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Halotolerant streptomycetes isolated from soil at Taif region, Kingdom of Saudi Arabia II: RAPD-PCR analysis and salt tolerance-gene isolation

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The genus *Streptomyces* is represented in nature by the largest number of species and varieties among the family Streptomycetaceae. This study aimed at extracting the DNA of four halotolerant *Streptomyces* strains followed by determination of DNA fingerprinting of them using a molecular tool. A trail to isolate salt tolerance gene(s) from their DNA was also aimed. RAPD-PCR technique was applied using seven RAPD-PCR primers. Results show that a total 71 fragments (65 polymorphic and six monomorphic) were amplified from the DNA of the four identified *Streptomyces* strains. The fragments were divided into polymorphic and monomorphic fragments. Three primers named OPA11, OPB15 and OPC07 did not show any monomorphic fragments. A number of 43 (7, 7, 8, 2, 9, 6 and 4) representing 60.56% were considered as unique DNA markers, and were amplified using OPA11, OPB15, OPC07, OPC18, OPD04, OPE05 and OPO14, respectively. Data shows similarity matrix between the four identified *Streptomycs* strains based on RAPD-PCR analysis ranged from 21.8 to 40.0. Results show that the *P5CR* gene was detected in the DNA extracts of three species, namely, *Streptomyces cirratus*-02; *Streptomyces rishiriensis*-04; *Streptomyces luteogriseus*-08, while, *mtlD* gene was only found in the DNA extracts of *S. cirratus*-02.

Key words: *Streptomyces*, halotolerant, salt tolerance genes, RAPD-PCR, Taif, KSA.

INTRODUCTION

Streptomycetes are widely distributed in terrestrial and aquatic habitats (Mayfield et al., 1972). Alvarez-Mico et al. (2013), Bhave et al. (2013) and Su et al. (2013) reported that halotolerant *Streptomyces* isolated from soil and/or marine were considered as sources of new compounds from their metabolites.

In Kingdom of Saudi Arabia (KSA), Altalhi and Mohamed (2010) identified a halotolerant streptomycete isolate able to produce pectin methyl esterase enzyme (PME) and appeared to be close to *S. nigrifaciens.* Al-Askar et al. (2011) isolated 128 strains of actinomycetes which were from different soils in Riyadh region, Saudi Arabia. Shori et al. (2012) identified seven streptomycete isolates able to grow in the presence of 7% NaCl in the starch nitrate

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agar medium from soil samples of Western region (Taif, Makah and Jeddah). At concentration of 10.5% NaCl, four isolates grew with weak growth (+) and three isolates showed in doubt growth (±). The antagonistic activities of the isolated actinomycetes were also tested against seven microorganisms including bacteria and fungi. The highest active isolates were identified as strains of *S. polychromogenes* (isolate 08), *S. chattanoogensis* (isolate 14), *Streptomyves lucensis* (isolate 20), *Streptomyves violaceus* (isolate 21), *S. violans* (isolate 32), *S. griseorubiginosus* (isolate 34), and *S. antibioticus* (isolate 35). Jose et al. (2011) isolated a moderately halophilic *Streptomyces* strain, designated JAJ06, from saltpan soil collected at Tuticorin, India. Several reports have been studied using the RAPD-PCR technique for screening the genetic variation within several species of fungi (*Plasmopara viticola*) (Seidel et al., 1999); *Candida albicans* (Pesti et al., 2001) and streptomycetes (El-Domyati and Mohamed, 2004; Abdel-Fattah, 2005; Saleh et al., 2011; Shash, 2011; Mohamed et al., 2012; Shori et al., 2012).

Streptomycetes are bacteria able to grow in soil, sea water and aquatic habitats due to their ability to tolerate salt. Moreover, some streptomycetes were recorded as halophilic streptomycetes. The scientists succeeded in the isolation of some salt tolerant genes from a few different kinds of bacteria (Serrano, 2004) including actinomycetes (Mohamed and Chaudary, 2005).

This study aimed at determining the DNA fingerprinting of four selected halotolerant *Streptomyces* strains using a molecular tool (that is, RAPD-PCR) and isolating a salt tolerance gene(s) of a halotolerant selected *Streptomyces* strains).

MATERIALS AND METHODS

Streptomyces strains

Four halotolerant *Streptomyves* strains isolated from sea water, Jeddah, KSA and named *S. cirratus*-02; *S. rishiriensis*-04; *S. alboflavus*-06; *S. luteogriseus*-08 were used.

DNA preparation

The DNA extracts were prepared from the pulverized streptomycete cells as described by Mahfouz and Mohamed (2002). The concentration and purity of DNA extracts of the *Streptomyces* strains were determined as recommended by Brown (1990).

DNA fingerprinting using RAPD-PCR

A number of seven RAPD-PCR primers (OPA11: 5'CAATCGCCGT3'; OPB15: 5'GGA GGG TGT T3'; OPC07:5'GTC CCG ACG A3'; OPC18:5'TGA GTG GGT G3'; OPD04: 5'TCT GGT GAG G3'; OPE05: 5'TCA GGG AGG T3' and OPO14: 5'AGC ATG GCT C3') were used for the determination of DNA fingerprinting of DNA-halotolerant *Streptomyces* strains.

Amplification reaction was carried out in a total volume of 25 µl based on the method of Bagheri et al. (1995). Each reaction mixture contained: 2.5 µl 10x PCR buffer (500 Mm KCl, 100 mM Tris-HCl (pH = 9.0) and 1% Triton-100); 1.5 µl $MgCl_2$ (1.5 mM); 0.5 µl dNTPs mix (0.2 mM dATP, dCTP, dTTP, dGTP); 1 µl DNA template (100 ng genomic DNA); 1.5 µl RAPD-PCR primer (0.4 µM); 0.5 µl *Taq* DNA polymerase (two units of *Taq* DNA polymerase -Promega Crop, Madison, WI, USA) and 17.5 µl d.H_2O.

PCR amplification as reported by Mahfouz and Mohamed (2002) was conducted in a GeneAmp 2400 PCR machine using the following program: denaturation for 5 min at 94°C (1 cycle); 35 cycles each of denaturation for 1 min at 94°C; annealing for 1 min at 36°C and elongation for 2 min at 72°C. The primer extension segment was extended to 5 min at 72°C in the final cycle.

The amplification products were resolved by electrophoresis (Sambrook et al., 1989) in a 1.2% agarose gel at 60 v for 2.5 h with 1X TAE buffer. The bands were checked by visualising the gel under the UV light transilluminator. The presence or absence of each sizes class was scored as 1 (amplified) or – 0 (not amplified), respectively. Bands of the same mobility were scored as monomorphic. The similarity coefficient (F) between isolates was defined by the formula of Nei and Li (1979). A dendrogram was derived from the distance by un-weighted paired-group method (Rohlf, 1990).

PCR-isolation of salt tolerance gene(s) from some halotolerant *Streptomyces* strains

The DNA of four identified halotolerant *Streptomyces* strains was used as a template in a trail to isolate some salt tolerance genes using PCR. For *P5CR* gene, two primers named F1: 5'GGA GAT CTA ACA ATG GAG ATT CTT CCG ATT CCG GCG G3' and R2: 5'GGG ATA TCT TAG CTC TGT GAG AGC TCG CGG3' flanking a size of about 831 bp were used. Regarding *mtlD* gene F2: 5'CGA GAT CTA ACA ATG AAA GCA TTA CAT TTG GCG C3' and R2: 5'GGG ATA TCT TAT TGC ATT GCT TTA TAA GCG G3' flanking 1150 bp were used. Amplification was performed as reported by Mohamed and Chaudary (2005) in GeneAmp PCR System 2400 Thermocycler for 35 cycles after initial denaturation for 4 min at 95°C. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. The primer extension was extended to 7 min at 72°C in the final cycle. The PCR amplified products were detected by electrophoresis on 1.5% agarose gel in 1X TAE buffer at 80 v for 1 h (Sambrook et al., 1989). PCR fragments were visualized as mentioned before.

RESULTS AND DISCUSSION

Molecular analysis of four halotolerant *Streptomyces* strains

DNA fingerprinting using RAPD-PCR

RAPD-PCR technique was used in some studies to determine the DNA fingerprinting of actinomycetes (Mohamed et al., 2001; Mahfouz and Mohamed, 2002; Abdel-Fattah, 2005; Saleh et al., 2011; Shash, 2011, Mohamed et al., 2012; Shori et al., 2012).

Data given in Table 1, Figures 1, 2 and 3 show that a total 71 fragments (65 polymorphic and six monomorphic) were amplified from the DNA of the four identified *Streptomyces* strains. These fragment distributed among the seven RAPD-PCR primers used were as follows: 11, 10, 12, 5, 12, 11, and 10 for OPA11, OPB15, OPC07, OPC18, OPD04, OPE05 and OPO14, respectively. The fragments were divided into polymorphic and monomer-

Table 1. DNA fragments (DFs) amplified by RAPD-PCR of four *Streptomyces* strains (*S. cirratus*-02, *S. rishiriensis*-04, *S. alboflavus*-06 and *S. luteogriseus*-08) using seven RAPD-PCR primers.

Fragment	*Streptomyces* strain				Type of fragment
	***S. cirratus*-02**	***S. rishiriensis*-04**	***S. alboflavus*-06**	***S. luteogriseus*-08**	
OPA11-1	1	0	0	0	Unique (+)
OPA11-2	0	1	0	0	Unique (+)
OPA11-3	1	0	1	0	Polymorphic
OPA11-4	1	0	0	1	Polymorphic
OPA11-5	1	1	0	1	Unique (-)
OPA11-6	0	1	0	1	Polymorphic
OPA11-7	1	1	0	0	Polymorphic
OPA11-8	1	0	0	0	Unique (+)
OPA11-9	1	1	1	0	Unique (-)
OPA11-10	0	0	0	1	Unique (+)
OPA11-11	0	0	0	1	Unique (+)
OPB15-12	1	1	0	0	Polymorphic
OPB15-13	1	0	0	0	Unique (+)
OPB15-14	0	1	0	0	Unique (+)
OPB15-15	0	1	0	0	Unique (+)
OPB15-16	1	0	0	1	Polymorphic
OPB15-17	1	1	0	0	Polymorphic
OPB15-18	0	1	1	1	Unique (-)
OPB15-19	0	1	0	0	Unique (+)
OPB15-20	0	0	0	1	Unique (+)
OPB15-21	0	0	0	1	Unique (+)
OPC07-22	1	0	0	0	Unique (+)
OPC07-23	0	0	0	1	Unique (+)
OPC07-24	1	0	1	1	Unique (-)
OPC07-25	0	0	1	0	Unique (+)
OPC07-26	1	0	1	0	Polymorphic
OPC07-27	0	1	0	1	Polymorphic
OPC07-28	1	0	0	1	Polymorphic
OPC07-29	0	0	1	0	Unique (+)
OPC07-30	1	1	0	0	Polymorphic
OPC07-31	0	1	0	0	Unique (+)
OPC07-32	1	1	1	0	Unique (-)
OPC07-33	0	0	1	0	Unique (+)
OPC18-34	1	1	1	1	Monomorphic
OPC18-35	1	1	1	1	Monomorphic
OPC18-36	1	1	1	1	Monomorphic
OPC18-37	0	1	1	1	Unique (-)
OPC18-38	0	1	1	1	Unique (-)
OPD04-39	0	0	1	0	Unique (+)
OPD04-40	1	0	0	0	Unique (+)
OPD04-41	0	0	1	0	Unique (+)
OPD04-42	0	0	1	0	Unique (+)
OPD04-43	1	1	0	1	Unique (-)
OPD04-44	1	1	1	1	Monomorphic
OPD04-45	1	0	0	0	Unique (+)
OPD04-46	0	0	0	1	Unique (+)
OPD04-47	1	0	1	0	Polymorphic
OPD04-48	0	0	1	1	Polymorphic
OPD04-49	1	1	0	1	Unique (-)

Table 1. Contd.

Fragment	*Streptomyces* strain				Type of fragment
	S. cirratus-02	*S. rishiriensis*-04	*S. alboflavus*-06	*S. luteogriseus*-08	
OPD04-50	0	0	1	0	Unique (+)
OPE05-51	1	0	0	0	Unique (+)
OPE05-52	0	1	0	1	Polymorphic
OPE05-53	1	0	0	1	Polymorphic
OPE05-54	1	1	1	0	Unique (-)
OPE05-55	0	0	1	0	Unique (+)
OPE05-56	1	0	1	0	Polymorphic
OPE05-57	0	0	0	1	Unique (+)
OPE05-58	1	0	0	1	Polymorphic
OPE05-59	1	1	1	1	Monomorphic
OPE05-60	1	1	0	1	Unique (-)
OPE05-61	0	0	1	0	Unique (+)
OPO14-62	0	0	1	0	Unique (+)
OPO14-63	1	0	1	0	Polymorphic
OPO14-64	1	0	0	1	Polymorphic
OPO14-65	0	0	1	0	Unique (+)
OPO14-66	1	1	1	1	Monomorphic
OPO14-67	0	0	1	0	Unique (+)
OPO14-68	0	1	0	1	Polymorphic
OPO14-69	1	0	1	0	Polymorphic
OPO14-70	1	1	1	1	Monomorphic
OPO14-71	0	0	1	0	Unique (+)

+, Present; -, absent; 1, amplified; 0, not amplified.

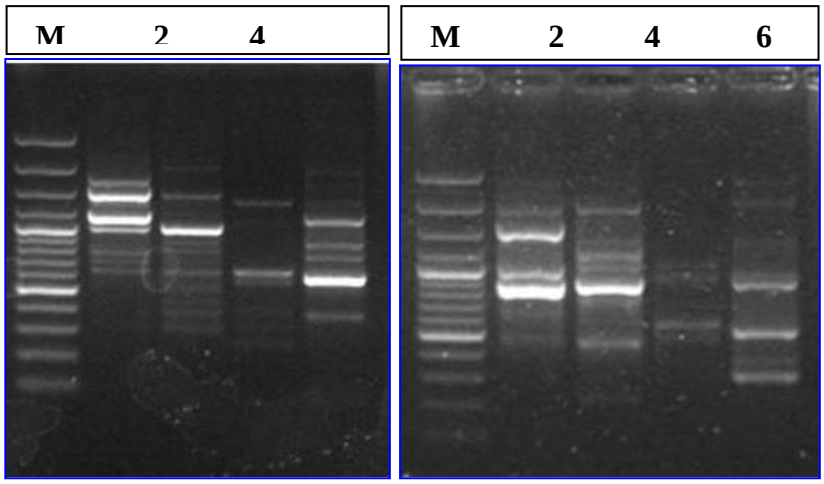

Figure 1. Electrophoresis of RAPD-PCR using agarose gel (1.2%) stained with ethidium bromide showing DNA polymorphisms of *Streptomyces* strains (*S. cirratus-02*; *S. rishiriensis-04*; *S. alboflavus-06* and *S. luteogriseus-08*) using OPA11 and OPB15 RAPD-PCR primers. M, 100 bp DNA ladder.

phic fragments. Three primers named OPA11, OPB15 and OPC07 did not show any monomorphic fragments. Using the seven RAPD-PCR primers, 43 (7, 7, 8, 2, 9, 6 and 4) representing 60.56% were considered as unique DNA markers, and were amplified using OPA11, OPB15, OPC07, OPC18, OPD04, OPE05 and OPO14, respectively.

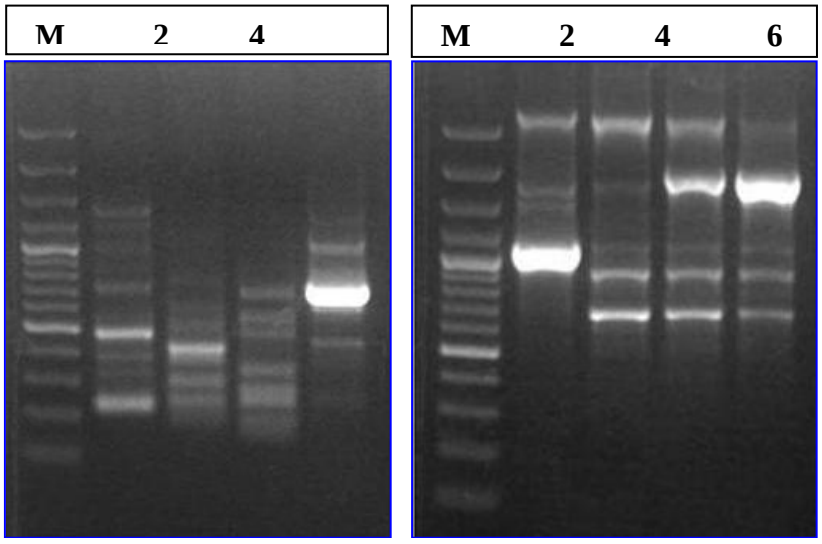

Figure 2. Electrophoresis of RAPD-PCR using agarose gel (1.2%) stained with ethidium bromide showing DNA polymorphisms of *Streptomyces* strains (*S. cirratus-02*; *S. rishiriensis-04*; *S. alboflavus-06* and *S. luteogriseus-08*) using OPC07 and OPC18 RAPD-PCR primers. M, 100 bp DNA ladder.

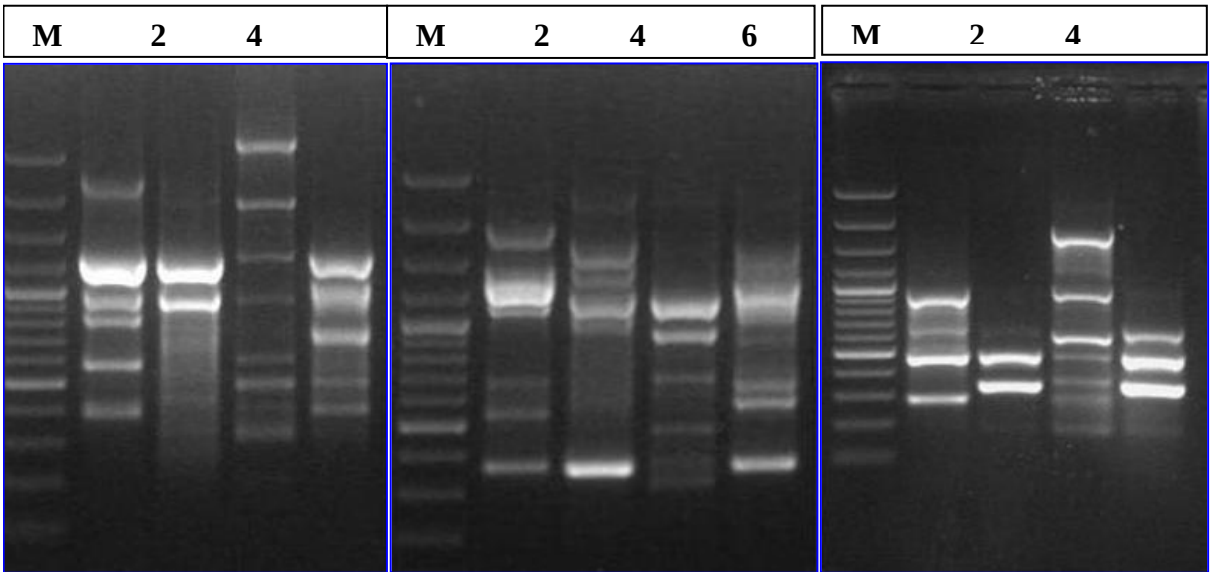

Figure 3. Electrophoresis of RAPD-PCR using agarose gel (1.2%) stained with ethidium bromide showing DNA polymorphisms of *Streptomyces* strains (*S. cirratus-02*; *S. rishiriensis-04*; *S. alboflavus-06* and *S. luteogriseus-08*) using OPD04; OPE04 and OPO14 RAPD-PCR primers. M, 100 bp DNA ladder.

A number of 32 unique fragments (5, 6, 6, 7, 4, and 4 belonging to OPA11, OPB15, OPC07, OPD04, OPE05 and OPO14, respectively) were recorded as present DNA markers, while 11 (2, 1, 2, 2, 2 and 2 belonging to OPA11, OPB15, OPC07, OPC18, OPE04 and OPE05, respectively) were recorded to be absent DNA markers. Data given in Figure 4 shows that three clusters were found in the dendogram deduced from the statistically analysis of the data. The first included both of *S. albofla-*

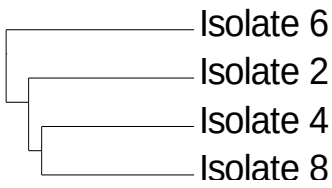

Figure 4. Dendrogram based on RAPD-PCR analysis of the four *Streptomyces* strains (*S. cirratus-02*; *S. rishiriensis-04*; *S. alboflavus-06* and *S. luteogriseus-08*) using 7 RAPD-PCR primers.

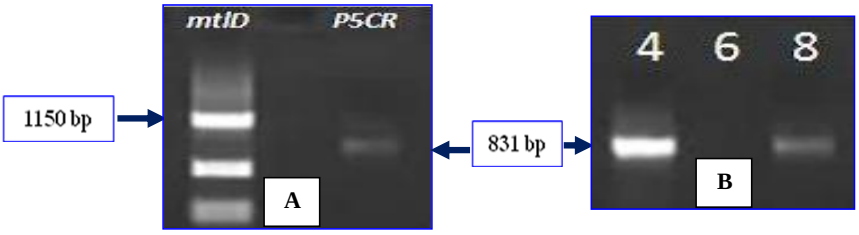

Figure 5. Agarose gel electrophoresis of amplified mtlD and P5CR genes from the DNA extracted from *S. cirratus-02* (A) and P5CR gene from the DNA extracted from *S. rishiriensis-04*; *S. alboflavus-06* and *S. luteogriseus-08* (B).

Table 2. Similarity matrix between the four identified *Streptomyces* strains (*S. cirratus*-02, *S. rishiriensis*-04, *S. alboflavus*-06 and *S. luteogriseus*-08) based on RAPD-PCR analysis.

***Streptomyces* strain**	***S. cirratus*-02**	***S. rishiriensis*-04**	***S. alboflavus*-06**	***S. luteogriseus*-08**
S. cirratus-02	100	36.0	30.9	34.0
S. rishiriensis-04		100	25.5	40.0
S. alboflavus-06			100	21.8
S. luteogriseus-08				100

vus-06 and *S. luteogriseus*-08.

The two *S. cirratus*-02 and *S. alboflavus*-06 lied in a separate cluster for each of them. This data is in harmony with the cultural characters of the *Streptomyces* isolates, as the strains (*S. cirratus*-02, *S. rishiriensis*-04 and *S. luteogriseus*-08) of the gray series were found to be too related to each other, while that of yellow (*S. alboflavus*-06) series lied in a separate cluster. Data given in Table 2 show similarity matrix between the four identified *Streptomyces* strains based on RAPD-PCR analysis ranging from 21.8 to 40.0.

PCR-isolation of a salt tolerance gene(s) from some halotolerant *Streptomyces* strains

In this study, the DNA of the four halotolerant *Streptomyces* stains of this study, isolated from sea water and tolerated a salt range of 14 to 21% NaCl in the growth medium were used. A trial was conducted to detect two salt tolerance genes (*P5CR*, and *mtlD*) in the DNA extracted from the applied *Streptomyces* strains using PCR.

Results in Table 3 show that the *P5CR* gene was detected in the DNA extracts of three species, namely, *S. cirratus*-02; *S. rishiriensis*-04; *S. luteogriseus*-08 (Figure 5), while, *mtlD* gene was found in the DNA extracts of *S. cirratus*-isolate 2. These results are sup-ported by the results of Mohamed and Chaudary (2005), which showed that such results indicate the differences in the genetic makeup of different *Strepto-myces* species. The results also indicate that different mechanisms are involved in conferring salt tolerance in these *Streptomyces* species.

Table 3. PCR detection of two salt tolerance genes from the DNA extracts of four *Streptomyces* strains (*S. cirratus*-02; *S. rishiriensis*-04; *S. alboflavus*-06; *S. luteogriseus0* 8) isolated from sea water.

***Streptomyces* strain**	**PCR detected gene**	
	P5CR	***mtlD***
S. cirratus-02	+	+
S. rishiriensis-04	+	-
S. alboflavus-06	-	-
S. luteogriseus-08	+	-

+, Detected; -, not detected.

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