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Occurrence and extracellular enzyme potential of Actinomycetes of a thermoltalerant, northern region of Karanataka, Inida

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Abstract

Occurrence and extracellular enzyme activities of a thermotalerent actinomycetes isolated from thermal spring of compost pit area, hair, feathers dumping places and municipal wastes of Gulbarga and Bellary (Northern part of Karnataka) and tested their degradability against hair and feather. A total of 67 actinomycetes were isolated, out of these 57 actinomycetes screened exhibited amylase, protease, chitinase, cellulase and keratinase activity determined in the petriplate using on starch casein agar (SCA) and nutrient agar media (NA). Out of 57 actinomycetes, 50 isolates showed amylase, 54 protease, 50 chitenase, 52 cellulase and 41 isolates showed keratinase activities respectively. The Maximum keratinase activity shows VSAC-12, this study shows that the isolates were found to be capable using keratin substrates as sole source of carbon and energy for further investigation.

Keywords: Actinomycetes, Extracellular enzymes, Keratinase

INTRODUCTION

Actinomycetes play an important role in decomposition of plant and other materials especially in the degradation of complex and relatively recalcitrant polymers. They degrade lignin, cellulose and lignocellulose. There is evidence that actinomycetes are involved in the degradation of many other naturally occurring polymers in soils such as, hemicellulose, pectin, keratin, chitin and fungal cell wall materials (Ali and Royman, 1984). Among the microorganisms, actinomyctes gained special importance due to their capacity to produce bioactive secondary metabolites and enzymes. They are known from many habitats: Soil, compost, sludge, freshwater, marine and atmosphere (Lacey, 1973; jenson, 1995; Dhevendaran and Annie, 1999; Seng *et al*., 1999). Actinomycetes, at one time were considered to be microbiological curiosities of no great economic importance. Now it has become the subject of intensive search for sources of new biologically active compounds. Actinomycetes have always been regarded as a strange group of prokaryotes with respect to the diversity of their morphology and of their metabolic products such as, antibiotics, antitumor agents, enzymes, enzyme inhibitors and growth promoting substances (Lechevalier and Lechevalier, 1967). They have been found in all ecological niches such as, soil, water (marine and fresh), compost, extreme environments, marshy places etc. Among them soil has the greatest population density. Extreme environments such as, alkaline, acidic, high temperature, high pressure, complex ecological system, polluted environments, rhizospheres, can be looked for screening new actinomycetes (Gray and Willams, 1975; Goodfellow, 1983). Most of the interest in actinomycetes group of microorganisms lies in their ability to produce a divers group of bioactive compounds, having application in medicinal, industries and agricultural fields. Many genera, notably *Streptomyces* produce commercially important antibiotics (Goodfellow and O'Donnell, 1994) and an array of other secondary metabolites; these include aliphatic alcohols, lactones, biogenic sulphides, ketones, esters, thioesters, furanones and isoprenoids (Gerber, 1983; Jachymova *et al.,* 2002). Actinomycetes also produce secondary metabolites that show bioactivities other than antibiotics such as, enzyme inhibitors, immunosuppressors, phytotoxins and pesticides (Berdy, 1995; Park *et al.,* 2002). We report here the occurrence and enzymatic activities of actinomycetes isolated from a thermal spring situated at the North Karnataka region.

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MATERIAL AND METHODS

Collection of soil samples

The soil samples were collected from different places like, compost pit area, hair and feather dumping places and municipal wastes of Gulbarga and Bellary (Northern part of Karnataka). Top layer of the soil was removed to a depth of about 8-10 cm with a clean spade and then using clean stainless steel scoop and plastic spoon. About 50 g of soil sample was collected in sterile polythene bags and the bags were labeled with date, place of collection and the sample number.

Isolation of Actinomycetes

The standard serial dilution plate culture method (Nakeeb and Lechevalier, 1963) was employed to isolate the pure culture of actinomycetes. Adequate serial dilution (10^{-1}–10^{-5}) was prepared from the enriched samples. 0.1 ml of the sample from the respective dilution was plated on starch casein agar. The inoculated plates were incubated at 30^{0}C for one week. The growth of actinomycetes was observed on the medium at regular interval of 24 h. After isolation of the colonies in pure culture on the slants, they were preserved in refrigerator.

Morphological characterization

A morphological study including substrate mycelium, aerial mycelium, sporulation and pigmentation status of the actinomycetes (Williams and Welington, 1980), after their adequate growth on starch casein agar was carried out as per the procedure prescribed in Goodfellow (1989) Bergey's manual of Systematic Bacteriology. The growth characters including aerial mycelium, pigmentation and growth activity of all the 67 isolates were studied. The typical colonies of actinomycetes were re-inoculated on starch casein agar in which cover slips were placed in inclined position at an angle, 45^0 and incubated for 3 days. After the growth of actinomycetes, the cover slip was removed and observed for the substrate mycelium and aerial mycelium (Shinobu, 1958; Kawato and Shinobu, 1959).

Screening of Extracellular Enzymes

The selected 62 actinomycete isolates strains were screened for the production of different extracellular enzymes namely, amylase, protease, chitinase, cellulase, and keratinase. Amylase activity was assayed using agar diffusion method by growing the isolates on nutrient agar (peptone, 5 g; yeast extract, 1.5 g; beef extract, 1.5 g; NaCl, 5 g; agar, 16 g; distilled water 1000 ml; pH 7.2) supplemented with 1% iodine in 2% KI. The clear zone developed around the colony was observed and inhibition diameter was measured. Protease activity was assayed by growing the isolates on the nutrient agar medium supplemented with 1% casein as a carbon source. After incubation, the plates were flooded with 0.1 ml of hydrochloric acid. The formation of clear zone around the colony was observed and measured. The Chitinase activity was determined by growing the isolates on colloidial chitin agar medium (CCA). After incubation the clear zone around the colonies was considered as positive for chitinase production and using scale. The cellulase activity was determined by growing the isolates on the medium containing ($NaNO_3$ 2 g; K_2HPO_4, 1 g; $MgSO_4$ $7H_2O$ 0.05 g; KCl, 0.05 g; $FeSO_4$ $7H_2O$ 0.01 g; Agar 16 g; distilled water 1000 ml; pH 6.8), supplemented with 1% CMC–Na salt as carbon source. After incubation, the plates were flooded with 5ml of 1% KI. The formation of a clear zone around the colony against reddish brown background was observed and measured using scale. The keratinase activity was assayed by growing the isolates on NA (peptone, 5 g; yeast extract, 1.5 g; beef extract, 1.5 g; NaCl, 5 g; agar, 16 g; distilled water 1000 ml; pH 7.2) medium with 4% keratin as carbon source. After the incubation, the plates were flooded with 5 ml of 1% $HgCl_2$. The formation of a clear zone around the colony against white precipitate was seen and the diameter was measured using scale.

RESULT

Screening of Isolates for Extracellular Enzymes

A total of 67 thermotalerent actinomycetes were isolated (Plate-I). The qualitative activity of five different enzymes *viz.,* amylase, protease, chitinase, cellulase and keratinase were determined in the petriplate using SCA and NA medium. Out of 57 actinomycete isolates, 50 isolates showed amylase activity, 54 isolates showed protease, 50 isolates showed chitinase, 52 isolates showed cellulase and 41 isolates showed keratinase activity (Table-1).

Table 1. Enzyme activity in thermotalerent actinomycetes isolates from soils, composts pits of Gulbarga and Bellary District

Organisms	Zone of digestion of of substrate (mm) Amylase	Protease	Chitenase	Cellulase	Keratinase
VSAC-1	44.7	22.3	34.3	19.0	16.3
VSAC-2	31.6	31.0	24.3	20.0	15.3
VSAC-3	47.6	15.3	17.0	32.3	22.0
VSAC-4	34.3	18.6	24.3	0	19.3
VSAC-5	59.6	39.6	29.6	35.0	44.3
VSAC-6	29.3	41.6	32.0	25.6	0
VSAC-7	39.6	22.3	45.6	28.3	0
VSAC-8	26.3	28.6	49.0	0	0
VSAC-9	21.3	50.6	32.6	19.3	0
VSAC-10	35.6	26.3	28.6	45.6	33.0
VSAC-11	41.6	29.0	20.0	32.0	18.0
VSAC-12	**81.3**	**74.3**	**78.0**	**69.0**	**82.6**
VSAC-13	63.6	53.2	68.4	56.0	63.0
VSAC-14	41.3	29.6	32.3	19.3	21.0
VSAC-15	32.3	24.3	0	21.0	15.0
VSAC-16	24.6	38.3	41.0	0	26.0
VSAC-17	75.3	41.1	18.0	0	0
VSAC-18	0	18.3	21.6	26.3	0
VSAC-19	0	22.0	35.6	19.0	0
VSAC-20	0	26.3	16.0	45.6	32.0
VSAC-21	32.3	54.3	48.0	47.3	44.0
VSAC-22	14.3	0	22.0	32.0	51.0
VSAC-23	27.6	0	19.3	24.0	0
VSAC-24	54.6	36.3	41.3	41.0	19.0
VSAC-25	41.3	31.6	54.0	51.6	49.0
VSAC-26	48.3	28.3	43.3	39.6	28.0
VSAC-27	37.6	42.3	27.0	32.5	15.0
VSAC-28	18.6	34.3	20.6	0	0
VSAC-29	21.3	18.0	38.3	45.0	32.6
VSAC-30	0	14.6	0	25.6	0

VSAC-31	61.6	39.6	49.0	19.3	44.0
VSAC-32	22.6	25.6	35.6	36.3	31.0
VSAC-33	29.3	21.3	29.3	34.3	22.0
VSAC-34	59.6	51.6	16.0	28.0	48.0
VSAC-35	46.6	19.6	19.6	50.3	0
VSAC-36	29.3	23.0	36.3	29.3	0
VSAC-37	35.3	31.6	51.6	49.6	39.0
VSAC-38	34.6	27.3	0	21.0	19.3
VSCA-39	29.6	19.5	22.0	35.3	25.9
VSAC-40	41.6	16.3	19.0	45.0	32.0
VSAC-41	35.3	27.6	26.3	27.3	32.6
VSAC-42	39.6	0	21.0	41.0	26.3
VSAC-43	0	18.3	32.0	21.9	0
VSAC-44	61.6	51.3	54.5	51.0	53.0
VSAC-45	59.3	44.3	19.0	21.3	0
VSAC-46	28.3	39.0	0	0	19.0
VSAC-47	22.6	25.3	49.6	50.3	28.3
VSAC-48	44.3	18.6	0	29.3	0
VSAC-49	64.8	12.0	0	19.0	32.0
VSAC-50	37.3	21.3	32.0	25.3	19.6
VSAC-51	0	41.9	45.0	39.3	24.0
VSAC-52	44.6	53.6	39.0	49.6	18.0
VSAC-53	49.6	38.6	18.3	26.3	14.0
VSAC-54	58.3	51.0	50.0	52.0	59.0
VSAC-55	27.6	39.3	0	22.0	0
VSAC-56	44.6	34.3	16.6	32.0	16.2
VSAC-57	0	19.6	25.3	29.3	21.0

Isolates on Starch Casein Agar Media, Mean of 3 values.

Maximum amylase activity was seen in isolate VSAC-12 followed by VSAC-13, VSAC-17, VSAC-24, VSAC-31, VSAC-34, VSAC-44, VSAC-45, VSAC-49 and VSAC-54 and lowest activity in isolate VSAC-22. No activity was observed in VSAC-18, VSAC-19, VSAC-20, VSAC-30, VSAC-43, VSAC-51 and VSAC-57 isolates. Highest protease activity was observed in isolate VSAC-12 followed by VSAC- 13, VSAC-21 VSAC-34, VSAC-44, VSAC-52 and VSAC-54 and lowest activity in VSAC-30. No activity was observed in VSAC-22, VSAC-23 and VSAC-42 isolate. Similarly, the highest chitinase activity was seen in isolate VSAC-12 followed by VSAC-13, VSAC-25, VSAC- 37, VSAC-44 and VSAC-54 and lowest in VSAC-20. However, no activity was observed in VSAC-15, VSAC-30, VSAC-38, VSAC-46, VSAC-48 and VSAC-55 isolates. The cellulase activity was maximum in isolate VSAC-12 followed by VSAC-13, VSAC-25, VSAC-35, VSAC-44 and VSAC-47. No activity was recorded in isolates VSAC-4, VSAC-8, VSAC-16, VSAC-17, VSAC-28 and VSAC-46. The keratinase activity was maximum in isolate VSAC-12 fallowed by VSAC-13, VSAC-22, VSAC-44 and VSAC-54. No activity was recorded in isolates, VSAC-6, VSAC-7, VSAC-8, VSAC-9, VSAC-16, VSAC-18, VSAC-19, VSAC-23, VSAC-28, VSAC-30, VSAC-35, VSAC-36, VSAC-43, VSAC-45, VSAC-48 and VSAC-55. The VSAC-12 actinomyctes for further study in our laboratory.

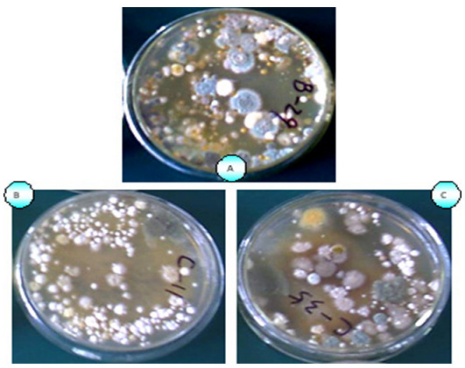

Fig 1. Keratinolytic actinomycete isolates from soils, composts pits of Gulbarga and Bellary District: (A) B-29; (B) C-11; (C) C-35.

DISSCUSSION

The biological activity of Indian subcontinent is one of the richest (second highest population in the world) owing to its vast geographical area, varied topology, climate, and the juxtaposition of several bio geographical regions. India is recognized as one of the twelve mega diversity regions in the world because of its richness in overall species diversity, Thus, India's contribution to the global diversity is around 8% (Manish kapoor and Rakesh kumar, 2004). It is important to study microbial diversity not only for basic scientific research, but also to understand the link between diversity and community structure and function. Human influences such as, pollution, agriculture and chemical application could adversely effect microbial diversity and perhaps also above and below ground ecosystem functioning (Jennifer *et al*., 2004). Actinomycetes, at one time considered being microbiological curiosities of intensive search for the sources of new, biologically active compounds. The demonstrated ability of these organisms to produce useful antibiotics and to carry out other transformations of commercial interest has focused attention on factors bearing on their isolation form their natural habitat, the soil (Hesseltine *et al*., 1954).

In the present study, different, compost pit areas, hair and feather dumping places and municipal wastes of Northern part of Karnataka were studied for the diversity of actinomycetes. The distribution of actinomycetes was observed to be predominant (2.4-7.80 X 10^5 cfu/g) in black soil when compared to red soil (1.90 – 3.55 x 10^3 cfu/g). The soils obtained from the alkaline habitat showed considerably very high predominance of actinomycetes than forest and salty soils. Abussaud and Saadoun (1991) were also reported 10^5–10^7 cfu/g of actinomycetes in dry soils of Jordan. A 10^3–10^6 cfu/g of actinomycetes were reported in acidic soils by Khan and Williams, (1975). Balagurunathan *et al*., (1996) while studying diversity of soil actinomycetes from south India and south China recorded progressive increase in the number of actinomycetes from forest to vegetable farm lands, composts, municipal wastes which is in confirmation with observations made in this study. Soil is the most common habitat of actinomycetes, these bacteria can be found

in all kinds of soils (Kutzner, 1986 and Williams *et al.,* 1983). *Streptomyces* were found (Lacey and Crook, 1988), in composts and fooder, especially in self-heated hay or grain. During the early stages of composting or self-heating, mesophilic species were present, but these were replaced by thermotolerent species like, *Streptomyces albus* or *Streptomyces griseus*, and with increasing temperature, the real thermophilic species take their place (Goodfellow and Simpson, 1987). It is well known that bacteria, fungi and actinomycetes play an important role in decomposition of complex organic substrate found in nature. Bacterial and fungal growth rate is faster than actinomycetes, so they bring about faster degradation of these compounds. The complex organic compounds such as, chitin and keratin are not easily degradable by bacteria and fungi. Although slow growing actinomycetes decompose recalcitrant organic compounds. It is also seen that the percentage of biochemically active actinomycetes possessing amylase, protease, chitinase, cellulase and keratinase. A number of workers have been reported the presence of actinomycete isolates from soil with different enzymatic activity. Amylase activity has been reported by (Abraham and Hen, (1964): Chaphalkar and Dey (1993): Nawami, *et al.,* (2003) and Ammar *et al.,* (2002). Desai and Dhala, 1969: Ellaiah and Srinivasulu, *et al.,* 1996: Kulkarni and Deshmukh, 1999) have detected protease activity, and Shejul and Kapadnis, 1998: Nawami, *et al.,* 2003: Hiroshi Tsujibo, *et al.,* (2003) have detected chitinase activity. Cellulase activity reported by (Stutzenberger, 1972: Ali and Roymon, 1984: Shejul and Kapadnis, 1998: George *et al.,* (2001). Keratinase activity has been reported by Nickerson and Durand, 1963: Nadanishi and Yamamoto, 1974: Bockle, *et al*., 1995: Muhin and Hadi, 2002: Suneetha and Lakshmi, (2003).

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