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Short Communication

A report on extracellular enzyme production potential of actinomycetes isolated from sediments of river Godavari, India.

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ABSTRACT

50 actinomycetes isolated from sediment samples of river Godavari were primarily screened to assess their extracellular amylase, protease and gelatinase production potential through inoculation on plates containing medium supplemented with starch, skim milk and gelatin as substrates respectively. 31 (62%) isolates were capable of producing amylase, 34 (68%) showed a positive activity for protease production and 29 (58%) isolates were gelatinase producers. 12 (24%) of the isolates were producers of all the three enzymes.

Key words: actinomycetes, Godavari, amylase, protease, gelatinase.

INTRODUCTION

Actinomycetes are excellent sources of biotechnologically important compounds and that still makes them one of the most sought after microbes to research on, despite decades of research dedicated to unravel their bioprospects. A new dimension to this research was added when habitats other than soil were searched for presence of actinomycetes and reports pointed towards a promising future. The first step towards any detailed research into industrial prospects and applicability of a microbial product is the screening of the microbes in question for their bioactivity. This paper reports a simple study on actinomycetes from river Godavari, Nanded, India, with reference to a preliminary investigation to gain insights into their extracellular enzyme production potential. Since the need to explore the aquatic habitats to uncover the presence and bioactive potential of actinomycetes has been expressed time and again (Cheng and Jiang, 2006; Jiang and Xu, 1996; Rifaat, 2003), this paper, also aims to contribute to the knowledge available on the enzyme production potential of actinomycetes from this relatively less-studied habitat.

MATERIALS AND METHODS

Isolation of actinomycetes: Sediments collected from River Godavari were serially diluted and spread on Actinomycetes Isolation Agar and Starch Casein Agar plates. These media were supplemented with 25% sediment extracts and 10 µg/ml each of Nalidixic Acid and Cycloheximide to minimize bacterial and fungal contamination (Rizvi et. al, 2012). The plates were incubated for 14 days at 28^0 C, and colonies showing characteristics of actinomycetes were picked up, purified and maintained on the same medium (un-supplemented) on which they were isolated.

Primary screening for enzyme production: Since colonies of actinomycetes are hard and cannot be scraped easily (except when producing aerial mycelial spores, which can be scraped with relative ease), a nichrome wire needle was used to pick colonies or spores from an actively growing culture, followed by a gentle stabbing into the agar plates. The plates were then incubated for 4 days at 28^0 C. The screening was done in triplicates and the incubation of one of the sets was continued for up to 7 days, before being recorded as negative for an enzyme production.

Amylase: For the purpose of testing amylase production potential, cultures were gently stabbed into starch casein agar plates (HiMedia, Mumbai). Following incubation, the plates were flooded with Grams iodine solution and a zone of clearance around colonies of test isolates against a dark blue stained lawn of starch interpreted as a positive result.

Protease: Skim milk agar composed as peptone – 5.0g, yeast extract – 3.0g, skim milk powder – 1.0g in 1000ml of distilled water (Fulzele, 2011) was used for testing protease production. An isolate was recorded as being a producer of protease if a zone of clearance was observed around its colony post incubation.

Gelatinase: Gelatinase production was tested through inoculation on gelatin agar plates composed as gelatin - 15.0g, peptone – 4.0g, yeast extract – 1.0g, agar – 15g, distilled water - 1000ml. To visualize the results for gelatinase production, the plates were treated with acidified mercuric chloride solution with help of a dropper (precautions taken to avoid skin contact with the solution) until gelatin on the plate precipitated to form a white lawn, the potential gelatinase producing isolate in this case, being the isolate with a clear zone around its colony, post flooding and precipitation (Balan *et al.*, 2012).

RESULTS AND DISCUSSION

Out of the 50 tested isolates 31 (62%) showed a positive activity for amylase production, 34 (68%) were positive for protease whereas 29 (58%) were gelatinase positive. 8 (16%) isolates were capable of producing both amylase and protease, 2 (4%) isolates produced both amylase and gelatinase whereas 9 (18%) isolates produced both protease and gelatinase enzymes. 12 isolates (24%), were producers of all the three enzymes (figure 1). The bioactive potential of actinomycetes from aquatic habitats has been unlocked by many researchers (Jiang and Xu, 1996; Mane and Deshmukh, 2009; Rifaat, 2003; Tawiah *et al.,* 2012) however; comparatively a little knowledge on presence and bioactivity of these marvellous microbes from Indian rivers is available. Further studies in the direction of herein reported research are currently underway.

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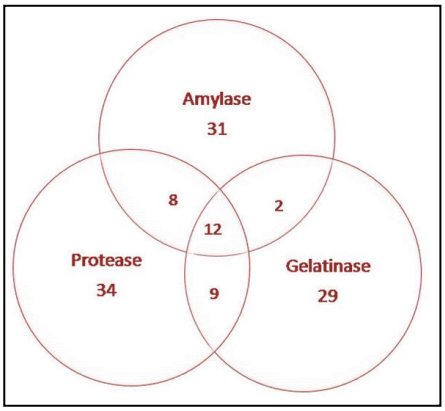

Figure 1: A Venn diagrammatic representation of the number of isolates producing the three enzymes.

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