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RAPD-PCR Analysis of *Streptomyces* sp. with Novel Antibacterial Activity

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Random amplified polymorphic DNA (RAPD) analysis was applied to ten bioactive and non bioactive *Streptomyces* isolates (RW series) recovered from Syrian soils. The *Streptomyces* isolates were characterized with different patterns of antibacterial activities against Gram positive and negative bacteria. Nine of 22 RAPD decamer primers which were used in the study were found to amplify *Streptomyces* genomic DNA, and revealed interesting DNA polymorphism in the *Streptomyces* genomes of the isolates. RAPD- PCR products generated twenty six DNA fragments with 290 to 1081 base pairs. RAPD-PCR analysis of *Streptomyces* sp. RW6/2 genomic DNA showed that the PCR products of primer OPZ-19 gave one specific fragment of 521 base pairs which was not detected in other *Streptomyces* isolates.

Key words: Antibacterial activity, DNA polymorphism, RAPD-PCR, *Streptomyces*, *Vibrio fluvialis*.

Bacterial isolates of genus *Streptomyces* have been considered the most important of family streptomycetaceae for their ability to produce a wide variety of antibiotics currently used in therapy[1]. It has been reported that 45-55% of known antibiotics are produced by various species of this genus[2]. Recent studies have shown that this group of bacteria still remains an important source of antibiotics [3,4,5].

Random amplified polymorphic DNA (RAPD) analysis provides a very useful tool for genomic analysis in bacteria and other organisms. For example RAPD analysis was used as a tool to study particular strains of *Nocardia farcinica*[6], differentiation of thermophilic anaerobic gram-positive bacteria[7], identifying unique DNA polymorphisms in *Streptomyces*[8] and to investigate polymorphic markers in phylogenetic studies [9].

In this study an investigation was carried out to study DNA polymorphism of *Streptomyces* isolates recovered from Syrian soils and to ascertain the possibility of identifying unique RAPD-PCR fragments with potential specific use in identifying novel *Streptomyces* isolates.

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MATERIALS AND METHODS

Microorganisms and cultural conditions

Streptomyces isolates series RW and test bacteria (*Vibrio fluvialis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Bacillus subtilis*) were obtained from Microbial Collection of Syrian National Commission of Biotechnology. Test bacteria were maintained on nutrient agar at 37°C, whereas *Streptomyces* isolates were maintained on yeast extract-malt extract (YEME) agar at 30°C. The antibacterial activities of *Streptomyces* isolates were ascertained by criss cross method on inorganic salts-starch agar at 37°C[10,11]. Antibiotic resistance of test bacteria and *Streptomyces* isolates were performed according to reported methods[4].

DNA isolation and RAPD amplification reaction

Genomic DNA was isolated from *Streptomyces* isolates according to the reported method[12]. RAPD-PCR reactions were carried out using 22 (10-mer) oligonucleotides random primers provided from Operon kits (Operon Technology, Alameda, CA).

Amplification reactions were performed in a total volume of 25 µl consisting of 12.5 µl of PCR master mix (Fermentas, EU), 2.5 µl primer (10 pmol), 2 µl genomic DNA (50 ng) and 8 µl sterile distilled water.

Amplification program was started with two minutes at 94°C for initial denaturation followed by 40 cycles of 94°C for one minute (denaturation), 56°C for one minute (annealing) and 72°C for 2 min (extension). The final cycle was 72°C for 1 min (final extension). The RAPD reaction was performed using the thermocycler (Apollo, ATC 401, USA).

The amplification products were separated on 1.5 % agarose gel and visualized under UV light after staining with ethidium bromide.

Data analysis

The bioinformatics toolkit in MATLAB was used for the statistical analysis of amplified DNA banding patterns and for creating phylogenetic tree to investigate the relationships among *Streptomyces* isolates.

RESULTS AND DISCUSSION

Antimicrobial activity

The antimicrobial activities of the *Streptomyces* isolates grown for five days on inorganic starch agar media at 37°C were ascertained against four Gram negative bacteria (*Vibrio fluvialis, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*) and two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). Bioactive *Streptomyces* isolates (RW1, RW4, RW8, and RW10) showed usual antibacterial activity against test bacteria except *Streptomyces* RW6/2 which is derivative of RW6. In previous study 4, *Streptomyces* sp. RW6 showed novel activity against multiple antibiotic resistant *Vibrio fluvialis*, in this respect RW6/2 showed almost similar pattern of antibacterial activity. The test bacterium *Vibrio fluvialis* can grow on second Mueller Hinton agar layer only above *Streptomyces* RW6 growth, but cannot grow away from streak of *Streptomyces*; whereas *Vibrio fluvialis* showed growth of isolated colonies away from the streak of RW6/2 (Fig. 1), indicating that the test bacteria has high mutation rates toward the antibiotic produced by RW6/2. This phenotype was not shown with original isolate RW6, which was able to give complete inhibition of test bacteria away from *Streptomyces* streak[4].

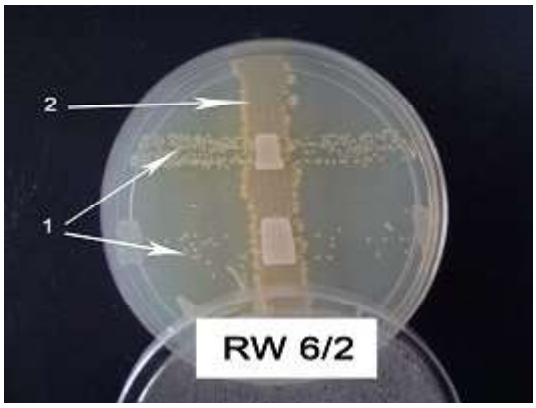

Fig. 1. Antimicrobial activity of *Streptomyces* isolate RW 6/2 against *V. fluvialis.*
The *Streptomyces* isolate showed unusual antibacterial activity on inorganic starch agar media at 37°C. 1 and 2 indicate *V. fluvialis* and *Streptomyces* RW 6/2 respectively.

RAPD-PCR analysis

In this study twenty two random Operon decamer primers were used, nine primers (Table 1) were able to amplify genomic DNA of *Streptomyces* isolates and gave DNA fragments ranged from 290 to 1081 base pairs. The DNA profiles obtained for the nine primers showed 25 polymorphic bands and one monomorphic band of 521 base pairs. The scored DNA amplified fragments were subjected to phylogenetic analysis using MATLAB bioinformatics toolbox to create phylogenetic tree and dendogram was developed (Fig. 2). In the dendogram the ten isolates were distinctly divided into two major clusters with subgroups. Three bioactive isolates RW1, RW4 and RW6/2 were found in one major cluster, whereas the other major cluster include the five non bioactive isolates (RW2, RW3, RW5, RW7 and RW9) and two remaining bioactive isolates (RW8 and RW10).

Electrophoresis of PCR products of one RAPD primer showed that the *Streptomyces* sp. RW6/2 genomic DNA gave specific DNA fragment not found in other *Streptomyces* isolates. PCR products of primer OP-Z19 (Fig. 3) gave one specific DNA fragment (521 base pairs). On the other hand, other amplifying RAPD primers do not show specific DNA fragments in RW series isolates.

The obtained results might suggest that the RAPD-PCR products of *Streptomyces* genomic DNA might find application for specific detection of novel *Streptomyces* isolates. In this respect, the available procedures for discovery and identification of novel antibiotic producing *Streptomyces* sp. are considered time consuming and require extensive screening protocols[13,14]. Thus, investigators are looking for new methods for detecting and selecting novel *Streptomyces* strains or variants from natural habitats to enrich culture collection and help further strain development through genetic manipulation techniques. Random Amplified Polymorphic DNA analysis (RAPD-PCR) in bacteria has shown potential promise for such investigations[8,12]. The obtained results in the present investigation with RAPD primer OPZ-19 support the possibility of using this approach for identifying novel *Streptomyces* isolates.

Table 1. Sequences of RAPD primers which were used for PCR amplification of *Streptomyces* genomic DNA

RAPD Primers	Sequences
OPA-11	5¯ CAATCGCCGT 3¯
OPA-15	5¯ TTCCGAACCC 3¯
OPB -11	5¯ GTAGACCCGT 3¯
OPB -12	5¯ CCTTGACGCA 3¯
OPB -15	5¯ GGAGGGTGTT 3¯
OPJ -05	5¯ CTCCATGGGG 3¯
OPW -17	5¯ GTCCTGGGTT 3¯
OPZ-02	5¯ CCTACGGGGA 3¯
OPZ-19	5¯ GTGCGAGCAA 3¯

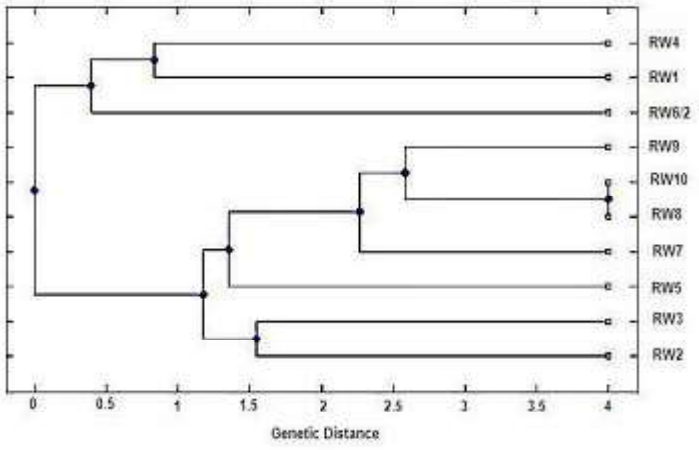

Fig. 2. Phylogenetic analysis based on similarity data from nine amplifying RAPD primers, showing genetic relationship for ten *Streptomyces* isolates

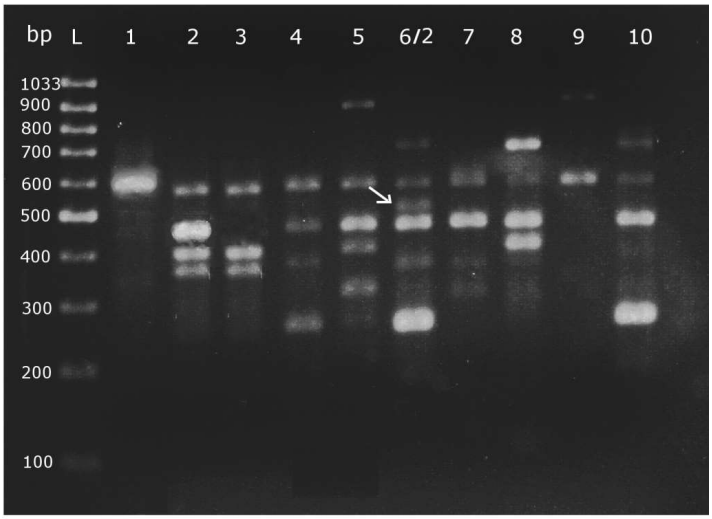

Fig. 3. Patterns of RAPD-PCR DNA fragments of ten *Streptomyces* isolates (RW) produced by primer OPZ-19. The arrow indicates the presence of specific DNA fragment in genomic DNA profile of RW 6/2. L, Ladder marker; numbers indicate the ten RW series *Streptomyces* isolates

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