JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2008. Vol. **2**(1), p. 19-22

Novel Antibacterial Activity of *Streptomyces* sp. Isolated from Syrian Soil

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(Received: 01 January 2008; accepted: 12 February 2008)

The aim of the study was to screen and identify novel activities of *Streptomyces* isolates from Syrian soil. *Streptomyces* isolate designated RW6 showed unusual inhibition pattern against *Vibrio fluvialis*. On the other hand , *Streptomyces* RW6 showed no antibacterial activities against *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Bacillus subtilis.*

Possible involvement of plasmid content of *Streptomyces* isolate RW6 with its novel antibacterial activity against *Vibrio fluvialis* was studied. Treatment of the isolate with acridine orange resulted in curing of the plasmid as demonstrated by agarose gel electrophoresis, and subsequently the loss of novel pattern of antibacterial activity against *Vibrio fluvialis.*

Key words: *Streptomyces, Vibrio fluvialis*, plasmids, antibiotic resistance, antibacterial activity.

Members of various genera within the family streptomycetaceae have been known for their commercial importance, since they produce over 50% of 10000 known antibiotics and many other bioactive substances with valuable clinical, agricultural, industrial and other applications[1]. The genus *Streptomyces* within this family is considered the most important genus in this respect, since members of this genus produce most of antibiotics and bioactive substances. It has been reported that 45-55% of known antibiotics are produced by various species of this genus[2]. Recent studies have indicated that genus *Streptomyces* continues to be an active area of exploration of new antimicrobial products[3]. In this regard, antibiotic resistance of pathogenic bacteria is one of important concerns facing health sector, since frequent appearance of antibiotic resistant mutants decreased the usefulness of several antibiotics used in therapy, moreover, arising multiple resistance of pathogenic bacteria have been increasing and causing therapeutic problems[4]. Thus, a research program has been initiated to explore the antibacterial substances produced by *Streptomyces* isolates recovered from Syrian soils with novel activities against certain pathogenic bacteria showing multiple antibiotics resistance. In this study we report an interesting antibacterial activity of the local *Streptomyces* isolate RW6 against multiple antibiotic resistant strain of *Vibrio fluvialis*. It had been shown that this bacterium is gaining more attention in gastroenteritis infections[5].

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MATERIAL AND METHODS

Microorganisms

Streptomyces isolate RW6 and test bacteria (*Vibrio fluvialis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Bacillus subtilis*) were obtained from Microbial Collection of National Commission of Biotechnology. *Streptomyces* isolate RW6 was originally obtained from Deir-azoor (eastern Syria) soil on selective medium (inorganic salt-starch agar with 100 µg/ml cycloheximide) following the standard methods[6,7]. The isolate was characterized according to the methods of International *Streptomyces* Project (ISP)[8].

Antibacterial activity

The antibacterial activity of the isolate RW6 and recovered *Streptomyces* colonies after acridine orange treatment was carried out in Petri dishes by two agar layers screening test. The first layer (inorganic salt-starch agar) was for growth of *Streptomyces* streak, whereas the second layer (Mueller Hinton agar) was for cultivation of test bacteria streaks. The plates were first inoculated with *Streptomyces*, and after five days of incubation at 37°C, the second layer of molten Mueller Hinton agar was poured on the already grown *Streptomyces* streak, then test bacteria were streaked above RW6 streak growth in a cris cross pattern according to the method adopted by Ali *et al.,* 1998[9].

Antibiotic sensitivity tests

Disc assay tests were carried out on Mueller Hinton agar plates against *Streptomyces* RW6, colonies recovered from RW6 after acridine orange treatment, and *Vibrio fluvialis* test bacteria. Plasmid curing experiment: Acridine orange (Fluka AG) at various concentrations (4, 8, 16, 32 and 64 µg/ml) were used for curing of plasmid(s) of RW6 isolate according to the method reported by Ali *et al.,* 1998[9].

Plasmid detection

Modified alkaline lysis method[10] was used to prepare plasmids from RW6 and colonies recovered after acridine orange treatment. Fifty milligrams of *Streptomyces* mycelia were grounded in sterilized porcelain dish with aid of mortar in presence of suitable amount of liquid nitrogen, then 600 µL of LETS buffer [0.1 M LiCL, 10 mM EDTA, 10 mM Tris-Cl(pH 8), 0.5% SDS] were added to the mycelial powder, transferred to eppendorf tube , mixed well by vortex mixer and incubated for 10 minutes at 65°C. The following steps are the same as reported by Sambrook *et al.,* 1989[10]. Plasmid detection was carried out on agarose gel (0.8%) electrophoresis[10].

RESULTS AND DISCUSSION

The growth characteristic of the *Streptomyces* isolate RW6 is typical of *Streptomyces* sp., this isolate was originally obtained from Deir-azoor soil , north east of Syria. It showed discrete colonies with weak growth of substrate mycelia and good growth of aerial mycelia. The aerial mycelia showed non veticillate type of sporophores , this character distinguishes the genus *Streptomyces* from genus *Streptoverticillium.*

The color of aerial mycelia varied from light pinkish gray at 37° C to yellowish at 28°C. At these temperatures the substrate mycelia varied from pinkish to yellowish respectively, while the color of the soluble diffusible pigment was light yellow at both temperatures. Fig. (1) shows the retinaculum-apertum morphology of the spore chain of RW6, and the number of spores in the chain exceeds 15 spores, this is another important characteristic of genus *Streptomyces*. Isolate RW6 is also a producer of amylase since clear zones appeared around colonies when grown on inorganic salt- starch agar, moreover urease activity was also observed .The isolate RW6 showed no antibacterial activities against five test bacteria used in this work, three Gram negative bacteria (*Pseudomonas aeruginosa , Escherichia coli and Klebsiella pneumoniae*) and two Gram positive bacteria (*Staphylococcus aureus and Bacillus subtilis*). On the other hand, *Streptomyces* RW6 showed unusual inhibition pattern against *Vibrio fluvialis*. , in this respect, antibacterial screening test of RW6 grown on inorganic salt-agar plates showed that *Vibrio fluvialis* can grow on second Mueller Hinton agar layer only above *Streptomyces* RW6 growth, but cannot grow away from *Streptomyces* RW6 (Fig. 2) . This phenomenon might be explained as a result of production of two compounds by

The reported results in this study in respect of loss of streptomycin resistance of isolate C13 and gain of the usual antibacterial activity along *Streptomyces* streak against test bacteria, besides the loss of plasmid as indicated by gel electrophoresis experiment lead to a conclusion that production of compound (B) is controlled by plasmid, and isolate C13 is no more a producer of this compound. Therefore, *Streptomyces* C13 is expressing its antibacterial activity without interference by compound (B). Plasmid involvement in antibiotic production has been suggested, biosynthetic genes coding for methylenomycin , an antibiotic produced by *Streptomyces coelicolor*, are known being coded by plasmid[11]. Lankacidin production which is produced by *Streptomyces* sp. is controlled by linear plasmid[12]. As far as compound (B) activity is concerned, it is worth to mention the observation made by Mamber and his group[13], on the antibacterial activity of himastatin which is produced by *Streptomyces hygroscopicus*. In their investigations they demonstrated that the markedly decreased antibacterial activity of himastatin was not enzymatic in nature but was related to the presence of certain fatty acid salts. Their study revealed that saturated fatty acids sodium salts with a carbon chain number of 8 or more reduced the antimicrobial activity of himastatin 50 to 100 times. Moreover, they reported that if antibiotics such as ampicillin, bacitracin, chloramphenicol, and tunicamycin were used in place of himastatin, no meaningful reduction in antibacterial activity occurred. However, the antibacterial activity of the membrane-active peptide antibiotic polymyxin B, but not that of polymyxin E (colistin), was reduced in a manner similar to that of himastatin .

The reported results demonstrated the novel antibacterial activity conferred by *Streptomyces* RW6 isolated from Syrian soil against multiple antibiotic resistant strain of *Vibrio fluvialis*.

ACKNOWLEDGMENTS

The authors would like to thank Professor M. F.Azmeh (Director General of NCBT) for support during the preparation of this manuscript. Prof. Mohammed A. Ibrahim is grateful to Al-Nahrain University / Baghdad for the sabbatical leave.

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