Molecular genetic characterization of some biosurfactant-producing bacteria isolated from Egyptian red sea mangrove forests

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ABSTRACT

Biosurfactant are important biotechnological products, with a wide range of applications in many industries. In the present work, four soil bacterial strains were isolated, purified and tested for their natural ability to produce biosurfactants. Results of this experiment showed that the tested bacterial isolates were capable of producing biosurfactant. Traditional microbial characterization methods revealed that these bacterial strains belong to Bacillus subtilis, Bacillus coagulans, Bacillus amyloliquefaciens and Pseudomonas pseudoalcaligene. Moreover, molecular genetic characterization of the tested bacterial strains has been carried out via polymerase chain reaction-restriction fragment length polymorphism (PCR-16S rRNARFLP) and random amplified polymorphic DNA- PCR (RAPD-PCR). No genetic variations among the strains has been detected by the use of PCR-RFLP analysis. However, genetic dissimilarities have been detected via RAPD-PCR. In addition, phylogenetic tree has been constructed based on RAPD-PCR analysis in which results indicated the discrimination of the three bacillus strains from the pseudomonas strains.

Key words: *Biosurfactant, Molecular genetic characterization, Red sea, Mangrove forest, RAPD, RFLP.*

INTRODUCTION

Microbial surface active agents are surface-active substances synthesized by living cells. Biosurfactants are important biotechnological products, with a wide range of applications in many industries. Advantages of biosurfactants include biodegradability, generally low toxicity, biocompatibility, digestibility, availability of raw materials, acceptable production economics, use in environmental control, specificity and effectiveness at extreme temperature, pH and salinity (Yin *et al.*, 2009).

Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions and potential applications in environmental protection (Rahman *et al.*, 2002). Types of biosurfactants include lipopeptides synthesized by many *Bacilli* and other species, glycolipids synthesized by *Pseudomonas* species and *Candida* species, phospholipids synthesized by *Thiobacillus thiooxidans*,

polysaccharides-lipid complex synthesized by *Acinetobacter* species or even the microbial cell surface itself (Van Dyke *et al.*, 1991, Bodour and Maier, 2002). Biosurfactants can also be used in bioremediation of soil or sand (Van Dyke *et al.*, 1991) or in the cleanup of hydrocarbon contamination in groundwater (Ron and Rosenberg, 2001). Bacterial communities are difficult to study due to their immense complexity and the potential problems in culture ability of many of the members (Abou-Shanab, 2007). Serological and bacteriological methods are not sensitive enough to differentiate all bacterial isolates (Taghi *et al.*, 2008). Therefore, DNA-based analyses can contribute significantly to the characterization of bacteria that have been successfully isolated from these environments. Genomic DNA fingerprinting using random amplified polymorphic DNA– polymerase chain reaction (RAPD-PCR) was found to be useful in differentiating among closely related bacteria (Williams *et al.*, 1990). Besides being easier and cheaper, these methods are as effective as the more labor intensive restriction fragment length polymorphism (RFLP) techniques for establishing genetic relation-nships and identifying bacterial genomes (Selenska-Pabell *et al.*, 1996). Also, RAPD-PCR is an effective technique for typing microbial isolates such as *Brucella* spp. (Behroozikhah *et al.*, 2005), *Leptosphaeria maculans* (Goodwin *and Annis*,1991), *Histoplasma capsulatum* (Kersulyte *et al.*, 1992), *Cryptococcus neoformans* (Brandt *et al.*, 1995) and *Salmonella enteritidis* (Lin *et al.*, 1996).

Bacterial 16S rRNA is a common target for taxonomic purposes and identification, largely due to the mosaic composition of phylogenetically conserved and variable regions within the gene (De Rijk *et al.*, 1992; Gurtler and Sanisich, 1996).

The present study aims at screening biosurfactant-producing bacteria, besides evaluating their biosurfactant productivity and genetic diversity.

MATERIALS AND METHODS

Sampling and media used

Soil and water samples were collected from Mangrove sediment samples of the Red Sea beaches in sterilized bottles from eight different petrochemical sites. A 10 ml (water) or 10 g (soil) aliquot of each sample was transferred aseptically to 100 ml mineral salt medium (MSMA), of the following composition: (g/l):NH_4Cl,1;Na_2HPO_4,0.38;NaH_2PO_4,0.38;$MgCl_2.6H_2O$,0.08;$CaCl_2$,0.07;1mg $FeSO_4$. $7H_2O$ and 2.5 ml trace element solution. The trace salt solution comprised (mg/l): MnCl2.4H2O, 27;H_3BO_3,31; $CoCl_2.6H_2O$,36;$CuCl_2.2H_2O$, 10;$NiCl_2.6H_2O$,20;$Na_2MoO_4.2H_2O$,30 and $ZnCl_2$, 50. The MSM was supplemented with 0.2 % (w/v) crystalline phenanthrene, anthracene, naphthalene, salicylate, nitrophenol, α-naphthol, β-naphthol and catechol and 0.2 %(v/v) cyclohexane and toluene.

While MSMB was composed of (g/l) $(NH_4)_2SO_4$,10; KCl,1.1; NaCl, 1.1 ;$FeSO_4.7H_2O$,0.028 ;KH_2PO_4,3.4 ;$K_2HPO_4.3H_2O$,4.4 ;$MgSO_4$,0.5;yeast extract, 0.5, distilled water 1000 ml and trace salt solution,0.5 (ml/l) with 0.2 % (w/v or v/v) as the sole carbon source. Trace salt solution contained $ZnSO_4$, 0.2g; $CaCl_2$, 0.24 g; CuSO4, 0.25 g; MnSO4, 0.17 g/l was filter- sterilized. The pH of the medium was adjusted to 7.0 before sterilization.

The MSM cultures were incubated aerobically at 30ºC for 24 h. Following visible growth, aliquots of the cultures were transferred to fresh MSM containing 0.2% (w/v or v/v) of the previously mentioned

hydrocarbon compounds. After several subculturing, different hydrocarbon degrading strains were isolated by repeated streaking on solid media until individual colonies were obtained.

Table (1): List of primers, their nucleotide sequences and total number of bands for each isolate produced by six primers.

Primer code	Primer sequence	Isolates				Total Bands	Amplified bands	Polymorphic bands	Monomorphic bands
		R4	R2	R5	R41				
A3	5'-AGTCAGCCAC-3'	7	7	4	3	21	13	13	0
B2	5'-TGATCCCTGG-3'	4	6	6	4	20	12	12	0
B3	5'-CATCCCCCTG-3'	4	8	5	6	23	8	3	5
C2	5'-GTGAGGCGTC-3'	5	7	5	4	21	14	14	0
C4	5'-CCGCATCTAC-3'	8	7	7	7	29	9	4	5
C5	5'-GATGACCGCC-3'	4	9	4	9	26	11	3	8
	TOTAL	32	44	31	33	140	67	49	18

Monitoring of hydrocarbon degradation on solid media

Hydrocarbon degrading bacterial members were isolated from the enrichment cultures. The bacterial isolates exhibiting good growth were selected for further studies.

Identification of biosurfactant- producing bacterial isolates

The four most potent biosurfactant-producing bacterial isolates able to grow on the different hydrocarbon compounds as the sole source of carbon and energy were characterized by traditional microbiological methods, e.g. by plating on selective media and microscopic examination. Biochemical characterization was carried out according to standard microbiological techniques.

Production of biosurfactants

Bacterial strains were grown at 30°C for 48 h in α-naphthol, MSMA and B media. Biosurfactant activities were determined by oil spread technique. The diameter of the clear zone on the oil surface was measured in triplicate for each sample. Biosurfactant activity was defined as the diameter of the clearing on the surface in centimeters. Hemolytic activity was carried out in which Isolated strains were screened on blood agar plates containing 5% (v/v) blood and incubated at 45°C for 24-48h. Hemolytic activity was detected as the presence of a clear zone around a colony.

Molecular genetic fingerprinting
DNA extraction

Bacterial strains were grown in 5 ml of liquid LB medium. A portion of 1.5 ml of the culture was centrifuged at 8000 rpm for 2 min. Pellet washed in 1ml of SET buffer (20% sucrose, 50 mM EDTA, 50 mM Tris Hcl, pH : 8.0) and recentrifuged as mentioned above. One hundred µl SET buffer and 200 µl of SL buffer (SET buffer contains 25 mg/ml lysozyme) were added to the pellet. The tubes inverted several times gently and placed on a water bath on 37 ^{0}C for 60 min. An equal volume of phenol/ chloroform/isoamyl alcohol was mixed thoroughly and centrifuged for 5 min in a microcentrifuge. Aqueous viscous supernatant was removed to a new microfuge

tube, leaving the interface behind. An approximately equal volume of chloroform/ isoamyl alcohol was added and samples were centrifuged for 5 minutes at 8500 rpm. Supernatant was transferred to a fresh tube and equal volume of isopropanol was added to precipitate the nucleic acids. The DNA pellets were washed with 70% ethanol and the pellets were dissolved in 100 µl double destilled deionized (dd) H2O.

16SrRNA PCR-RFLP analysis

Primer sequences used to isolate the 16S rRNA gene fragment were: F-968 GC (59-CGCCCGGGGCGCGCCCCGGGCGGGGCG GGGGCACGGGGG GAACGCGAAGAACCTTAC-39) and R-1401 (59-CGGTGTGTACAAGACCC-39). The PCR master mix contained 0.01nM primers, 50 nM dNTPs, 1 U Taq DNA pol., 3µl of 10x buffer, mixed with 1µl (50 ng) of DNA template. Sterile d.H2O was added to a final volume of 25 µl. Thermal cycler (Uno II , Biometra, Germany) program was 94 °C for 4 min., 94 °C for 1 min., 55 °C for 1 min., 72 °C for 1.5 min, the number of cycles was 35 cycle and the post PCR reaction time was 72°C for 5 min.

Analysis of the PCR product

The PCR products were resolved by 1.5% agarose gel electrophoresis and stained with ethidium bromide.

Restriction enzyme digestion

Three restriction enzymes were used in order to charectirize the bacterial strains under study. These enzymes; e.g *EcoRI* and *HindIII* (Sigma, Germany), *CfoI* (Jena Bioscience, Germany) were used as described by the manufacturer.

Gel visualization and documentation

The DNA bands representing the 16SrRNA gene fragment were visualized under the UV light and the documentation was carried out using the UV band gel analysis software (Jena lab., UK) in the presence of a 100 bp molecular weight DNA standard .

RAPD-PCR

RAPD reactions were conducted in Biometra T-personal Thermal Cycler. RAPD-PCR amplification was performed as described by Selenska-Pobel *et al.* (1996) using six arbitrary 10-mer primers namely AO3, BO2, BO3, CO2, CO4 and CO5 (Table 1) (Operon Tech., Inc., Germany). The thermal cycler machine program was a 35-cycle PCR (initial denaturation, 94°C for 2.5 min; subsequent denaturation, 94 °C for 45 sec; annealing temperature, 37°C for 30 sec; extension temperature, 72 °C for 2 min and final extension, 72 °C for 10 min). The PCR products were analyzed on 1.5% agarose gel and visualized by ultraviolet illumination after staining with 0.5 µg/ml ethidium bromide.

Statistical analysis

The presence / absence RAPD data were analyzed using the NTSYSpc ver 2.10 software program. Pair-wise comparisons between isolates were used to calculate the genetic similarity values (F) derived from the Dice similarity coefficient.

RESULTS AND DISCUSSION

Soil samples screened for different hydrocarbon compounds were collected from Mangrove sediment samples taken from Egyptian Red Sea beaches. The initial screening on two types of media viz., MSMA and MSMB yielded four bacterial isolates capable of growing on phenanthrene, cyclohexane, toluene, anthracene, naphthalene, salicylate, nitrophenol, α-naphthol, β-naphthol and catechol as only source of carbon and energy (Table 2). The most potent

biosurfactant producing bacterial isolate *Bacillus amyloliquefaciens* R5 capable of growing on the previously hydrocarbon compounds was tested for biosurfactant production quantitatively with hemolytic activity and oil spread technique (Table 3). The isolates were also examined for cell shape, cell arrangement, relation to oxygen and nutritional requirements.

Cells of R2 were Gram-positive rods, spore former, motile, produced catalase and capable of growing under facultative anaerobic conditions. Strain R2 could ferment D-fructose, D-glucose, glycerol, mannose, starch and sucrose but not D- arabinose, D(-) lactose, D(-) mannitol and rhamnose. Strain R2 could produce amylase, pectinase and cellulase while do not produce gelatinase. Strain R2 could utilize citrate, reduce nitrate, produce H_2S and form levan. Strain R2 could grow well on blood agar medium with β-heamolysis and gave positive result in MR test but negative with V-P test. Temperature growth range for strain R2 was 8-40 °C. The R2 strain grew at NaCl concentrations ranging from 0 to 2%. According to Bergey's Manual of Determinative Bacteriology (Sneath *et al.*, 1986) this isolate is belonging to *Bacillus subtilis*, thus it could be given the tentative name *Bacillus subitilis*-R2.

Table (2): Growth of the four bacterial strains in two types of cultural media containing ten different hydrocarbon compounds at 30°C for 48h.

Bacterial code no.	Hydrocarbon compounds 0.2 % (w/v or v/v).									
	Phenanthrene		Cyclohexane		Toluene		Anthracene		Naphthalene	
	A*	B**	A	B	A	B	A	B	A	B
R2	-	+	-	+	-	+	+	+	±	+
R4	-	+	-	+	-	+	-	+	+	+
R5	-	+	+	+	+	+	+	+	±	+
R41	±	+	-	-	-	+	-	+	+	+
	Hydrocarbon compounds 0.5 % (w/v or v/v).									
	Phenanthrene		Cyclohexane		Toluene		Anthracene		Naphthalene	
	A	B	A	B	A	B	A	B	A	B
R2	-	-	-	-	-	+	-	+	-	±
R4	-	±	-	-	-	+	-	+	±	-
R5	-	-	+	-	+	+	+	+	+	-
R41	+	-	-	-	-	+	-	+	±	-

P.: Pseudomonas. * and ** are MSM A and B, respectively.

Table (3): Detection of biosurfactant production on different organic compounds by hemolytic activity and oil spread technique.

Compounds	Hemolytic activity (mm)	Oil spread technique (mm)
Galactose	0	0
Tryptone	0	6
Mannitol	2.3;2:2	-
Salicylate	1.2	3;3.5;5
Cyclohexane	2;2.5	1.5
Sugar cane molasse	0	1
Anthracene	1.5;1.2;1.2	-
Catechol	1.5	4;2
Hexane	0	3
Nitrophenol	0	5;6
Phenanthrene	0	3.5;2
Toluene	1.7;1.8;1.5	2;1
Fructose	1.5;1.5;1.7	1
Glucose	-	1
Crude oil	1.5;1.5;1.7	2.5;3
B-naphthol		2
α-naphthol		5;3;2.5

Cells of R4 were Gram-positive rods, motile, produce catalase, spore former and capable of growing under facultative anaerobic conditions. The isolate could ferment D-fructose, D-glucose, glycerol, mannose, starch, D(+) sucrose while do not ferment D-arabinose, D(-) lactose, D(-) mannitol, rhamnose. Strain R4 could reduce nitrate to nitrite, citrate utilized, do not produce H_2S and formation of levan, utilize of glucose under both oxidative and fermentative conditions. Strain R4 could produce amylase and cellulase while do not produce gelatinase and pectinase. This strain also grow well on blood agar medium with β-heamolysis while failed to grow on MacConkey agar medium and failed to produce any diffusible pigment during incubation. Temperature growth range of strain R4 is between 8 and 40°C.

Cells of R5 are Gram-positive rods, motile, spore former, produce catalase and capable of growing under facultatively anaerobic conditions. Strain R5 could ferment D-fructose, D-glucose, starch and D-sucrose but do not ferment of D-arabinose, D(-) lactose, glycerol, D(-) mannitol, rhamnose and mannose. Strain R5 reduce nitrate, citrate utilized, do not produce H_2S, levan formation, do not resist KCN and utilize glucose under fermentative conditions. Strain R5 could produce amylase and cellulase but do not produce of gelatinase and pectinase. Temperature growth range for strain R5 was 8-50°C, with the maximal growth rate at 30°C. Strain R5 could grew well on blood agar with β-heamolysis but do not grew on MacConkey agar medium.

Table (4): Specific markers for biosurfactant-producing bacterial strains by RAPD-PCR analysis.

Marker		Bacterial strain				Total
primer	Band MW (bp)	R4	R2	R5	R41	
C2	520			+		6
	750			+		
	900				+	
	950				+	
	1000				+	
	2200		+			
C5	650				+	2
	1800	+				
B2	650				+	4
	700	+				
	1500			+		
	1800		+			
B3	480				+	4
	700		+			
	1500		+			
	2000		+			
A3	200		+			6
	250	+				
	350			+		
	400	+				
	600	+				
	2200	+				
Total		6	6	4	6	22

Table (5): Similarity coefficients among the studied bacterial strains.

	R4	R2	R5	R41
R4	1.00			
R2	0.69	1.00		
R5	0.57	0.76	1.00	
R41	0.11	0.08	0.02	1.00

Cells of R41 are negative short rods, motile, produced catalase and capable of growing under facultatively anaerobic conditions. Strain R41 could ferment D-fructose, D-glucose, mannose and D (+) sucrose while do not ferment of D-arabinose, D(-) lactose, glycerol, D(-) mannitol, rhamnose and starch. Strain R41 could not produce amylase, gelatinase, pectinase and cellulase. Strain R41 could reduce nitrate, utilize citrate, form levan and do not resist KCN. Strain R41 grow well on blood agar medium with β-heamolysis, while it did not produce any diffusible pigment during incubation on any media used during this study. Temperatures growth range for strain R41 was 8-60°C, with the maximal growth rate at 30°C. In view of the previously recorded results, the four bacterial isolates could be identified as *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus amyloliquefaciens*, and *Pseudomonas pseudoalcaligenes*, respectively.

Molecular genetic characterization

Because bacterial strains under study have not been characterized before on the molecular genetic level, this experiment aimed at investigating the genetic relationships of these bacterial strains and to obtain a specific RAPD marker for production of biosurfactant among studied bacterial strains. PCR-RFLP analysis of the 16SrRNA gene and random amplified polymorphic DNA (RAPD - PCR) were employed using a bacterial universal pair of oligonucleotide primers as well as three restriction enzymes, *EcoRI, HindIII* and *CfoI.*

PCR – 16S rRNA RFLP analysis

Two universal oligonucleotide primers were used for the detection of the 16S rRNA gene fragment as a characterization tool for bacterial taxonomy. However, those two universal primers generated one common band of the same molecular weight of 500 bp (Fig.1). This result is expected since the used primers are universal for bacteria. Also, this result is in accordance with the results reported by Watanabe *et al.* (2001), who stated that the universal primers used in this study generates usually a common DNA band.

Restriction enzyme digestion

Three restriction enzymes were tested for cutting the generated PCR product; Ec*oRI, HindIII* and *CfoI*. Restriction enzyme digestion pattern of the generated PCR fragment with *CfoI* restriction enzyme produced two common bands with all tested bacterial strains (Fig. 2). The first band has a molecular weight of 200 bp, while, the second has a molecular weight of 300 bp. However, the other two restriction enzymes *EcoRI,* and *HindIII* did not produce any restriction fragments after treating the generated PCR product with either one. This is the first record to describe the employment of the restriction enzyme *CfoI* to cut the 16SrRNA PCR fragment generated from the described oligonucleotide primers with the tested bacterial strains. However, no molecular genetic variations have been detected among the studied strains. Therefore, additional molecular marker analysis such as RAPD-PCR and DNA sequencing approaches was suggested to be carried out in order to investigate the similarity and dissimilarity among the studied bacterial strains.

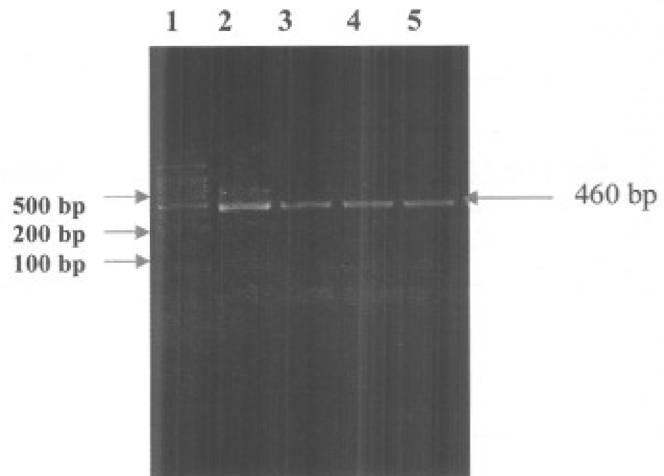

Fig. (1): The 16SrRNA gene fragment, lane 1: 100 bp ladder molecular weight standard, lane 2-4: samples R2, R4, R41 and R5, respectively.

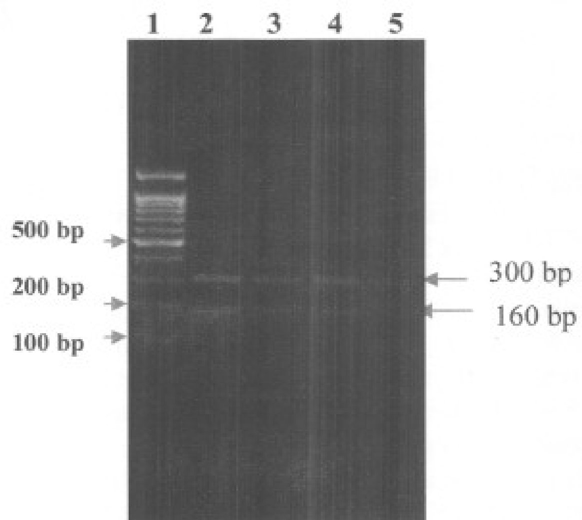

Fig. (2): The CfoI restriction pattern of 16SrRNA gene fragment, lane 1: 100 bp ladder molecular weight standard, lane 2-4: samples R2, R4, R41 and R5, respectively.

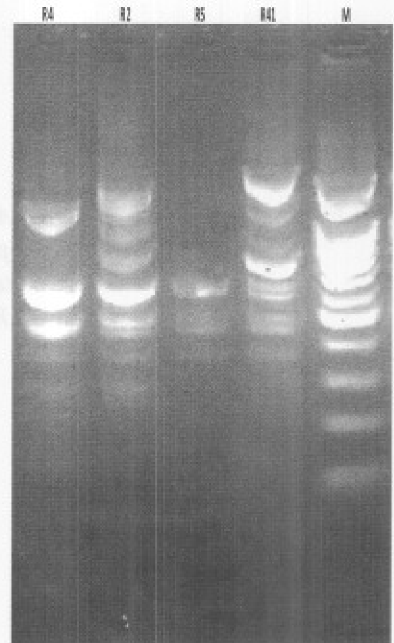

Fig. (3): The RAPD-PCR banding pattern obtained from bacterial strains generated by random primer C5.

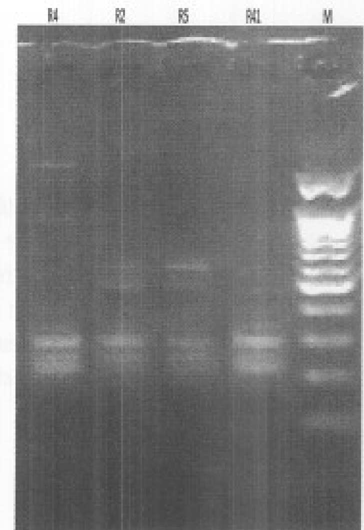

Fig. (4): The RAPD-PCR banding pattern obtained from bacterial strains generated by random primer C2.

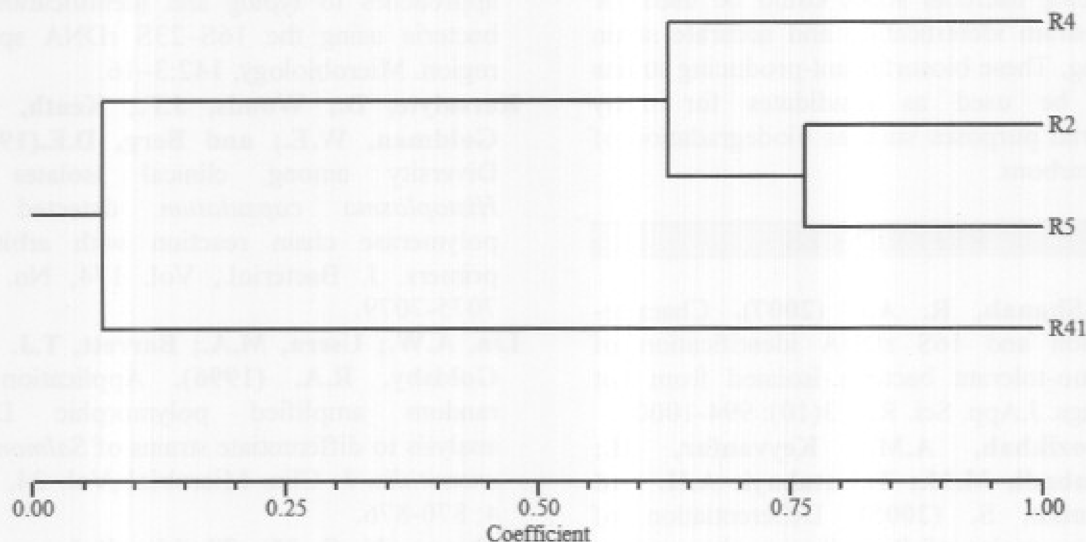

Fig. (5): Phylogenetic tree of the four tested bacterial strains using RAPD-PCR analysis.

RAPD- PCR analysis

In general, RAPD-PCR technique provide genetic relationship and genetic similarity between studied bacterial stains. All bacteria were subjected to RAPD-PCR using six primers: A3, B2, B3, C2 (Figure 3), C4 and C5 (Figure 4). The used primers have amplified 67 bands with 140 total number of bands, out of them 49 bands were polymorphic bands with a percentage of 73 % and 18 bands were monomorphic bands (27%).The obtained banding patterns showed a great differentiation between the examined strains. The phylogeny tree (Figure 5) obtained from statistical analysis of the RAPD-PCR banding pattern showed that the dendrogram was divided into two subgroups. The first contains R2, R5 and R4 isolates. The closest genetic distance was found between R2 and R5 strains, which were first clustered together and then with R4. The second subgroup includes R41 strain. Data illustrated in Table (5) showed that, the highest genetic similarity was between R2 and R5 (76%), while the genetic similarity between R5 and R41 was the lowest one (2%).These results confirmed that R2, R5 and R4 strains belong to different *Bacillus* species (*Bacillus subtilis*, *Bacillus coagulans* and *Bacillus amyloliquefaciens* while R41 bacterial strain belongs to other bacterial genus (*Pseudomonas pseudoalcaligenes*) . On the other hand, five primers produced useful molecular genetic markers which could be used to distinguish bacterial strains and characterize each biosurfactant- producing bacterial strain (Table 4). The maximum number of produced markers was 22 specific markers. The number of specific markers varied among studied primers, the highest primers produced specific markers were C2 and A3 primers, whereas both of them produced six specific markers, while the lowest number of specific markers produced was resulted from C5 primer which produced two specific markers. The R4, R2 and R41 had the highest specific marker producing bacterial strains; each of them produced six specific markers while R5 bacterial strain had 4 specific markers. These

specific markers for each biosurfactant-producing bacterial strain could be used for rapid strain identification and accurate strain tracking. These biosurfactant-producing strains could be used as candidates for many industrial purposes such as biodegradation of hydrocarbons.

REFERENCES

Abou-Shanab, R. A.I. (2007). Characterization and 16S rDNA identification of thermo-tolerant bacteria isolated from hot springs. J.App. Sci. Res, 3(10): 994-1000

Behroozikhah, A.M.; Keyvanfar, H.; Feizabadi, M.M.; Tabatabayi, A.H. and Alamian, S. (2005). Differentiation of Iranian strains of *Brucella spp*. by random amplification of polymorphic DNA. Arch. Razi Inst., 59: 13-23.

Bodour, A. A. and Maier, R. M.(2002): Biosurfactants :types, screening methods and applications,P.750-770.*In* G.Bitton(ed.), Encyclopedia of environmental microbiology.1st ed.John Wiley and Sons,Inc., Hoboken.

Brandt, M.E.; Hutwanger, L.C.; Kuykendall, R.J. and Pinner, R.W. (1995). The cryptococcal disease active surveillance group.Comparison of multilocus enzyme electrophoresis and random amplified polymorphic DNA analysis for molecular subtyping of *Cryptococcus neoformans*. J. Clin. Microbiol., Vol. 33, No. 7: 1890-1895.

De Rijk, P; Neefs, J-M; Van de Peer, Y. and De Wachter, R. (1992). Compilation of small ribosomal subunit RNA sequences. Nucleic Acids Res., 20:2075–2089

Goodwin, P.H. and Annis, S.L. (1991). Rapid identification of genetic variation and pathotype of *Leptosphaeria maculans* by random amplified polymorphic DNA assay. Appl. Environ. Microbiol., 57:2482-2486.

Gurtler, V. and Stanisich, V A. (1996). New approaches to typing and identification of bacteria using the 16S–23S rDNA spacer region. Microbiology, 142:3–16.

Kersulyte, D.; Woods, J.P.; Keath, E.J. Goldman, W.E.; and Berg, D.E.(1992). Diversity among clinical isolates of *Histoplasma capsulatum* detected by polymerase chain reaction with arbitrary primers. J. Bacteriol., Vol. 174, No. 22: 7075-7079.

Lin, A.W.; Usera, M.A.; Barrett, T.J. and Goldsby, R.A. (1996). Application of random amplified polymorphic DNA analysis to differentiate strains of *Salmonella enteritidis*. J. Clin. Microbiol.,Vol. 34, No. 4: 870-876.

Rahman, K. S. M.; Thahira-Rahman, J.; McClean, S., Marchant, R., and Banat, I.M. (2002). Rhamnolipid biosurfactants production by strains of *Pseudomonas aeruginosa* using low cost raw materials. Biotechnol Prog. 18 1277−1281.

Ron,E.Z. and Rosenberg,E.(2001). Natural roles of biosurfactants. J. Environ. Microbiol. 3(4),229-236.

Selenska-Pobell, S.; Evguenieva-Hackenberg E.; Radeva G.; and Squartini, A. (1996). Characterization of *Rhizobium* 'hedysar' by RFLP analysis of PCR amplified rDNA and by genomic PCR fingerprinting. Journal of Applied Bacteriology, 80: 517- 528.

Sneath, P.H.A., Mair N.S. and Sharpe M. E. (1986). Bergey's Mannal of Systematic Bacteriology, Vol. 2: 1208-1234. Baltimore: Williams & Wilkins.

Taghi Z. S.; Seyed A. M.; Vahid K.; and Fatemeh A. K. (2008): Molecular genetic differentiation of avian *Escherichia coli* by RAPD-PCR. Braz. J. Microbiol. 39:494-497

Van Dyke, M. I.; Lee H. and Trevors, J.T.(1991). Application of microbial surfactants. Biotechnol. Adv.9,241-253.

Williams, J.G.K.; Kubelik A.R.; Livak K.J.; Rafalski J.A.; and Tingey S.V.; (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research, 18: 6531-6535.

Watanabe K.; Kodama Y. and Harayama S. (2001). Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting. Journal of Microbiological Methods, Vol. 44: 253–262.

Yin, H.; Qiang, J.; Jia, Y.; Ye, J.; Peng, H.; Qin, H.; Zhang, N.; and He, B. (2009). Characteristics of biosurfactant produced by *Pseudomonas aeruginosa* S6 isolated from oil-containing wastewater. Process Biochemistry, 44:302-308.

الملخص العربي

التوصيف الوراثي الجزيئي لبعض سلالات البكتيريا المقللة للتوتر السطحي والمعزولة من غابات المانجروف بالبحر الأحمر

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تمثل المواد الحيوية المقللة للتوتر السطحي أحد أهم المنتجات في مجال البيوتكنولوجي حيث تدخل في العديد من التطبيقات الصناعية ، تم في هذا البحث عزل وتنقية عدد أربعة سلالات بكتيرية وإختبار مدى قدرتها على إنتاج المواد الحيوية المقللة للتوتر السطحي حيث أظهرت النتائج قدرة هذه السلالات البكتيرية الأربعة على إنتاجها بكمية كبيرة. كما تم تعريف السلالات البكتيرية الأربعة باستخدام الطرق البكتيريولوجية التقليدية حيث أظهرت النتائج أن السلالات محل الدراسة تتبع أربعة أنواع هي *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus amyloliquefaciens* and *Pseudomonas pseudoalcaligene* كما تم إجراء التعريف على المستوى الوراثي والجزيني للسلالات الأربعة محل الدراسة باستخدام تفاعل البلمرة المتسلسل بتقنيتي PCR-RFLP و RAPD PCR حيث اتضح من التقنية الأولى عدم وجود أي اختلافات وراثية بين الأنواع البكتيرية الأربعة بينما أوضحت التقنية الأخرى وهي RAPD-PCR وجود فروق وراثية بينهم وهو ما أدي إلى تحديد درجة القرابة الوراثية بين السلالات البكتيرية محل الدراسة باستخدام الطرق الإحصائية المناسبة. بالاضافة الى استخلاص معلمات وراثية لكل سلالة من السلالات محل الدراسة يمكن استخدامها فيما بعد لتتبع تلك السلالات.