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SHORT COMMUNICATION

Usefulness of *strb1* and 16S rDNA-targeted PCR for Detection of *Streptomyces* spp. in Environmental Samples

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Abstract

In this study, we revealed rapid detection of streptomycin-producing *Streptomyces* spp. by extraction of total soil DNA from 14 soil samples using a modified lysis method followed by PCR amplification of a genus-specific sequence in the *Streptomyces'* 16S rDNA gene. DNA band of the expected size (438 bp) was seen with all the samples. Additionally, specific amplification of the streptomycin-coding gene (*strb1*) directly from soil revealed the presence of a single DNA band of 940 bp. These results indicate that PCR-amplification of *Streptomyces* specific genes could be used for direct detection of streptomycin-producing *Streptomyces* species from soil.

Key words: *Streptomyces* spp., PCR, 16S rDNA, *strb1*, streptomycin

Streptomyces species have always been a unique group of prokaryotes in respect to their morphological diversity and their metabolic products, antibiotics and many enzymes of industrial interest (Tanaka and Omura, 1990). They are natural inhabitants of soil and they together with the other genus *Nocardia* are the most abundant actinomycetes found in the soil (Williams *et al*., 1989).

Traditional detection and identification of *Streptomyces* spp. specially antibiotic-producers is time-consuming, requires multiple procedures and involves extensive experimental work (Hain *et al.*, 1997; Mehling *et al.*, 1995; Williams *et al.*, 1983).

The economic importance of the antibiotic-producing *Streptomyces* and the importance of *Streptomyces* in controling soil-born pathogens by antibiosis (Saadoun and Al-Momani 1997; Tulemisova and Nikitina 1989; Weller and Thomashow, 1990) have promoted several workers to detect and characterize these organisms by simple and rapid procedures. Detection and identification of these organisms in their natural habitats by rapid and sensitive tests such as PCR-based methods are needed to demonstrate their potential for antagonism against pathogens in soil.

This study attempted to rapidly detect streptomycin-producing *Streptomyces* spp. in Jordanian soils by PCR-based method using DNA isolated directly from soil. Streptomycin was chosen to be screened because its coding gene *strb1* is highly conserved between streptomycin producers (Retzlaff *et al.*, 1993).

A total of 21 soil samples were collected from different locations in northern, eastern and southern Jordan (Saadoun and Gharaibeh, 2002). These were from Northern Region (Ajlun: AJ1, AJ3, AJ5; Sammer: S1, S2, S7), Jordan Valley (Ash Shuna: JV1, JV3; Deir Allah: D1), Eastern Region (Badia: N3, Bb, PR, Aa, Aa7; Zarqa: Z2; Azraq: AZ2, AZ4), Southern Region (Aqaba: AQ1, AQ2, AQ5, AQ7). Only 14 soil samples were selected for this study and were mostly from cultivated and uncultivated areas in Jordan. They were collected then processed as described by Saadoun and Gharaibeh (2002).

As isolates of *Streptomyces* were recovered (Saadoun and Gharaibeh, 2002) Streptomyces-like colonies were purified by repeated streaking then tested for streptomycin production (Gharaibeh *et al*., 2003) using streptomycin sensitive (*Escherichia coli* and *Bacillus subtilis*) and resistant (*Klebsiella pneumoniae* and *Staphylococcus aureus*) bacteria. An isolate was considered to be active streptomycin-producer if it inhibited the growth of *E. coli* with inhibition zone diameter of 18 mm or greater. Streptomycin-producing *Streptomyces* isolates were characterized morphologically and physiologically following the directions given by

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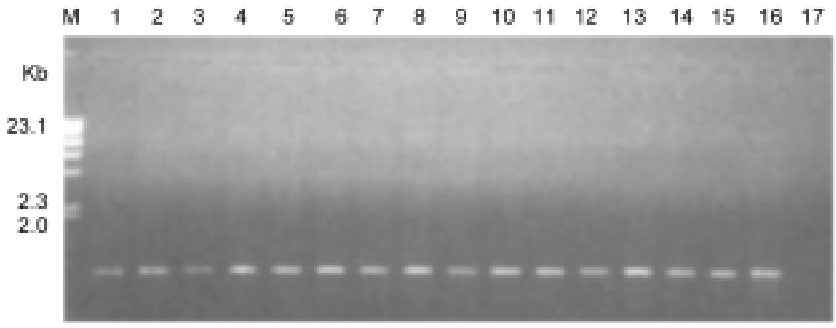

Fig. 1. Direct PCR amplification of streptomycete 16S rDNA from soil DNA using RI7/RI8 primers and 2% agarose. Lane M: 100 bp DNA ladder molecular weight marker; lanes 1–15: soil samples AJ 5, AJ 3; JV 1; AJ 5; N 3; Bb; PR; Z 2; Aa; AQ 1; AQ 5; S 1; S 2; S 7; AZ 4; lane 16: *Streptomyces coelicolor* ATCC 10147; lane 17: PCR negative control.

the International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966).

Total DNA was extracted from 14 untreated soil samples known to harbor streptomycin producers by a modification of the direct lysis method (Ogram *et al.*, 1987, Wellington *et al.*, 1992) as follows: 1 gram of soil was suspended in 10 ml sterile distilled water, incubated for 1 hour at 28°C with shaking at 200 rpm. After settling, the supernatant was centrifuged at 3000 rpm for 10 min. The pellet was resuspended in 50 mM EDTA and SDS (Promega) was added to a final concentration of 2%, then incubated in a water bath (GFL, Germany) at 100°C for 10 min, after that the mixture was centrifuged at 10 000 rpm for 10 min. The supernatants were transferred to sterile Eppendorf tubes containing isopropanol (GCC, UK) (3:1 ratio), the tubes were inverted several times, and centrifuged at 10 000 rpm for 1 min. The supernatant was discarded and 100% ethanol (Fluka, Germany) was added, the tubes were gently inverted, followed by centrifugation at 13 000 rpm for 2 min. Tubes were drained for 15 min to remove all of the ethanol then the DNA pellet was rehydrated by adding 50 ml of sterile TE buffer (10 mM Tris-HCl pH = 7.4 0 and 1 mM EDTA pH = 8.0).

Three sets of primers were used in this study. The first (RI7 and RI8) and second (AM45 and AM47) sets of primers were taken from Gharaibeh *et al.*, (2003). Both sets amplify 16S rDNA conserved regions found only in *Streptomyces* spp. The third set represents the forward and reverse primers of *strb1*, a biosynthetic gene that codes for streptomycin amidinotransferase (Distler *et al.*, 1992) according to Huddleston *et al.*, (1997) using the following primers: forward primer 5'-TGAGCCTTGTAAGCGTCCAC-3' and reverse primer 5'-TTCATGCCGTGCTTCTCCAG-3' (OPERON Technologies, USA) to yield a 940 bp product. Primers were synthesized by Operon Technologies (Operon, USA).

Detection of streptomycin-producers was carried out by the amplification of *strb1* gene. PCR amplification was carried out as mentioned above except that the annealing temperature was 55°C. Nuclease free water was used as negative controls.

PCR products were checked for DNA by standard electrophoresis procedures (Sambrook *et al.*, 1989). Gels were viewed using Fotodyne UV illuminator (Fotodyne Inc., USA) and photographed with Polaroid MP4$^+$ Instant Camera System (Polaroid corp., USA).

Results of dilution series and plate count of the samples that harbored most of the active *Streptomyces* isolates in addition to the streptomycin producers revealed an average streptomycetes count ranged between 3×10^4 and 2×10^6 colony forming unit (cfu) per 1 g of dry soil (data not shown). The colony morphology of the *Streptomyces* isolates on starch casein nitrate agar plates (SCNA) after 10 days of incubation at 27°C indicated that they were small (1–10 mm diameter), discrete and leathery, initially relatively with smooth surface but later developed a weft of aerial mycelium that appeared granular, powdery and velvety.

The ability of the constructed (RI7/RI8) and the control primers (AM45/AM47) to detect *Streptomyces* directly from soil is indicated by the presence of a single DNA band of 438 bp. (Fig. 1 and Fig. 2, respectively). PCR products are of similar size to those obtained when DNA from pure cultures was used as a template (Fig. 1 and 2, lane 16). No bands were seen with the negative control (Fig. 1 and 2, lane 17).

Detection of the presence of streptomycin producers directly from soil using crude DNA extracted from soil samples that contained streptomycin producers as a template for PCR to amplify the s*trb1* gene; is clearly indicated by a single band with 940 bp in size for all of the 14 soil samples tested (Fig. 3). No PCR products were observed in the negative control that contained nuclease free water (Fig. 3, lane 16). The genus *Streptomyces* as a major antibiotic producing group among microorganisms is the most abundant actinomycetes found in the soil (Williams *et al.*, 1989). Based on that, only 14 soil samples were selected for this study as they contain streptomycin producers (Gharaibeh *et al.*, 2003). These organisms have been advocated as promising biocontrol agents against several phytopatho-

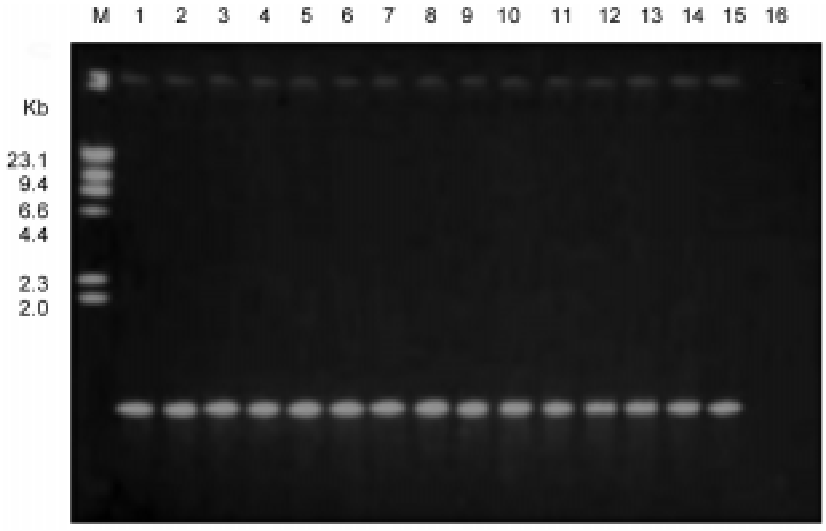

Fig. 2. Direct PCR amplification of streptomycete 16S rDNA from soil DNA using AM45/AM47 primers and 1% agarose.
Lane M: l Hind III molecular weight markers; lanes 1–15: soil samples AJ 5, AJ 3; JV 1; AJ 5; N 3; Bb; PR; Z 2; Aa; AQ 1; AQ 5; S 1; S 2; S 7; AZ 4; lane 16: *Streptomyces coelicolor* ATCC 10147; lane 17: PCR negative control.

genic fungi and bacteria (EL-Tarabily *et al.*, 1997; Saadoun and AL-Momani 1997; Saadoun *et al.*, 2000; Tahtamouni *et al.*, 2006).

Direct extraction of DNA from soil was of a great value, since it provides a sufficiently pure DNA for PCR within short time and without the need for further purification steps. Both primer pairs AM45/AM47 and RI7/RI8 were used and evaluated in detection of *Streptomyces* directly from soil. Taking into consideration the fact that 1 gram of soil may contain at least 4000 various genomes (Torsvik *et al.*, 1990), the two primers showed a high specificity in detecting *Streptomyces* spp. The presented work did not check for detection limits of the available streptomycetes in soil samples. We indicated that these soil samples under investigation revealed an average streptomycetes count less than the numbers that have reported before from other locations in Jordan (Saadoun *et al.*, 1999).

The protocol described by Ogram *et al.* (1987) and Wellington *et al.* (1992) for extraction of DNA from environmental samples along with PCR detection of *Streptomyces* supports the results presented here and mean that PCR can be used for direct detection of *Streptomyces* in such samples. This could be useful for tracking or quantifying *Streptomyces* in ecological studies; *i.e.* for monitoring the population of this bacterial genus in soil specially when *Streptomyces* are used for bio-controlling of fungal root and seed pathogens in soil.

The use of specific primers for the direct detection of streptomycin producers from soil represent a leap in the screening strategies for active *Streptomyces* isolates since *strb1* is highly conserved between streptomycin producers (Retzlaff *et al.*, 1993). It provides a shortcut to the preliminary studies on screening for the production of such compounds from environmental samples or pure *Streptomyces* isolates.

This study suggests that there is a resident population of *Streptomyces* in Jordan with the capacity for streptomycin-production. Further studies are needed to demonstrate the potential of streptomycin production for antagonism against soil bacteria in Jordanian soils.

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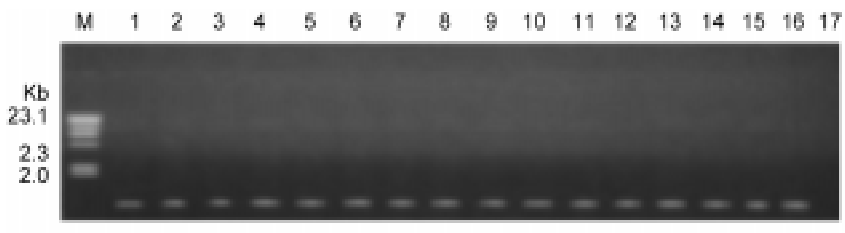

Fig. 3. Direct PCR amplification of *strb1* gene using soil samples DNA as a template and 1% agarose.
Lane M: l Hind III molecular weight markers; lane 1: *Streptomyces* isolate no. 20; lanes 2–15: soil samples AQ 5; AQ 2; AQ 1; AQ 7; S 1; AJ 3; D 1; AJ 5; JV 3; AZ 2; Aa 7; N 9; AZ 4; S 9; lane 16: PCR negative control.

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