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Enzymatic Activities of Actinomycetes from the Soil Samples of Jammu and Kashmir

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Abstract:
The present study focused on isolation of actinomycetes from the soil samples of jammu and Kashmir can provide rich source of enzyme producing actinomycetes. Totally, 52 different actinomycetes were isolated from the soil samples of jammu and Kashmir. 90 actinomycetal isolates were identified up to generic level. It was found that these actinomycetal isolates were belonging to *Streptomyces, Micromonospora, Intrasporangium, Saccharopolyspora, Streptosporangium, Rhodococcus, Saccharomonospora* and *Nocardia.*It has been found that out of 40 actinomycetes , 32 (80%), 27 (67.50%), 34 (85%) and 25 (62.5%) number of actinomycetes possessing amylase, protease, lipase and cellulose activity respectively.

Keywords: actinomycetes, amylase, protease, lipase, cellulose, oil samples.

I. INTRODUCTION

The name "Actinomycetes" was derived from Greek words 'aktis' (a ray) and 'mykes' (fungus). Actinomycetes are true bacteria (related to Corynebacteria and mycobacteria) but they form long branching filaments that resembles the hyphae of fungi, yet possess sufficient distinctive features to classify them into a separate actinomycete. Actinomycetes are best known for their ability to produce antibiotics and are gram positive bacteria which comprise a group of branching unicellular microorganisms.

II. MATERIAL AND METHODS

Take 250 ml of CSA broth was put it in 1 litre of distilled water. PH was adjusted to 7 and then 2% agar was added. The medium was autoclaved at 121°C, 15 pounds/inch for 15 minutes.

Sterility testing of Medium

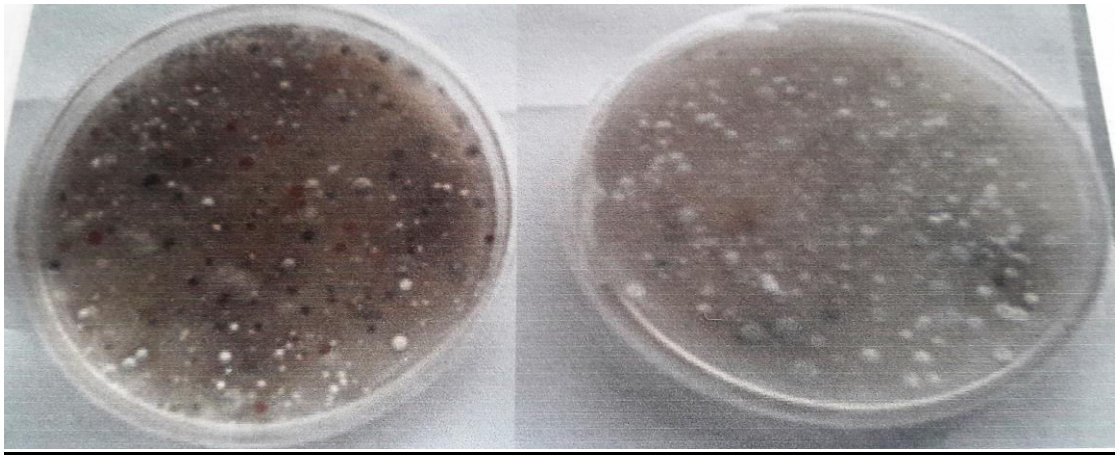
The autoclaved media were incubated at 37°C for overnight for sterility testing.

Isolation of actinomycetes strain from soil samples of Jammu and Kashmir

CSA media was prepared, sterilized and cooled. The media was poured on the Petri plates and incubated for overnight at 37° C to check sterility. Prepared three distilled water test tubes (9 ml) each and autoclaved them. With the help of sterilized spatula, added 1g of soil samples in sterilized distilled water tube and mixed it and then from it made dilutions up to 10^{-3}. By using micropipette and sterilized tips 100 µl of inoculum was poured on CSA- plates and spread it with sterilized and cooled glass spreader. Incubated the plates at 28° C for few days and observed for the appearance of colonies. Picked up the colonies appeared different in color with the help of sterilized tooth picks and incubated on CSA plates at 28°C.

Purification of isolated colonies

Prepare CSA media sterilized and cooled. Poured the Petri plates and incubated for overnight at 37 C to check sterility. Then streak the inoculum from master plates (cyps). Incubate Petri plates at 28°C for 5days. After 5th day growth appears on plates.



III.ENZYMATIC ACTIVITY OF ACTINOMYCETES

The actinomycetes isolates were inoculated on suitable medium by streak or spot inoculation method in order to check different enzymatic degradative activities. For amylase activity, casein starch agar with 1% starch was prepared. The 10 day old grown culture plugs were spot inoculated on to the medium and incubated at 28° C for 72 hours. The gram's iodine stain was spread on plate and left for 5 minutes. The organism which secretes amylase, produce zone of clearance around the growth. For protease activity, casein starch agar with 1% skimmed milk (SM) was prepared. The 10 day old grown culture plugs were spot inoculated on to the medium and incubated at 28° C for 72 hours. The organism which secretes protease, produce zone of clearance around the growth. For lipase activity, in one flask take 98ml 50mM Tris Hcl, pH 6.8, and 1.2 gm agar autoclave it. In another flask take 2ml Tris Hcl and autoclave it and add 300µ l of tributyrin (TB) and 30µ l of tween 20 after autoclaving and then mix both flasks and pour on the plates. The 10 day old grown culture plugs were spot inoculated on to the medium and incubate at 28° C for 72 hours. The organism which secretes lipase, produces zone of clearance around the growth.

For cellulose activity, casein starch agar with 1% carboxymethyl cellulose (CMC) was prepared. The 10 days old grown culture plugs were spot inoculated on to the medium and incubated at 28° C for 72 hours. The congo red stain (2%) was spread on plate and left for 5 minutes. Plates were distained with 1M Nacl. The organism which secretes cellulose, produce zone of clearance around the growth.

Table.1. Studies on the Enzymatic Activities on Actinomycetes

Sr. No	Enzyme	Medium	Incubation period (days)	Criteria of positive activity
1	Amylase	CSA with 1% starch	7	Zone of clearance
2	Protease	CSA with 1% skimmed milk	7	Zone of clearance
3	Lipase	98 ml of 50 mM Tris Hcl+ 1.2 gm in 1 flask & 2ml Tris+ 300 µl Tribyturin+30 µl Tween 20	7	Zone of clearance
4	Cellulose	CSA with 1% CMC	7	Zone of clearance

IV. RESULT AND DISCUSSION:

The enzymatic activity of actinomycetes is shown above and only few culture shown good enzymatic activity. Those culture which shown good zone of clearance will show good enzymatic activity. Actinomycetes have been a source of a numerous useful products including pharmacerticals, agrochemicals, enzymes for use in a number of industrial applications from food industry to paper making.

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