Morphology, Physiology and Taxonomy of *Streptomyces verticillatus*

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Abstract

The antibiotic research from the discovery of Fleming to present time has been an exciting, fascinating, continuously changing and developing adventure. Antibiotic drug discovery is an indispensable process to combat aggressive ability of pathogenic microorganisms and emerging infectious diseases against health and well-being of people through-out the world. The search for new antibiotics has stimulated a variety of different approaches for identification of novel producers throughout the world. Actinomycetes, as potential candidates, continue to be isolated by mix of traditional and modern methods from a wide variety of soils and substrates. The lists of new antibiotics and new actinomycetes species suggest that the careful exploration of new soils and habitats might continue to be useful. Therefore, in this regard, distribution, isolation, identification and detection of active *Streptomycetes* microflora of Kashmir, India were investigated. In preliminary screening of soil inhabitant Actinomycetes, we isolated (*Streptomyces verticillatus*) from soils of an apple orchard in Budgam District, J&K India. It revealed high antagonistic activity against wide range of pathogenic bacteria. Identification to the genus level was based mainly on morphological characters. Microscopy and direct observations revealed the morphological criteria as: color of aerial mycelia was grey, reverse color was Yellow and spore chains were spiral with smooth spore surface. Physiological characterizations indicated that it can utilize inositol, glucose, fructose, rhamnose and raffinose but not arabinose, xylose and sucrose and without melanoid or soluble pigments.

Keywords: Garden soil, actinobacteria, antimicrobial activity, fermentation, *Streptomyces plicatus*, melanoid pigments

Introduction

Actinomycetes are the most economical and biotechnologically valuable class of prokaryotes producing bioactive secondary metabolites notably antibiotics (Blunt and Prinsep, 2006) anti tumor agents, immunosuppressive agents (Mann, 2001) and enzymes (Berdy, 2005; Cragg and Newman, 2005; Strohl, 2004). Goodfellow and Haynes (2005) reviewed the literature on isolation of actinomycetes and suggested that only 10 % of actinomycetes are isolated from nature. Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi. Although soils have been screened by pharmaceutical industry for about 50 years, only a small fraction of actinomycetes taxa have been discovered.

The actinomycetes are gram positive, high G+C (>55%) organisms that tend to grow slowly as branching filaments. Actinomycetes encompass a wide range of bacteria. They have universal occurrence and play an active part in the cycle of nature. The class Actinobacteria holds some of the resilient species, capable of growing in extreme, hostile and polluted environments. Their adaptation has been the outcome of several chemical entities which are answers to a number of medicinal and industrial questions of today.

Kashmir offers a favorable environment as well as rich soil diversity for microbial research studies. Very little research has been carried out in Kashmir regarding Actinomycetes except some stray reports. But the studies on soil actinobacteria in Kashmir are largely unexplored. Hence we have taken an initiative to isolate and identify soil actinomycetes from different soil types and to characterize them.

The isolation and identification of novel Streptomycetes can be a laborious process and can best be justified by subjecting the newly isolated test organism to as many meaningful test systems as is feasible (Nolan and Cross,

1988). The Waksman in 1940s showed that actinomycetes are not only capable of producing medically useful antibiotics, but also stimulated the intensive search for new active strains. Since the systematic screening programs in the search for antimicrobial metabolites were first performed by Waksman, microbial cultures have been a major source of antibiotic substances (Okami and Hotta, 1988). Actinomycetes have been screened widely, but it would be no gross oversimplification to state that their world-wide economic importance centers around their pharmacologic activities; especially their bioactivity against infectious agents.

The Streptomycetes have been the source of the majority of antibiotics, but in recent years, interesting products have been also isolated from species belonging to non Streptomycetes genera. Consequently, there has been a growing trend from actively pursuing research centre's that have made their screening programmes more intensive, resulting in a growing number of reports of novel active compounds (Suzuki *et al.*, 1994). Their characterizations are based on morphological and physiological criteria revealed by many laboratory tests. In this research streptomyces strain was isolated from agricultural soils (Apple Garden) of Budgam District and showed prominent biological activity against wide range of pathogens was identified as (*Streptomyces verticilatus*).

Materials and Methods

Sample collection: Soil samples (approx. 500 g) were collected by using clean, dry and sterile polythene bags along with sterile spatula, marking pen and other accessories. The site selection was done by taking care of the point where widely varying characteristics as possible with regard to the organic matter, moisture content, and particle size and colour of soil and to avoid contamination as far as possible. Samples were stored in sterile polybags and transported to the laboratory where they were kept in refrigerator until analysis.

Isolation: Soil sampling was employed as used by Valan *et al*., (2009) using standard dilution plate technique. The samples were taken for the serial dilution upto the 10^7 dilution, 0.1 ml of each dilution was inoculated in duplicate plates of the Starch casein Agar media for the isolation of actinomycetes by the spread plate technique. After incubation all plates incubated at 30^oC in the incubator for 3 weeks. Nystatin and chloramphenicol were used as antifungal and antimicrobial agent in media. Pure strains of actinomycetes were isolated by streak plate method. Strains were identified on the basis of their phenotypic, physiological and biochemical characteristics.

After proper incubation period, most of the Streptomycetes produced colonies visible to the naked eye (Nolan and Cross, 1988). Selection of candidate colonies was performed by using a stereomicroscope. Streptomycetes colonies were picked on the basis of some morphological features.

Culture Media for Isolation, Screening and Identification: Different bacteriological media were employed for isolation and identification of isolates according to International Streptomyces Project. Oatmeal agar (ISP-medium No. 3) is a standard medium for morphological studies and color determination of all cultures (Shirling and Gottlieb, 1966). Trace salts solution was used in Oatmeal agar medium. Its application is complementary of other media wherever be necessary. Starch Casein glycerol agar was used for isolation, identification and supporting Streptomycetes strains (Kuster and Williams, 1964). Pepton-yeast extract iron agar (ISP- medium No. 6) is standard medium for physiological studies such as determination of melanin production (melanoid pigments) in cultures (Shirling and Gottlieb, 1966). Carbon utilization medium (ISP-medium No. 9) is a standard medium for physiological studies such as determination of ability of Streptomycetes to use different carbon sources in cultures (Shirling and Gottlieb, 1966 and Dietz and Thayer, 1980).

Morphological Characters: The morphological characteristics of Streptomycetes as described by Cross and Goodfellow (1973), were determined as follows:

Morphology of spore bearing hyphae: Characteristics of spore-bearing hyphae were determined by direct examination of the culture surface (21 days old) on opened dishes of the crosshatched cultures under light microscope using 100 x magnification. Using these criteria, species were divided into sections as Rectus or straight, flexible or flexuous, Retinaculum-Apertum and spiral.

Color determination: Observation was made after 15 days and was limited to mature cultures with heavy spore mass surface using code for determining the color of aerial mycelium of Streptomycetes composed by Prauser (1964) for color tabs of Baumann Farbtonkarte Atlas. The color of substrate mycelium was viewed from the reverse side.

Morphology and Ultrastructure of Spores: Spore morphology was determined using slide culture Technique. Thin blocks of agar were cut and placed on sterile glasses and inoculated with isolates all over the agar block surface. A cover slip was then placed over each inoculated agar block and the slides were placed in moist chamber and incubated until good growth of isolate observed. The coverslips were removed from agar blocks, mounted on glass slide, stained properly and observed under oil immersion to study arrangement of spores.

Physiological Characters: The most important physiological criteria used for taxonomical characterizations were: Melanin pigment-production, chitinase degradation activity and use of carbon sources as described by Korn-Wendisch and Kutzner, 1992 as follows: **1- Melanin Production:** Peptone iron agar was used for the detection of deep brown to black diffusible pigment (+). Absence of the color was recorded as negative (-). **2- Carbon Utilization:** Utilization of sugars as L-arabinose, D-xylose, meso- inositol, D-mannitol, D-fructose, rhamnose, raffinose and sucrose was investigated as described in the ISP (Shirling and Gottlieb, 1966).

Results

Taxonomy: Based on morphological, physiological and biochemical characterization, the active isolate was identified as (*Streptomyces verticilatus*). Table 1 shows identification criteria of this strain. It can utilize Xylose, Inositol, Mannitol, Fructose and Rhamnose but not Arabinose, Sucrose and Raffinose.

Table 1. Biochemical and physiological features of strain

S. No.	Test	Result
1	Gelatin liquefaction	+
2	Citrate Utilization	+
3	Nitrate reduction	-
4	Gram staining	+
5	Indole production	-
6	Methyl red	+
7	Voges prauskouer	+
8	Urease production	-
9	**Sugar utilization**	
	Xylose	+
	Arabinose	-
	Sucrose	-
	Fructose	+
	Mannitol	+
	Raffinose	-
	Inositol	+

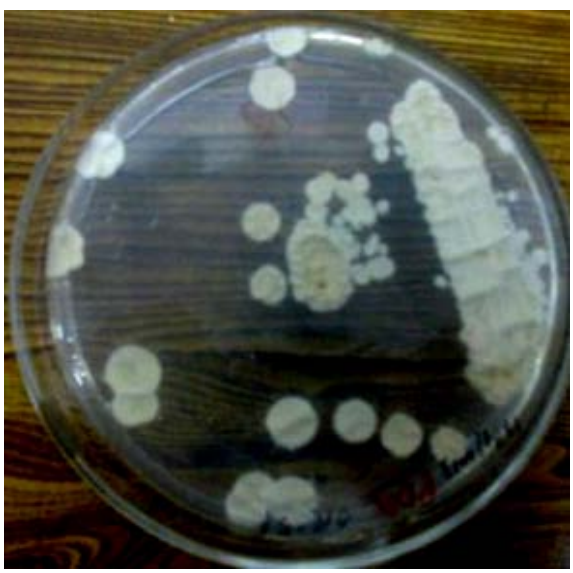

Fig. 1. Streptomyces species on starch casein agar

Discussion:

For decades, microbial natural products have been one of the major sources of novel drugs for pharmaceutical companies, and today all evidence suggests that novel molecules with potential therapeutic applications are still waiting to be discovered from these natural sources, especially from actinomycetes. Any appropriate exploitation of the chemical diversity of these microbial sources relies on proper understanding of their biological diversity and other related key factors that maximize the possibility of successful identification of novel molecules. The strain was identified as Streptomyces verticilatus. Our study correlates with the results found by Anderson *et al.,* 2001. Arunachalam *et al.,* (2010) identified streptomyces using cellulose production. The morphological and biochemical characteristics of 71 *Streptomyces* spp. isolated from soil samples collected at different places of Venezuela were studied by Taddei *et al.,* (2006). Our results are confirmed by studies carried out by Kalyani *et al*., 2012. The future investigation of this strain should be focused on its capability in gene transfers, use as biofungicide or biofertilizer, determination of its spectrum of activity on human and animal pathogens and its chitinase activity on insect's body wall in biological control of insect pests.

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