CHARACTERIZATION OF ACTINOMYCETES FOR SOME ENZYMES THAT ARE IMPORTANT IN INDUSTRIES

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ABSTRACT

Actinomycetes are well known for their ability to produce antibiotic. The role of actinomycetes in organic compound degradation is also considerable. However the potential of this group for enzyme production is not given much attention. In present study we have characterized actinomycetes from various soil samples. We collected soil samples from various decomposing soils. Systematic screening methods were employed for the isolation of actinomycetes. A characteristic feature of actinomycetes on star casein media is formation of white powdered colonies. On this basis preliminary selection was done for actinomycetes. Predominance of *Streptomyces* species was observed. Though the genera are similar varied species are reported on the basis of biochemical characteristics. Around ten different species of actinomycetes are observed. A comparative study of the enzyme was carried with respect to three important enzymes viz. amylase, deoxyribonuclease (DNase) and L-asparaginase. Regarding enzyme production it was observed that most species could produce amylase. DNase and L-asparaginase were produced by very few species. The enzymes produced by these species are also abundant. Purification of enzymes from these group as compared to other prokaryotic organisms is also expected to be easier.

KEY WORDS: actinomycete, enzyme production , Soil samples,

INTRODUCTION

Actinomycetes are aerobic gram positive bacteria which predominate the soil. The variation of the species in the soil is because of the nature and conditions of the soil (Arifuzzaman *et al,* 2010). This group resembles the fungi being filamentous. The name 'Actinomycetes' was derived from Greek 'aktis' (a ray) and 'mykes' (fungus) and given to these organisms form initial observation of their morphology. These group is also helpful in degradation of organic compounds and synthesis of bioactive compound as well (Naikpatil and Rathod, 2011). Apart from antibiotic production the ability of actinomycetes in sludge digestion is taken quite seriously. These group causes the foaming of the sludge thereby enhancing the digestion of the sludge (Davenport *et al,* 2000). Similar studies were carried by Madoni *et al,* (2000) in Italy dealing with foaming and bulking. The foam production and stability was also carried by Heard *et al,* 2008.

Because of their ability for degradation of various organic compounds viz. carbohydrates, proteins and aromatic compounds they have been used quite efficiently in the treatment of waste matter (Lemmer and Kroppenstedt, 1984; Lemmer, 1986). The first antibiotic of actinomycetes origin was streptomycin produced by *Streptomycin griseus*. More than 12,000 antibiotics have been discovered in the last 55 years of which actinomycetes constitutes 70% and 30% is by fungi and other microorganisms (Nanjwade *et al,* 2010). Various microorganisms capable of producing antibiotics includes filamentous fungi and the prokaryotic actinomycetes e.g., *Amycolatopsis*, *Nocardia* and *Streptomyces* are reported (Wezel *et al,* 2006). Organo pesticides are the major problem these days because these are used frequently and carelessly. The bioaccumulation is causing various health hazards. However some actinomycetes are found to degrade these pesticides quite efficiently. The study was initiated in Argentina by Fuentes (*et al,*2010). The pesticides studied were chlordane, lindane or methoxychlor. Indeed actinomycetes has wide range of application many fields, however the ability of actinomycetes for various enzyme production is given less concern which is focus of our study. In this study we have characterized actinomycetes from various soil samples and studied the potential of the same for industrially important enzymes like DNase, L-asparaginase and amylase.

MATERIALS AND METHODS

Collection and preparation of soil sample

Top 4 cm soil is considered a good source of microorganisms as most activities takes place in this region. Samples were collected from decomposing soil in Srinagar area of kashmir. Soil sample (approx. 10g) were collected using clean, dry and sterile polythene bags along with sterile spatula and were marked properly. Varied soil with regards to moisture, texture and content was selected. Samples were stored at 40°C until pretreatment. Microorganisms other than actinomycetes are degraded because of pretreatment (Arifuzzaman *et al*, 2010).

Isolation of Actinomycetes

The pretreated soil suspensions were spread over starch casein agar followed by incubation at 35^0 C up to 5 days. Dilution 10^{-7} gave well isolated white powdery growth on this agar surface a characteristic feature of actinomycetes (Reddy *et al*, 2011).

Identification of actinomycetes

Identification of isolates was based on cultural, morphological and biochemical characteristics. Motility has been performed according to the hanging drop method. The standard biochemical tests such as catalase, oxidase and

fermentation various sugars, methyl red reaction, Voges Proskauer test and citrate utilization on Simmon's citrate agar was performed. Further the enzymatic studies of these isolates have been studied.

Enzymatic study of isolates

The screened isolates were studied for the production of enzyme such as amylase, DNase and L-asparaginase respectively.

L-asparaginase production

A mineral base agar containing glucose as carbon source and L-asparagine as nitrogen source with phenol red as indicator system was used. The liberated ammonia after L-asparagine break down leads to change in color from yellow to pink around the colony. Pink coloration around the colony was noted (Prakasham *et al*., 2007).

Amylase Production Test

Starch Agar Medium containing soluble starch as carbon source was prepared and by the method of Marasabessy *et al*., (2011). The activity of amylase was studied by flooding the plates with iodine.

DNase Production Test

The isolates were spot inoculated on media supplemented with 0.2% DNA and indicator system as toludine blue. The decolorization of DNA from blue to colorless around the colony was noted (Schreier, 1969).

RESULTS AND DISCUSSION

Isolation of Actinomycetes

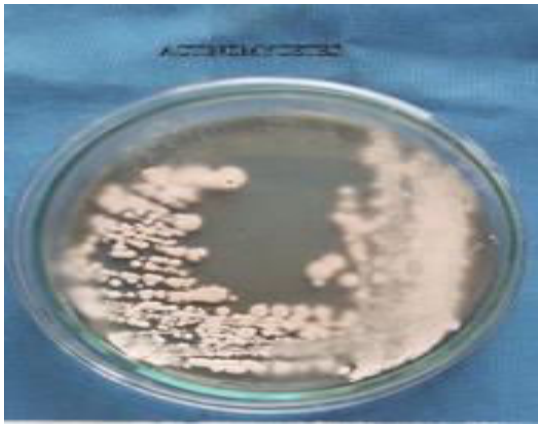
Tedious screening procedures were adopted for large number of soil samples. Finally obtained cultures were designated SA-1 to SA-10. Most colonies were either white or off white and size ranged from 3 to 4.5mm. Colonies on starch casein agar were irregular with filamentous margin rarely margin was wavy. Elevation was raised, in few cases it was convex and flat (Table 1).

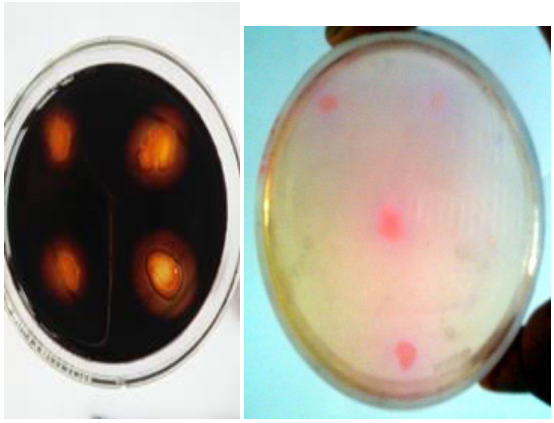
Table.1Characterization of actinomycetes

Isolate		Carbohydrate utilization									Morphological properties			
	Dextrose	Lactose	Trehalose	Citrate	Arabinose	Maltose	Salicin	Sorbitol	Mannitol	Raffinose	Colour	Shape	Gram reaction	Identified bacterial sp
SA1	-	-	-	+	+	-	-	-	-	-	white	filanentous	+	*Streptomyces*
SA2	-	-	-	+	+	-	+	-	-	-	white	Long rods	+	*Streptomyces*
SA3	-	-	-	+	+	-	-	-	-	-	Off white	Long rods	+	*Streptomyces*
SA4	-	-	-	+	+	-	-	-	-	-	White	filmentous	+	*Streptomyces*
SA5	-	-	-	+	+	-	-	-	-	-	Off white	filmentous	+	*Streptomyces*
SA6	+	-	-	+	+	-	+	-	-	-	Off white	filmentous	+	*Streptomyces*
SA7	-	-	-	+	+	-	-	-	-	-	White	filmentous	+	*Streptomyces*
SA8	+	-	+	+	+	+	-	-	+	+	White	Long rods	+	*Streptomyces*
SA9	-	-	-	+	+	-	-	-	-	-	White	filmentous	+	*Streptomyces*
SA10	-	-	-	+	+	-	+	-	-	-	Off white	Long rods	+	*Streptomyces*

Microscopic studies carbon sources utilization:

The peculiar properties of actinomycetes is that these bacteria are filamentous and gram positive. All bacteria were non-motile and spore forming. All strains were found to be oxidase and catalase positive. All strains utilized arabinose and citrate as carbon source. Cultures were negative for indole and VP test. Lactose and sorbitol was not fermented. Culture designated SA-8 was found to utilize maltose and raffinose but rest did not. Dextrose and trehalose was hydrolyzed by SA-6 and SA-8 only. Methyl red test was found to be positive except SA-4 and SA-8. Salicin was fermented by SA-2, SA-6 and SA-10 where as mannitol was fermented by SA-8 only (Table 1).





Isolation of actinomycetes and enzymatic activities

Cultures further were studied for production of three important enzymes viz. L-asparaginase, DNase and amylase. Both L-asparaginase and DNase are therapeutic enzymes. DNases are employed in genetic engineering processes also along with some industrial applications as well. Amylases are universally popular for use in textile and other industries. Therefore these enzymes were selected. Cultures designated SA-1, SA-2 and SA-3 were found to produce amylase only and other enzymes could not. SA-4, SA-7 and SA-8 were able to produce all three enzymes selected. SA-5 and SA-6 could produce amylase and DNase but could not produce L-asparaginase. SA-9 could only produce amylase whereas Act-10 could only produce DNase.The test was performed by spot inoculation though it was qualitative zones of enzymatic hydrolysis were measured which showed that largest amylase activity was found in case of Act-6. Act-10 was most efficient for DNase activity whereas largest L-asparaginase activity was found in case of Act-8 (Table2).

Isolate	Zone of Enzymatic Hydrolysis		
	Amylase	DNase	L-Asparginase
SA1	14	-	-
SA2	16	-	-
SA3	20	-	-
SA4	25	20	17
SA5	15	16	-
SA6	28	13	-
SA7	22	18	18
SA8	22	15	20
SA9	18	-	-
SA10	-	26	-

Table 2:Comparitive studies of enzymatic activities

CONCLUSION In conclusion actinomycetes are well known for antibiotic productions but other applications are given less concern. In the present study we have isolated ten strains of actinomycetes from decomposing soils from some areas of kashmir in which *Steptomyces* species are predominantly present in the soil. These strains are definitely preferable source for DNase, amylase and L-asparaginase which are quite important. Thus, these isolated strains of actinomycetes can be an economical and safe.

REFERENCES

Arifuzzaman M., Khatun M.R. and Rahman H. (2010). Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity. *African J.Biotech.* 9(29):4615-4619. Davenport R.J., Curtis T.P.,Goodfellow M., Stainsby F.M. and Bingley M. (2000).

Quantitative use of fluorescent in situ hybridization to examine relationships between mycolic acid-containing actinomycetes and foaming in activated sludge plants. *Appl. Env. Microbiol.* 66: 115-1166. El-Nakeeb M.A. and Lechevalier H.A. (1963).

Selective Isolation of Aerobic Actinomycetes. *Appl. Microbiol.*11:75- 77. Fuentes M.S., Benimel C.S., Cuozzo S.A. and Amoroso M.J., (2010).

Isolation of pesticide degrading actinomycetes from a contaminated site: Bacterial growth, removal and dechlorination of organochlorine pesticides. *Int. J. Biodeterioration Biodegradation.* 64(6): 434-441.

Heard J., Harvey E., Johnson B.B., Wells J.D. and Angove M.J. (2008). The effect of filamentous bacteria on foam production and stability. *Colloid Surface.* 63: 21-26. Lemmer H. (1986).

The ecology of scum causing actinomycetes in sewage treatment plants. *Water Res*, 20: 531-535. Lemmer H. and Kroppenstedt R.M., (1984).

Chemotaxonomy and physiology of some actinomycetes isolated from scumming activated sludge. *Syst. Appl. Microbiol.* 5: 124-135. Madoni P., Davol D. and Gibin G. (2000).

Survey of filamentous microorganisms from bulking and foaming activated-sludge plants in Italy. *Water Res*. 34: 1767-1772. Marasabessy A., Moeis M.R., Sanders J.P.M. and Weusthuis R.A. (2011).

Enhancing Jatropha oil extraction yield from the kernels assisted by a xylan-degrading bacterium to preserve protein structure. *Appl. Microbiol. Biotechnol*. 90: 2027-2036. Naikpatil S.V. and Rathod J.L., (2011).

Selective isolation and antimicrobial activity of rare actinomycetes from mangrove sediment of Karwar. *J. Ecobiotechnol.* 3(10): 48-53. Nanjwade B.K., Chandrashekhara S., Shamarez A.M., Goudanavar P.S. and Manvi F.V. (2010).

Isolation and morphological characterization of antibiotic producing Actinomycetes. *Trop. J. Pharmaceut. Res.* 9: 231-236.