Current Trends in Biotechnology and Pharmacy
Vol. 3 (2) 85-91, Apirl 2009. ISSN 0973-8916

Antibacterial activity of bacterial isolates of soil bacteria collected from Palestine

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A total of 51 *Actinomycetes* were isolated from different soil samples of Palestine. Preliminary screening by cross-streak method was carried out for all the 51 isolates. After preliminary screening, 17 isolates which showed antimicrobial (antibacterial, antifungal) activity were selected for further study. Among these 17 isolates tested, 5 isolates which were found to be promising were subjected to detailed taxonomic studies. A novel strain of *S. albovinaceus* (isolate 10/2) which was found to be maximum antibiotic producer and which has shown both broad spectrum antibacterial and antifungal activities was isolated and is been selected for further detailed optimization studies.

Key words
Actinomycetes / S. albovinaceus / Antibacterial / Isolation / Soil samples

Introduction

Ever since mankind started suffering from ailments, the quest for finding remedies to treat the diseases started. The science of antibiotics has remained and will remain for many years, one of the most interesting natural sciences, in both theoretical and practical aspects. Microbial natural products still appear as the most promising source of the future antibiotics that society is expecting (1). Antibiotics are produced by bacteria, fungi, actinomycetes, algae, lichens and green plants. Since the isolation of actinomycin in 1940 and streptomycin in 1944 by Waksman (2,3), the *Actinomycetes* have received tremendous attention of the scientists. The genus *Streptomyces* was proposed by Waksman & Henrici (4) for aerobic, spore-forming *Actinomycetes* . Members of *Streptomyces* are a rich source of bioactive compounds, notably antibiotics, enzymes, enzyme inhibitors and pharmacologically active agents (5-10). About 75 % of the known commercially and medically useful antibiotics are produced by *Streptomyces* (11,12). Waksman (13) recognized the natural substrates that are ideal sources for the isolation of *Actinomycetes*. Among these, they are quite commonly found in soil, water and other environments (14). In 1900 Beijerinck (15) established that *Actinonomycetes* occur in great abundance in the soil. The first quantitative enumeration of *Actinomycetes* in the soil was made by Hiltner and Stormer (16).There is now good evidence also for the growth of *Actinomycetes* in marine soils (14, 17, 18). Goodfellow and Haynes (19) reviewed the literature on the isolation of *Actinomycetes* from marine sediments. The screening programs for new *Actinomycetes* and for their antibiotics are

still proceeding at a very rapid pace. There is a need for the development of new antibiotics to overcome the problems associated with the existing antibiotics. To discover the new antibiotics it will be necessary to continue the use of conventional screening programs. Different soils all over the world had been exploited in search of bioactive *Actinomycetes*. So, in order to discover new antibiotics, our approach is to investigate unexplored regions of the world with the aim of isolating bioactive *Actinomycetes* from these regions and from those organisms whose potential was neglected through out the history. The aim of our work is to conduct intensive screening program on different soil samples of Palestine which is an unexplored territory and which is likely to yield purposeful results towards isolation of either new species of *Actinomycetes* and /or new antibiotics. As such, we felt that systematic screening of Palestine soil samples is very much necessary. The selected isolates were identified by morphological, biochemical and other criteria and finally screened for the production of novel antibiotics and pharmacological active compounds. This investigation may yield a few new species of *Actinomycetes* leading to the isolation of new antiviral and antibacterial antibiotics with desired antimicrobial spectrum and therapeutic use. It may lead to the isolation of new species producing already known and clinically useful antibiotics, but with high yield in a simple medium which facilitate the purification procedure and reduce the cost.

Materials and Methods

Several methods have been developed to identify *Streptomyces* species (20 – 27). In a systematic screening program for isolation of bioactive *Actinomycetes*, a total of 8 different soil samples were collected into sterile boiling tubes with a sterile spatula. Care was taken to see that the points of collection had as widely varying characteristics as possible with regard to the organic matter, moisture content, particle size and colour of soil.

Isolation of *Actinomycetes* from soil samples: About 1gm of sample was transferred to a sterile Erlenmeyer (E.M) flask containing 50 ml sterile water. The flasks were shaken on rotary shaker for 30 min for the detachment of the spore chains, if any. The flasks were kept aside for 30 min to settle down the particulate matter. The clear supernatant was diluted with sterile water. These dilutions (10^{-1}-10^{-3}) were used as inocula. One ml of each of these dilutions were pipetted out into the medium, plated into petridishes 6 inches diameters and incubated at 28 ^{o}C for 2-3 weeks. For the isolation of *Actinomycetes* from the above mentioned samples, the following media were used : starch casein agar medium (28) , potassium tellurite agar medium, oat meal agar medium , bennets agar medium.

Physiological, biochemical, and cultural (morphological) properties: Media used were those recommended by the International Streptomyces Project (ISP) (22) and by Waksman (29). Mycelium was observed after incubation at 28 ^{o}C for 2 weeks. Colors were determined according to Prauser (30). Reduction of nitrate and production of melanoid pigment were determined by the method of ISP (22). Carbohydrate utilization was determined by growth on carbon utilization medium (ISP 9) (22) supplemented with 1 % carbon sources at 28 ^{o}C. Liquefaction of gelatin was evaluated by the method of Waksman (29). Hydrolysis of starch and milk were evaluated by using the media of Gordon et al. (31). All cultural characteristics were recorded after 2 weeks.

Cell wall composition (chemotaxonomic analysis): Cells used for chemotaxonomic analysis were obtained after incubation at 28 ^{o}C for 3 days in yeast extract-glucose broth (pH 7.0) containing 10 g/l of yeast extract and 10 g/l of glucose. Isomers of diaminopimelic acid in the whole-cell hydrolysates were determined by thin-layer

chromatography according to the method of Hasegawa et al. (32). Whole-cell sugars were analyzed according to the method of Becker et al. (33).

Study of antimicrobial activity: All selected isolates were subcultured onto YEME agar slants and incubated at 28 °C for about 7-10 days. The following production medium was used to test antibiotic production: soyabean meal (1.0 %), corn steep liquor (0.5 %), soluble starch (1.0 %), dextrose (0.5 %), calcium carbonate (0.7 %) with pH (7.2) .Antimicrobial activity of the strain was determined by standard cup plate method using Gram (+) and (-) bacteria, fungi and yeast as test organisms. Assay plates were prepared by inoculating 20 ml of Mueller - Hinton agar medium with test organism. Agar-cups (6mm diameter) were filled with 50 ml of mycelia -free culture filtrate in triplicate and the plates were incubated at 37 °C for 24 h. Inhibition zone diameters were measured.

Results and Discussion

Isolation of *Actinomycetes* from soil samples: A total of 8 different soil samples were collected and used for screening and isolation of *Actinomycetes*. A brief description of soil samples is given in Table 1.The selected *Actinomycetes* isolates from the above soil samples are shown in Table 2. A total of 51 *Actinomycetes* were isolated from different soil samples of Palestine after discarding isolates with identical characteristics. The criteria chosen were the color of the aerial mycelium, substrate mycelium and the pigmentation. Preliminary screening by cross-streak method was carried out for all the 51 isolates. After preliminary screening, 17 isolates which showed antimicrobial (antibacterial, antifungal) activity were selected for further study. Among these 17 isolates tested, 5 isolates (7/2, 8/7, 10/2, 12/2 and 13/2) which were found to be promising were subjected to detailed taxonomic studies. The results of the taxonomic studies of the selected isolates are described in the following individual monographs.

Taxonomic studies on isolate no. 10/2

Morphological and cultural characteristics: Aerial mycelium is white in color. Raised growth was observed. Short straight sporophores were also seen. Detail taxonomic studies are shown in Tables 3 and 4**.**

Antimicrobial activity: Antimicrobial spectrum of the culture filtrate was studied and the results are shown in Table 5.

The above information indicates that this isolate belongs to the family Streptomycetaceae. As such the taxonomic characters of our isolate 10/2 is compared with that of the *Streptomyces* species reported in the existing literature and found to be closer to *S. albovinaceus* (34). The comparative data is given in Table 6. Our isolate 10/2 and reference strain have the following similarities: color of aerial mycelium, sporophore morphology, melanin pigmentation and utilization of glucose, fructose, arabinose, mannitol, xylose as a carbon source. Our isolate 10/2 differs from the reference strains in utilization of raffinose , sucrose , inositol and rhamnose . In view of the large number of similarities with the reference strain our isolate 10/2 is considered to be a strain closer to *S. albovinaceus*

Taxonomic studies on isolate no. 7/2

Morphological and cultural characteristics : Aerial mycelium was grey in color. Raised growth was observed. Short spirals with two to three turns' sporophores were also observed. Detail taxonomic studies are shown in Tables 3 and 4.

Antimicrobial activity: Antimicrobial spectrum of the culture filtrate was studied and the results are shown in Table 5.

The above information indicates that this isolate belongs to the family Streptomycetaceae. As such the taxonomic characters of our isolate 7/2 is compared with that of the *Streptomyces* species reported in the existing literature and found to be closer to *S. violaceoruber* (35). The comparative data is given in Table 7. Our isolate 7/2 and reference strain have the following similarities : color of aerial mycelium , sporophore morphology , melanoid pigments on ISP – 6 , utilization of D-glucose , D- fructose , L(+) arabinose , D- mannitol , xylose , inositol , rhamnose , gelatin , liquefaction , coagulation and peptonization of milk. Our isolate 7/2 differs from the reference strains in utilization carbon sources raffinose and sucrose. In view of the large number of similarities with the reference strain our isolate 7/2 is considered to be a strain to be close to *S. violaceoruber.*

Taxonomic studies on isolate no. 12/2

Morphological and cultural characteristics: Aerial mycelium was grey in color. Raised growth was observed. Long and straight sporophores were seen. Detail taxonomic studies are shown in Tables 3 and 4.

Antimicrobial activity: Antimicrobial spectrum of the culture filtrate was studied and the results are shown in Table 5.

The above information indicates that this isolate belongs to the family Streptomy cetaceae. As such the taxonomic characters of our isolate 12/2 is compared with that of the *Streptomyces* species reported in the existing literature and found to be closer to *S. tanashiensis* (20, 36 – 38). The comparative data is given in Table 8. Our isolate 12/2 and reference strain have the following similarities: color of aerial mycelium, sporophore morphology, utilization of glucose, arabinose , xylose , and rhamnose. Our isolate 12/2 differs from the reference strains in melanoid pigmentation on ISP – 6, utilization of fructose, mannitol , raffinose , sucrose , and inositol. In view of the large number of similarities with the reference strain our isolate 12/2 is considered to be a strain to *S. tanashiensis*

Taxonomic studies on isolate no. 13/2

Morphological and cultural characteristics: Aerial mycelium was whitish grey in color. Raised growth was observed. Long and straight sporophores were observed. Detail taxonomic studies are shown in Tables 3 and 4.

Antimicrobial activity: Antimicrobial spectrum of the culture filtrate was studied and the results are shown in Table 5.

The above information indicates that this isolate belongs to the family Streptomycetaceae. As such the taxonomic characters of our isolate 13/2 is compared with that of the *Streptomyces* species reported in the existing literature and found to be closer to *S. setonii* (13,20, 36, 39) . The comparative data is given in Table 9. Our isolate 13/2 and reference strain have the following similarities: color of aerial mycelium, sporophore morphology, melanoid pigments on ISP-1 and ISP-7, utilization of glucose, fructose, arabinose , mannitol , xylose , starch hydrolysis , gelatin liquefaction, coagulation and peptonization of milk and nitrate reduction. Our isolate 13/2 differs from the reference strains in melanoid pigments on ISP-6, utilization of raffinose , sucrose , inositol , and rhamnose. In view of the large number of similarities with the reference strain our isolate 13/2 is considered to be a strain to *S. setonii*

Taxonomic studies on isolate no. 8/7

Morphological and cultural characteristics: Aerial mycelium was light grey in color. Growth was not raised. Sporophore was short with very short branches. Detail taxonomic studies are shown in Tables 3 and 4.

Antimicrobial activity: Antimicrobial spectrum of the culture filtrate was studied and the results are shown in Table 5.

The above information indicates that this isolate belongs to the family Streptomycetaceae. As such the taxonomic characters of our isolate 8/7 is compared with that of the *Streptomyces* species reported in the existing literature and found to be closer to *S. longisporus* (20 , 36, 37). The comparative data is given in Table 10.Our isolate 8/7 and reference strain have the following similarities : color of aerial mycelium , morphological section, utilization of D-glucose , D-fructose , L(+) arabinose , D-mannitol , D-xylose , raffinose , and sucrose. Our isolate 8/7 differs from the reference strains in utilization of carbon sources like inositol and rhamnose. In view of large number of similarities with the reference strain our isolate 8/7 is considered to be a strain close to *S. longisporus*

Conclusion

In the present study effort was mainly directed towards the isolation of *Actinomycetes* from soil samples of Palestine, an unexplored territory for study of their morphological, cultural, physiological, biochemical and antimicrobial activities.

Detailed taxanomical studies were carried out and it was concluded that 5 different new strains were isolated of which one isolate (10/2) was found to be excellent antibacterial producer. Isolate no (10/2) *S. albovinaceus* which was found to be maximum antibiotic producer and which has shown both broad spectrum antibacterial and antifungal activities is been selected for detailed optimization studies. Further works on isolate no (10/2) like optimization studies, studies on anticancer activity and purification of the active principle which are under progress. Toxicology and commercial viability study will be submitted in a separate article.

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