Indian J. Innovations Dev., Vol. 1, No. 3 (Mar 2012) ISSN 2277 - 5390

Biochemical and physiological characteristics of actinomycetes isolated from high altitude shola soils of tropical Montane forest

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Abstract

Actinomycetes are gram-positive, free-living, saprophytic bacteria widely distributed in soil, water and colonizing plants showing marked chemical and morphological diversity. They are potential source of many bioactive compounds, which have diverse clinical effects and important applications in human medicine. In the present work, we have studied some of the physiological and biochemical characteristics of 36 actinomycete strains isolated from the shola soils of tropical montane forest; a relatively unexplored biodiversity hotspot. Ability of actinomycetes isolates to ferment and produce acids from various carbohydrate sources such as innositol, mannose, sorbitol, galactose, mannitol, xylose, rhamnose, arabinose, lactose and fructose were studied. Almost all the carbon compounds were utilized by one or other actinomycete isolates. The most preferred carbon sources were found to be xylose (94.44%) followed by fructose and mannose (91.66%). Only 41.76% of the isolates were able to ferment lactose. The ability of actinomycetes isolates to decompose protein and amino acid differ considerably. 72.22% of the isolates were able to decompose milk protein casein and 61.11% of the isolates decompose tyrosine. Only 8.33% of the strains were able to decompose amino acid hypoxanthine and none of them were able to decompose amino acid xanthine. Potential of the actinomycetes isolates to reduce esculin, urea and hippurate and to resist lysozyme was also checked. 91.66% of the isolates showed ability to decompose esculin and 63.88% of the isolates had the capacity to produce urease and to decompose urea. Only 25% of the isolate were able to decompose hippurate and 94.44% showed lysozyme resistance.

Key words: Actinomycetes; Tropical montane forest; Biochemical characteristics.

Introduction

Actinomycetes, the gram-positive filamentous bacteria, are well known as a good source of microbial secondary metabolites-producer in drug discovery programs. They are widely distributed in soil, water and other natural environments, the population and types of actinomycetes in an ecosystem are determined by numerous physical, chemical and biological factors. Actinomycetes are well known as a rich source of antibiotics and bioactive molecules, and are of considerable importance in industry. They produce branching mycelium, which may be of two kinds' *viz*. substrate mycelium and aerial mycelium. Among actinomycetes, the streptomycetes are the dominant and the non-streptomycetes are called rare actinomycetes, comprising approximately 100 genera (Holt *et al*., 2000).

After penicillin was discovered, the search for additional antibiotics focused on many fungi and bacteria. One particular group of microbe grabbed the attention of scientists, the actinomycetes. Most of the antibiotics are extra cellular secondary metabolites and serve as intermediates from primary metabolisms as precursors for their biosynthetic process (Vilches *et al*., 1990). At present, 4000 antibiotic substance obtained from bacteria and fungi have been applied in medicine, out of which about 75% are produced form gram-positive actinomycetes (Miyadoh, 1993). Thus the studies on actinomycetes are very important, in the present work we have studied some of the physiological and biochemical characteristics of 36 actinomycete strains from shola soils of tropical montane forest; a relatively unexplored biodiversity hotspot.

Materials and methods

Study area

The study area is located at the top areas of Eravikulam National park lies between 10º05'N - 10º20'N latitude and 77º0'E - 77º10'E longitude in Idukki district, Kerala, India (Anamudy Region) at an

Indian J. Innovations Dev., Vol. 1, No. 3 (Mar 2012) ISSN 2277 - 5390

altitude of 1900 m to 2400 m above MSL. Most of the land in this area is covered by grass lands and shola. Shola forests are considered as one of the biodiversity rich ecosystem. For the present study, we selected six sites in shola forest at different altitude for sample collection.

Collection of sample

Soil samples were collected from prefixed six sites (SH1 – SH6) of shola forest at different altitude or different microclimate. Samples were collected at a depth of 15 to 20 cm from the surface after removing the top layer. For each of the sampling sites, sub-samples of soil were collected from different locations, pooled together and homogenized so as to obtain representative sample. Sampling was carried out during post-monsoon, pre-monsoon and monsoon seasons. Samples were collected using a spade that is thoroughly cleaned and disinfected between sampling so as to prevent cross-contamination.

Isolation, Enumeration and maintenance of isolates

Isolation and enumeration of *Actinomycetes* were carried by standard serial dilution plate technique. 10 g of soil was transferred to in 90 ml sterile distilled water and agitated vigorously. Different aqueous dilutions, 10^{-1} to 10^{-4} of the suspensions were prepared and spread plated on Kusters Agar. Nystatin (50 µg/ml) or Amphotericin (75µg/ml) and Streptomycin (25µg/ml) were added to the isolation media in order to prevent fungal and bacterial contamination respectively. The plates were incubated at room temperature for 2 to 3 weeks. After incubation *Actinomycetes* colonies were count and separate colonies were streaked on to Kusters Agar plates and incubated at room temperature for 4-6 days to obtain pure cultures of *Actinomycetes*.

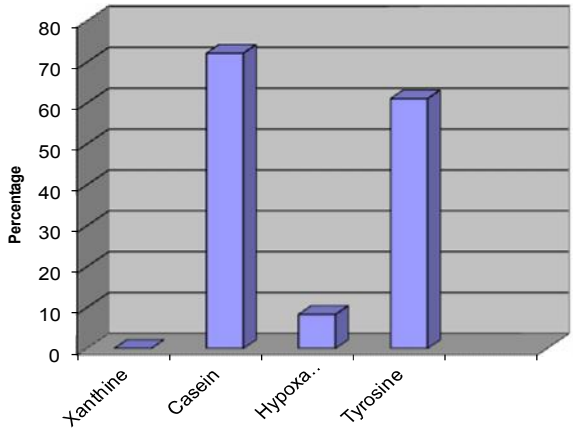

Fig.1. Comparison of the ability of actinomycetes isolates to decompose casein, xanthine, hypoxanthine and tyrosine

Biochemical Physiological studies of Actinomycetes isolates

Actinomycete strains, which are maintained as pure culture on Kusters Agar, were analyzed by morphological tests as per Bergeys Manual of Determinative Bacteriology (2000) and physiological tests (Gordon, 1967).

Results and Discussion

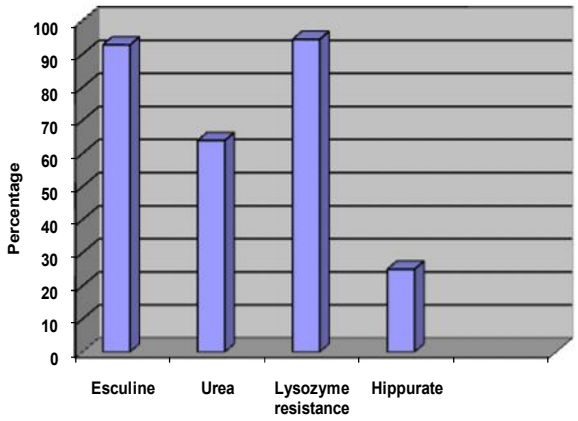

Fig.2. percentage of the ability of actinomycetes isolates to decompose Esculine, Urea, Hippurate and to resist Lysozyme

Biochemical tests such as decomposition of casein, xanthine, hypoxanthine and tyrosine were carried out. Of the isolated 36 strains of actinomycetes 72.22% showed positive result for casein decomposition (Fig.1). The ability of the isolate to decompose casein was determined by observing clear zone in the white opaque skim milk around the inoculums. Growths without clearing zone around the inoculums are considered negative (Barbara *et al*., 2006). 61.11% of the isolates decompose tyrosine and only 8.33% of the strains were able to decompose amino acid hypoxanthine. No isolate showed the ability to decompose amino acid xanthine. The work done by Berd (1973) on actinomycetes isolation showed that such physiological test plays a key role in the identification of actinomycetes strains.

Around 91.66% of the isolated strain showed ability to decompose esculin by producing esculinase enzyme and 63.88% of the isolates had the capacity to produce urease and to decompose urea (Fig.2). The actinomycetes utilize urea and liberate ammonia during the time of incubation, making the broth alkaline, which is indicated by a pink colour (Bergeye's Manual of

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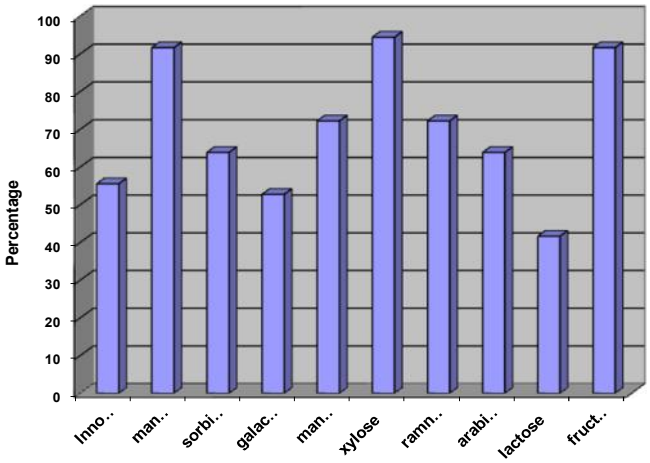

Fig. 3 Percentage of the ability of Actinomycetes to utilize different Carbohvdrates

Determinative Bacteriology). Only 25% of the isolate were able to decompose hyppurate and 94.44% shows lysozyme resistance.

The present study also check the ability of actinomycetes isolate to ferment and produce acids from various carbohydrate sources such as innositol, mannose, sorbitol, galactose, manitol, xylose, rhamnose, arabinose, lactose and fructose. Almost all the carbon compounds were utilized by one or other actinomycete isolates (Fig.3). The best carbon sources were found to be Xylose (94.44%) followed by fructose and mannose (91.66%). Least used one was fructose (41.76%). The result of the present study indicates that the actinomycetes are highly non-specific in their carbon requirements. Strzelczyk (1981) was also reported the non-specificity of actinomycetes carbon requirements.

Conclusion

Results of the present study concluded that *Actinomycetes* are extremely vague in their carbon requirements and almost all the carbon compounds were utilized by one or more actinomycete isolates. The best carbon sources were found to be xylose followed by fructose and mannose. The ability of actinomycetes isolates to decompose protein and amino acid vary significantly.

References

1. Barbara A, Elliot B and Brown JM (2006) Clinical and laboratory features of the *Nocardia spp.* Based on current molecular taxonomy. *Clin.Microbiol*., 19(2), 259-282.
2. Berd D (1973) Laboratory identification of clinically important aerobic actinomycetes. *Appl.Microbiol*. 25(4), 665-68.
3. Gordon RE (1967) In Gray TRG and Parkinson (ed.). The ecology of soil bacteria. Liverpool University Press, 293-321.
4. Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST (2000) Bergey's Manual of Determinative Bacteriology, Ninth edition. Williams and Wilkins, Baltimore.
5. Miyadoh S (1993) Research on antibiotic screening in Japan over the last decade. A producing microorganisms approach. *Actinomycetologica,* 9, 100-106.
6. Strzelczyk AB (1981) Microbial biodeterioration: stone. In: Rose AH (ed.), *Economic Microbiology*, Vol. 6. Academic Press, London, 62–80.
7. Vilches C, Mendez C, Hardisson C and Salas JA (1990) Biosynthesis of oleandomycin by Streptomyces antibioticus: Influence of nutritional conditions and development of resistance. *J. Gen. Microbiol*. 136, 447-1454.