Isolation and Screening of a Feather-Degrading Keratinolytic Actinomycetes from *Actinomyces sp*

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ABSTRACT: The Actinomycetes comprise a ubiquitous order of bacteria which exhibits wide physiological and morphological diversity. These microorganisms are particularly abundant in alkaline soils rich in organic matter. Keratin is an insoluble structural protein of skin, and its derivatives (e.g. feather, hair, wool and horn) are known for their high stability. Actinomycetes producing keratinases are having high applications in feed, fertilizer, leather and also for pharmaceutical and biomedical applications. *Actinomyces* species newly isolated, thermo tolerant feather degrading bacterial strain was investigated for its ability to produce keratinase enzyme. Maximum keratinolytic activity was observed at 28°C and pH 7.5. Keratin-containing materials (feather, hair, wool, etc.) are abundant in nature but have limited uses in practice since they are insoluble and resistant to degradation by the common proteolytic enzymes. Keratinous wastes represent a source of valuable proteins and amino acids and could find application as a fodder additive for animals or source of nitrogen for plants. Actinomycetes have the ability to break down many different varieties of organic compounds. The keratinase production by the thermophilic actinomycete strain *Actinomyces* was induced by chick feather as the sole source of carbon and nitrogen in the cultivation medium and characterization studies were carried out for the identification of the specific strain. [Journal of American Science. 2010;6(12):45-48]. (ISSN: 1545-1003).

Keywords: Keratin, Actinomycetes, Antibacterial activity, *Actinomyces*.

1. INTRODUCTION

Actinomycetes sometimes cause bio-deterioration of materials and are often responsible for spoilage of hay, straw, cereal grains, seed, wood paper, wool, hydrocarbon, rubber and plastics. In nature, biodegradation by acinomycetes plays an extremely useful role in waste removal and is an integral part of the recycling of materials. Most actinomycetes live in aerobic soils, where they biodegrade organic substrate. Number of actinomycetes living in soils often exceed in one million per gram. The keratin chain is tightly packed in the helix and then substrate turns into a supercoiled polypeptide chain resulting in mechanical stability and resistance to common proteolytic enzymes such as pepsin, trypsin and papain (Parry and North, 1998). Keratin is having more protein and aminoacid source Chicken feather mainly contains keratin, which is an insoluble protein with high stability and is indigestible by common proteases (Parry and North., 1998; Kreplak *et al.*, 2004). Keratinase enzyme are reported to produce by some species of bacteria especially Microbacterium sp (Thys *et al*., 2004) and by actinomycetes such as Bacillus and Streptomyces (Williams *et al.*, 1990)and by some keratinolytic Fungi (Yamamura *et al*., 2002). In this study, keratinolytic actinomycetes present in the soil environment were analyzed and isolation techniques were carried out.

2. MATERIALS AND METHODS

2.1 Collection of soil

The soil samples were collected from various poultry and chick farm.

2.2Cleaning of glassware

The glassware of borosil grade was used in all the experiments. The glassware was cleaned by soaking in chromic acid solution (100g potassium dichromate dissolved in one liter water with 500ml concentrate sulphuric acid) for two hours and washed in water.

2.3Chemicals used

All the chemicals were used of high purity and whenever necessary sigma grade chemicals were used.

2.4 Sterilization techniques

All the glass wares were sterilized in a hot air oven at 180^{o}C for two hours. All the prepared media and water blanks were sterilized in an autoclave at 1atm for 95 minutes. All the antibiotics were filter sterilized using sintered glass filter the isolation, purification, inoculation and other microbiological

works were carried out in a laminar Air flow chamber (Air Flow, India).

2.5. Isolation and screening of keratinolytic actinomycetes

A basal medium, containing 0.5g $M_gSO_4.7H_2O$, 1.0g K_2HPO_4, 3.0g $CaCO_3$, 20g agar per liter and 1ml trace element solution was used to test the ability of salt – loving actinomycetes to grow on sterile feathers. The feathers were supplied in small pieces on the surface of the solidified medium and were the sole source of carbon and nitrogen.

The basal medium was fortified with 100gm/LNacl to adjust salinity to 10%, and the initial pH of the medium was adjusted to 7.5-7.6. The plates were seeded with spore suspensions of the actinomycetes strains, and were incubated at 28^{o}C. Plates were examined for growth and colonization on feather pieces. Strains that showed visible growth on the feathers were considered to be potentially keratinophillic.

Keratinolytic potential of selected salt-loving strains

The keratinolytic potential of the two newly described salt-loving actinomycetes species, *Nocardiopsis halotolerans* and *Saccharomonospora halophila* (Al-Zarban *et al*.2002), were tested by a modification of the diffusion method of minimal agar medium containing 0.15% chicken feather flour (ball-milled) was poured in Petri plates. After solidification 4mm wells were cut in the agar, filled with culture fluid (initially grown in starch nitrate broth plus 10% Nacl) and incubated at 28^{o}C. The positive utilization of the feather flour as the sole source of carbon and nitrogen was assessed by the formation of a clear zone around the wells.

3.Physiological characteristics of Actinomycetes

3.1 Effect of pH

Modified Bennett broth was prepared and sterilized. The pH of broth was adjusted to 5, 7, and 8.5 using 0.1 N HCL and NaoH. The actinomycete cultures were inoculated in the broth. The tubes were incubated at room temperature for 7 days & 14 days after incubation the growth was recorded (Ivanko and Varbanets *et al*., 2004).

3.2 Effect of temperature

Modified Bennett broth was prepared and sterilized. The Actinomycetes cultures were inoculated in the broth. The tubes were incubated at 37° C & 45° C for 7 and 14 days, then at 4^0C & 10^0C for 2 and 4 weeks respectively. After incubation the growth was recorded. The pH and temperature optima were determined to be 8.6 and 70^{o}C (Ignatova *et al*., 1999).

3.3 Effect of Inhibitory compounds

Modified Bennett broth was prepared and sterilized. Inhibitory compound such as crystal violet (0.0001 gm/100ml), phenol (0.1 gm/100ml), sodium azide (0.01, 0.02 gm/100ml) sodium chloride (4, 7, 10, 13 gms/100ml) were added. Actinomycetes cultures were inoculated into the broth then the tubes were incubated at room temperature for 7 and 14 days. After incubation the growth was recorded.

3.4 Utilization of carbon source

Modified Bennett broth was prepared with carbon source like Dextrose, sucrose, Lactose, Maltose, Mannitol, L-arabinose, D-arabinose, D-Galactose, L-Rhamnose & starch. The tubes were sterilized at 121^0C for 15 lbs, 10 minutes Actinomycetes cultures were inoculated into the broth. Then tubes were incubated at room temperature for 7, 14 and 21 days after which the tubes were examined for the growth (Al-Musallam *et al*., 2003).

3.5 Utilization of Nitrogen source

Modified Bennett broth was prepared with Nitrogen source such as L-cysteine, L-histidine, L-phenylalanine, L-serine, L-Threonine, L-Glycine and L-valine Actinomycetes cultures were inoculated into the broth. Then the tubes were incubated at room temperature for 15 days. After incubation the growth was recorded (Chao *et al*., 2007).

3.6 RESISTANCE TOWARD SODIUM CHLORIDE:

The basal medium was prepared in 5 batches which are supplemented with NaCl (g/lit) 2, 7, 10, 30, 50, 70, and120 separately, the medium was autoclaved and poured into the plates.

An agar plate surface was divided into 4 sections. Each section was streaked with an actinomycete culture and incubated for 5 days at 30°C. Inhibition of growth from strong to moderate level was tested based on the observation of nature of growth compared to control.

Antibacterial property of the purified product was determined by overlay method. In this method spores were streaked on Petri plates containing 15ml of arginine glycerol salt agar medium and incubated for 5days at 30°C. 10ml (0.75% agar) of test bacteria were mixed with 0.1ml of cell/spores suspension (10^2 ml^{-1}) and overlaid on 5 days old growth of isolated actinomycetes. The plates were further incubated at 30°C for 24 hrs.

4. RESULTS

Streptomyces and *Actinomyces* partially degraded native chicken feather at 50°C (Bockle *et al*., 1995).

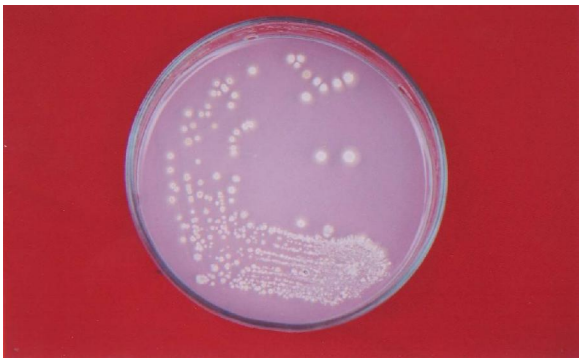

Figure: 4.1. Plate shows the Cultural characteristics of *Streptomyces spp* in Starch Caesin agar

5. DISCUSSION:

The soil samples were collected from various poultry and chick farm wastes was collected from Namakkal district and in and around Arcot, Tamil Nadu, India and the 64 isolates of actinomycetes were obtained from them is shown in Table 5.1.

Table 5.1. Total Actinomycetes population in different soil

S. No	Nameof The Soil	Number Of Actinomycetes Obtained
1	Poultry soil	28
2	Sheep soil	18
3	Cow soil	12
4	Buffalo soil	06
TOTAL		**64**

Total actinomycetes colonies were differentiated by cultural characteristics. The Actinomycetes isolates population was shown in Table 5.2, which were mainly Actinomyces and Streptomyces species.

Table 5.2. Selection of Keratinolytic Actinomycetes isolates

S. NO	NAMEOF THE SOIL	NUMBER OF DIFFERENT ISOLATES OBTAINED
1	Poultry soil	16
2	Sheep soil	12
3	Cow soil	8
4	Buffalo soil	3
TOTAL		39

SUMMARY AND CONCLUDING REMARKS

The present study mainly involved in the isolation of Actinomyces based on its morphology and identification based on the cultural characteristics. The Actinomyces species isolation was mainly based on the physiological characters and its potentiality were identified. Further work should be focused in species identification and on the keratinolytic potential of some dermatophytic fungi such as Trichophyton and Microsporum (Asahi et al., 1985, Qin *et al*., 1992, Filipello Marchisio 2000, Moallaei *et al*., 2006).

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