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Isolation of Thermophilic Actinomycetes from Banana Waste Compost and Their Biochemical Characteristics

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Abstract: ***In the present study 4 thermophilic actinomycetes were isolated from banana waste compost and were studied for their morphological microscopical and enzyme activity. Each isolates were subjected to starch hydrolysis to select high efficient genera for α-amylase production. Among the genera isolated genus Streptomyces sp. exhibited high α-amylase production. The observations showed that Streptomyces sp. was found to be the best producer of the enzyme α-amylase.***

Keywords: Banana waste, Compost, *Streptomyces sp*, Thermophils, α-amylase.

1. Introduction

Composting of organic residue is a complex, exothermic and dynamic ecological process. Organic matter decomposition involves mixed microbial population viz bacteria, actinomycetes, fungi and protozoans that bring about the hydrolysis of organic residues (Jenson etal., 1988, and Sivaramakrishna etal., 2006). Banana waste compost has been widely reported to favour microbial population.

Fungi are the chief decomposers during composting of organic residues Thermophilic actinomycetes usually form a significant component of the microbes in natural substrates when they attain a temperature in excess of 50 ° C such overheated substrates may be divided into groups.

Actinomycetes are noteworthy as antibiotic antibiotic producers, making three quarters of all known products (Waksman, 1961). *Streptomyces sp*. are the most common genus in soils, accounting up to 90% of the populations. However, new approaches for the isolation of actinomycetes from banana waste compost and their enzyme activity are significant during composting of banana waste at thermophilic state. Recently, the rate of discovery of new compounds from existing genera obtained from terrestrial sources has decreased, while the rate of reisolation of known compounds has increased.

Thus, it is critical that new groups of microbes from unexpected habitats particularly thermophilic actinomycetes during composting of banana waste. Keeping this point in view, the present study has been undertaken to isolate, screen and enzyme activity of thermophilic actinomycetes from banana waste compost.

2. Material and Methods

Banana samples (pseudo stems) were collected from market and were brought to the Department of botany Bangalore University Bangalore and composted in heaps and pits. The samples were later collected during thermophilic state of composting (50 - 55° C) in polythene bags and kept for further process. The thermophilic actinomycetes were isolated on starch casein agar.

2.1 Isolation of Actinomycetes

Starch casein agar (Kuster and Williams, 1964) medium (starch 1.0g; casein 0.03g; potassium nitrate 0.2g; sodium chloride 0.2g; di-potassium hydrogen phosphate 0.2g; magnesium sulphate 0.005g; calcium carbonate 0.002g; ferrous sulphate 0.007g; agar 2.0g; distilled water 1000 ml) was prepared and sterilized at 121°C in 15 lbs pressure for 15 minutes. Then it was added with amphotericin B 50mg/ml and tetracycline 20 mg/ml to prevent the bacterial and fungal growth. The medium was poured into the sterile petriplates. The collected soil samples were diluted up to 10^{-6} and 0.1 ml of the diluted samples was spread over the agar plates in triplicates. The inoculated plates were incubated at 50-55 ° C.

2.2 Characterization of actinomycetes

Colony characteristics (Burkholder etal., 1954)

Colony morphology of the isolates was recorded with respect to colour, size and nature of colony, and then the isolates were observed under the microscope.

2.3 Microscopic Characterization

Light microscopy cover slip culture technique, (Pridham and Tresner, 1974). Actinomycetes culture plate was prepared and 5 to 6 cover slips were inserted at the angle of 45°. The actinomycetes culture was slowly released at the intersection of medium and cover slip. The plates were incubated at 50-55 ° C for 4-8 days. The coverslips were removed and observed under the high power magnification of microscope. The morphological features of spores were observed and recorded (table 1).

2.4 Screening for α-Amylase production

The screening of thermophilic actinomycetes isolates for α amylase production was performed by spot inoculation

method. The actinomycetal isolate was spot inoculated on sterile starch agar supplemented with griseofulvin 50µg/ml. The plates were incubated at 50° C for 48 hours. After incubation the plates were flooded with iodine, zone of hydrolysis of starch around the colony indicated α-amylase production. The diameter of zone of hydrolysis and the diameter of colonies were recorded. The isolate showing maximum diameter of the zone was selected for further studies.

2.5 Extraction and Analysis of α-amylase

After the selection of the cultures that showed maximum zone of hydrolysis *Streptomyces sp.*[1] was selected for further enzyme activity. Culture of *Streptomyces sp.*[1] was fully grown on starch casein broth, the contents of the flask were harvested by adding 25 ml of sterile distilled water followed by shaking at 250 rpm for 1 hour, further, refrigerated for 2 hours. The contents of the flask were filtered through a cotton cloth. The filtrate was then centrifuged at 10,000 rpm for 15 minutes. The supernatant thus collected was subjected to analysis of α-amylase activity.

The α-amylase activity was determined as per (Okolo et al., 1995). The reaction mixture consisted of 1.25 ml of 1 % soluble starch, 0.5ml 0f 0.1M phosphate buffer (pH 7.0) and 0.25 ml of crude enzyme extract. After 10 minutes of incubation at 50 °C, the liberated reducing sugars (maltose equivalents) were estimated by Di-nitro salicylic acid (DNS) method (Miller 1959). The colour developed was read at 510 nm using spectrophotometer. Maltose was used as standard.

The blank contained 0.75 ml 0f 0.1M phosphate buffer (pH 7.0) and 1.25 ml of starch solution. One unit (1U) of α-amylase is defined as the amount of enzyme releasing one µmol of maltose equivalent per minute under the assay conditions.

3. Results and Discussion

Actinomycetes are prokaryotes with extremely metabolic potentiality. They produce numerous compounds essential for health such as antibiotics, enzymes, immunomodulators etc. In nature they play an important role in cycling of organic compounds and have also been associated with soil organic matter degradation Alexander (1961).

Banana plant waste contributes one of the major wastes that contain cellulose, lignin and α-amylase. In the present study a total of 4 actinomycetes isolates were isolated from banana compost during thermophilic state by using starch casein agar medium after 10 days of incubation. The actinomycetes were identified on the basis of colour of aerial mycelium, size of the colony and the microscopic morphology (sporophore morphology and formation of aerial substrate mycelium).

Among the four isolates two isolates were assigned to genus *Streptomyces sp*, one isolate *Actinomadura sp*. one of the genus *Nocardia sp.* (table 1). Several workers have reported actinomycetes from agricultural soil habitats (Kulkarni 2011)

Thermophilic actinomycetes usually form a significant component of the microbes in natural substrates which can withstand a temperature of 50 ° C and above because of their mycelial habit and prolific spore production. The thermophilic actinomycetes could have an ecological advantage over other bacteria in a suitable habitat giving rise to the very high spore numbers encountered in certain baled hays and composts.

The prelimnary screening of thermophilic actinomycetal isolates from banana compost the genus *Streptomyces sp* [1] isolate revealed the zone of hydrolysis on starch casein agar plates with distinct halo around the growth than other genera showed comparatively less growth.

The degree of α-amylase activity was indicated by width of the zone of hydrolysis. Thus, from the primary screening *Streptomyces sp*[1], gave maximum zone of inhibition were selected for further secondary screening. There was maximum α-amylase production (0.25 U/g) with increased enzyme activity and the results are in accordance with (Jenson B etal 1998) have screened and designed to isolate strains of *Thermomyces lanuginosus* producing high yield of α-amylolytic enzymes.

Their ability to produce antibiotics and bacteriolytic enzymes stable at high temperature (Mishra and Maheshwari, 1996) may give them an added advantage in molecular and biotechnological applications.

Thus, it is anticipated that the current attempt for the isolation and study of the enzyme activity α-amylase from *Streptomyces sp*[1] isolated from banana waste compost can be a milestone for the discovery of novel effective antibiotics against human pathogens.

Table 1: Morphological characteristic of actinomycetes isolates at 50 ° C during composting of banana waste

Name of the actinomycetes	*Colony size in mm*	*Microscopic characteristic*
Actinomadura	3	Chains of conidia on the aerial mycelium.
Streptomyces sp. [1]	14	Short chains on aerial mycelium.
Nocardia sp.	9	looped
Streptomyces sp. [2]	5	Spirally in chains on aerial mycelium.

4. Conclusion

Further investigations are needed for practical application in the starch industry on account of high purity of products formed their thermostability. In addition, the α-amylase of this strain would be useful in study of the structural organization of protein at high temperature.

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References

[1] Alexander, M., 1961. Introduction to Soil Microbiology. John Wiley and Sons Inc, New York.
[2] Burkholder, P.R., Sun, S.H., Ehrlich, J and Anderson, L., 1954. Criteria of speciation in the genus Streptomyces. Ann New York Acad. 60: 102-103.
[3] Jenson, B., Olsen, J and Allerman, K., 1988. Purification of extracellular amylolytic enzymes from the thermophilic fungus *thermomyceslanuginosus*. Can. J.Microbiol.34: 218-223.
[4] Kuster ,E., and Williams, S.T., 1964. Production of hydrogen sulphide by Streptomyces. Microbial Espanola, 16:193-202.
[5] Kulkarni, S, W., 2011. Biodiversity of soil actinomycetes of Solapur district.J.Microb..World 13(1) pp: 14-25.
[6] Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar.Anal.Chem.31:426-429.
[7] Okolo, B.N., L.I.Ezeogu, C.N, Mba 1995. Production of raw starch digesting amylase by *Aspergillus niger* grown on native starch sources. J.Sci.Food Agri.69:109-115.
[8] Pritham,T.G., 1974. Streptomycetaceae. In Bergey's Manual of Determinative Bacteriology (8th Edition), the Williams and Wilkins Co., Baltimore, U.S.A.pp747.
[9] Ravi, S., Mishra and Ramesh Maheshwari 1996. Amylases of thermophilic fungus *thermomyces lanuginosus*: their purification, properties, action on starch and response to heat. J.Biosci. Vol.21., pp653-672.
[10] Sivaramakrishnan, S. Gangadharan, D., Nampoothiri, K.M., Soccol, C.R and Pandey, A.2006. α- Amylase from microbial sources: An overview on recent developments.Food. Technol. Biotechnol.
[11] Waksman, S.A., 1961. The actinomycetes: classification, identification and descriptions of genera and species. Vol.II, Williams and Wilkins Co. Baltimore, U.S.A., pp363

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