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Actinomycetes: Isolation and Primary Screening for Alkaline Protease Production

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Abstract

Actinomycetes are Gram positive and filamentous microorganisms. They are thought to be a transition group between bacteria and fungi. Actinomycetes are widely distributed in different habitats such as fresh and marine water, agricultural and sediment soil and mangrove habitats. They are able to secrete industrially important extracellular multi-catalytic enzymes such as amylases lipases and proteases. Among the different classes of proteases, alkaline proteases are attracting attention of researchers and industrialists due to their special robust properties and applications. In present investigation, we had collected soil samples from local slaughter house of Nanded and isolated efficient alkaline protease producer actinomycete and designated as MK3. It was Gram positive long filamentous rod with many branches and spores. It was catalase, amylase, protease, and lipase positive. This isolate could be exploited in further research for production, purification and characterization of extracellular alkaline protease and could be used in different biotechnological applications.

Keywords: *Alkaline Protease Producer, Actinomycetes, Alkaline Soil, Protease Screening, Slaughter House*

1. Introduction

Actinomycetes are Gram positive and filamentous microorganisms. They belong to the phylum actinobacteria. They are thought to be a transition group between bacteria and fungi. Actinomycetes are widely distributed in nature. They are well known for their beneficial properties such as production and secretion of extracellular multi-catalytic enzymes, antimicrobial compounds, some specific secondary metabolites etc. In several studies, production of antibacterial and antifungal compounds from actinomycetes has been reported [1]. Moreover actinomycetes have been extensively studies under the broad area of biocontrol of plant diseases. Thermophilic actinomycetes are well known components of the microflora of composts [2, 3]. Composts are mainly for cultivation of button mushroom [4,5]. Soil actinomycetes act as potential source of biopesticides [6, 7]. Selected species of actinomycetes have applications in increased methanogenesis and odor control [8, 9]. Recently some important inferences about actinobacterial metabolites to combat Corona virus are known [10,11].

Proteolytic enzymes play a specific catalytic role in the hydrolysis of proteins. They are widespread in all living organisms as they are essential for cell growth and differentiation. Alkaline proteases are a physiologically and commercially important group of enzymes used in various branches of industries such as the detergent, food, leather tanning, pharmaceutical, and textile industries [12-21]. Many microorganisms produce proteases; however, the enormous extent of commercial alkaline proteases is generally obtained from *Bacillus* sp., as a result of its capacity to secrete large amount of alkaline proteases having significant proteolytic activity and stability at a wide range of pH. Number of studies has been reported on isolation, screening, production and applications of alkaline proteases. Alkaline proteases are attracting attention of researchers due to their special properties and applications [21-29].

Natural weathering of the soil particles, irrigation and flood are some causes of changing pH of the soil. Alkalinity of soil is associated with the presence of sodium carbonate and sodium bicarbonate in the soil. The soil to the close vicinity of slaughter house tends to have a diverse microbial population since soil of this area is often mixed with animal wastes.

Detergents are used to perform washing practices of slaughtering equipments and as such water mixes with the soil there, the pH of the soil is likely to be alkaline [30-32]. Hence in present study, we collected soil samples from local slaughter house of Nanded and five cultivable actinomycetes isolated. These isolates were screened for their proteolytic activity and an efficient alkaline protease producing isolate was characterized.

2. Materials and Methods

2.1. Soil sample collection

Soil samples were collected from local slaughter house, near Degluar naka region of Nanded, Maharashtra, India. The samples were collected in sterilized cotton bags and transported to the laboratory within 12 hours. 1 gm of composite soil sample was moistened with few drops of distilled water and pH was recorded by pH paper strips (HiMedia, Mumbai).

2.2. Isolation of Actinomycetes

One gram of this soil sample was transferred to a flask containing 100 ml sterilized distilled water and kept on rotary shaker at 30^{o}C and 120 rpm for 15 min. Serial dilutions from 10^{-1} to 10^{-10} were prepared and 0.1 ml of each dilution was inoculated and spread on Actinomycetes isolation agar plates (Hi media, Mumbai). The plates were incubated for 6 days at 30^{o} C and characteristics of morphologically different colonies appearing on the medium were isolated [36-39].

2.3. Screening of selected isolates for proteolytic activity

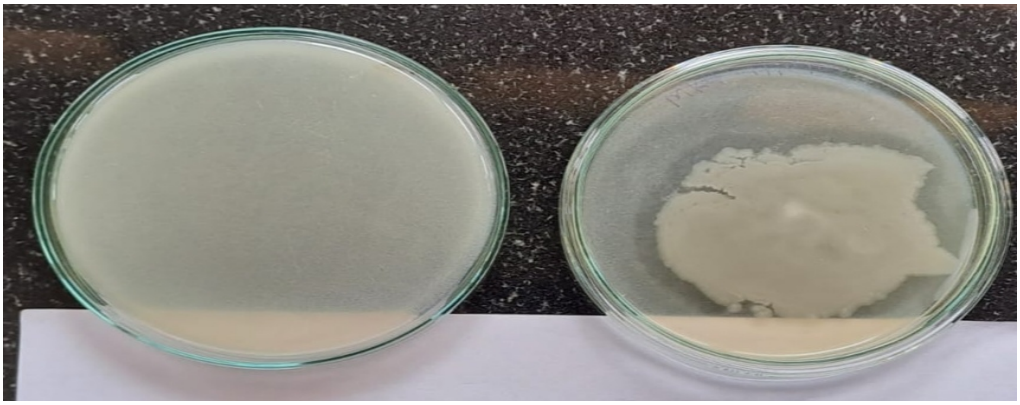
The selected colonies from Actinomycetes isolation agar plate were screened for proteolytic activity. In this screening method, selected colonies were spot inoculated on alkaline skimmed milk agar plates (pH 8) and incubated at 30^{o}C for 24 to 72 h [40-47].

2.4. Biochemical characterization

For the preliminary characterization of the selected promising isolate the biochemical tests were performed such as IMViC test, catalase production and hydrolysis of lipid, casein, starch, cellulose and urea. Carbohydrate fermentation tests were performed by using standard procedures [48-64].

3. Results and Discussion

The recorded pH of composite soil sample was near about 8.0. This indicated that, the collected soil sample was alkaline. Total 91 colonies were appeared in an average on actinomycetes agar plates, out of which 5 colonies were designated as MK1 to MK5. These colonies were sub-cultured on actinomycetes agar slants. Pure cultures of these isolates were obtained with great difficulty by performing repeated sub-culturing technique. Out of 5 isolates, the isolate MK3 has shown a zone of clearance around it's colony on alkaline skimmed milk agar plate (Figure 1). Morphological and microscopic characters of MK3 isolate are given in Table 1. The observed colony characters, microscopic filamentous view and cellular arrangement resembles that the MK3 belongs to actinomycetes group when compared to reference strain. Biochemical characters of MK3 isolate are given in Table 2.



A. B.

Fig. 1: Primary screening of Actinomycetes for extracellular alkaline protease secretion.

A: Skimmed milk agar plate (pH 8); B: Zone of clearance around the colony of actinomycetes on skimmed milk agar plate (pH 8)

Table 1: Morphological and microscopic characters of MK3 isolate

Character	***MK3 isolate***
Size	3 mm
Shape	Circular
Color	Off white
Margin	Irregular
Elevation	Raised
Surface	Smooth
Consistency	Sticky
Opacity	Opaque
Gram stain reaction	Gram Positive
Growth of colonies	Highly spreading

Table 2: Biochemical characters of MK3 isolate

Test	**Result**	**Enzyme profile**	**Results**	**Carbohydrate fermentation test**	**Results**
Catalase	+	Protease	+	Lactose	+
Indole production	-	Urease	-	Maltose	+
Methyl red	-	Amylase	+	Galactose	+
VP test	-	Lipase	+	Sucrose	+
Citrate utilization	+	Pectinase	+	Inulin	+
Nitrate reduction	-	cellulase	-	Raffinose	-
				Xylose	-
				Trehalose	+
				Melibiose	-
				Salicine	-

In the literature, isolation of actinomycetes has been reported from different sources such as humus layer of forest soil, corn field, cow barn yard, Antarctic Soil, Mitidja plain (Algeria), marine soil, stream sediments and lake muds, marine sediments, root and stem samples of *Cinnamomum zeylanicum*, *Zingiber spectabile*, *Elettariopsis curtisii* and *Labisia pumila*, mangroove sediments etc. However the most common source for the isolation of actinomycetes is agricultural soil sample. Malviya et al. (2013) have isolated actinomycetes from specific soil samples collected after fire operations at agricultural sites under shifting cultivation in northeast India [65]. Many actinomyces species are opportunistic pathogens. Actinobacteria also play an important role as symbionts and as pathogens in plant-associated microbial communities.

Researchers have used different media for isolation of actinomycetes such as starch-casein medium, humic acid-vitamin agar, starch casein nitrate agar (SCS), hair hydrolysate vitamin agar (HHVA), Bennet's agar (BA), arginine-glycerol salt (AGS) medium, chitin medium, modified Benedict's medium, soybean meal-glucose medium, Gauze's agar medium, Czapek's agar medium, egg albumen medium, glucose-asparagine medium, glycerol-asparaginate agar, chitin agar, coal-vitamin agar, mineral salt (MS) medium, yeast extract-malt extract agar, starch casein nitrate (SCN) agar medium, M3 agar medium, asparagine agar, glycerol-glycine agar, AIM medium, Actinomycetes isolation agar (AIA), tap water yeast extract agar (TWYE) and asparagine-glucose agar medium. The most common medium for cultivation of actinomycetes is starch-yeast extract agar [1,6,8,10,65].

4. Conclusion

The isolate MK3 has shown morphological, microscopic and biochemical characters almost same as with standard reference strains of actinomycetes. Hence it has been concluded that the isolate MK3 belongs to actinomycetes. MK3 has shown proteolytic zone of clearance around it's colony on skimmed milk agar plate (pH 8). This isolate could be exploited in further research for production, purification and characterization of extracellular alkaline protease.

Conflict of Interest

None.

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