2006; Rao and Narasu, 2007; Kasana and Yadav, 2007). The proteolytic activity of marine *Streptomyces sp* MMLI614 was detected in which the casein gelatin formed a clear zone around the colonies, the formation of clear zone hydrolytic zone indicates that the marine stain of *Streptomyces* M11VILL 1614 produce extra cellular protease (Dekleva *et al*., 1985). Our study showed that the pH, Temperature were the essential parameters for the enzyme activity. Protease yields vary considerably with temperature and pH (Maghsoodi *et al*., 2013). Strain numbers MB22, MB41, MB16, MB47, BN3, BN16, NK17, NK15 showed high level zone of protease activity. All the strains were stored at 4˚C for further protease production and fibrinolytic enzyme investigation (Table 2).

Table: 2 Protease Zone of inhibition

S.No	Strain Numbers	Zone of inhibition (mm)
1	MB22	20
2	MB41	19
3	MB16	17
4	MB47	19
5	BN3	14
6	BN16	11
7	NK17	18
8	NK15	16

In the present experiment, maximum protease zone of activity was obtained at pH7.0 and at a temperature of 28˚C.

Conclusion

Actinomycetes can be used for large scale production of protease to meet daily needs in the industrial sector. In the present study, casein was used with the skim milk agar medium to detect preotease activity. Based on the protease enzyme activity strains were selected for the study. Eight strains of maximum protease activity were observed at pH7.0 and at a temperature of 28˚C. Potential strains were further used for protease production and for the identification of fibrinolytic enzymes.

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