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Mutagenic effect of acridine orange on the production of EPS in *Bacillus sp*., its applications as anticoagulant and genetic diversity by ISSR and SCoT technique

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ABSTRACT

Many important substances are produced by some organisms, such as extracellular polysaccharides (EPS). The genus *Bacillus* is an important one in this field. As a result, in this study, a defined strain of *Bacillus sp*. BAB3450 (Gen Bank) was used to produce EPS with the goal of increasing productivity by introducing acridine orange mutations (AO). The results were obtained from three mutations that were more productive than the wild type. On the other hand, using primers Inter-Simple Sequence Repeat (ISSR) and Start Codon Targeted Polymorphism (SCoT), a comparison with the three mutants and wild type were made at the molecular level. Eleven ISSR primers were used. The results showed that ISSR-19, with 92.86% polymorphism, was the best primer. Using ten SCoT primers, the results showed that SCoT-2 was the best primer, with 90% polymorphism. For the ISSR primer and SCoT primers, the wild type and three mutants were divided into two major groups based on marker analysis with distance 60 to 75% and 55 to 65%, respectively. Cluster analyses were used to create a dendrogram of *Bacillus* strains and mutants, revealing high genetic variations between the wild type and mutants. Finally, extracellular polysaccharides (EPS) have a wide range of industrial applications in biomedicine, such as anticoagulant. The EPS from wild type and three mutants were tested for anticoagulant comparing with heparin. Mutant No.1 gave the best anticoagulant (15>%), while the wild type, mutant No.2, mutant No.3 and mutant No.4 gave 10>% comparing with heparin (80>%).

Key wards: Genetic Diversity, anticoagulant, Bacillus, acridine orange, ISSR and SCoT.

INTRODUCTION

Many gram-negative and gram-positive bacteria produce extracellular polysaccharides (Wingender *et al.*, 1999; Marvasi *et al*., 2010). They bind to the cell surface before being released into solution. Microbial EPS may represent a novel source of functional biopolymers for food, industrial and medical applications. Several approaches have been used to improve EPS from Bactria by using mutagenesis (Nassef *et al.*, 2002; Parekh *et al*., 2000). In this study, a relatively easy method was described in order to induce genetic changes in bacteria using acridine orange (AO). Acridine orange is an aromatic compound that acts as intercalate or DNA base pairs, promoting nucleotide base insertions and deletions during replication. The resulting mutation changes the translated reading frame of the information encoded in the mRNA transcript, resulting in a different amino acid sequence at the point of insertion and/or deletion (Carlton and Brown, 1981; Kapuscinski and Darzynkiewicz, 1984). In molecular biology, molecular markers are primarily used to identify a specific DNA sequence. It is defined as a potentially

detectable difference. A genetic marker can be long or short DNA sequence (Fraser *et al*., 2003). SCoT is easy and gene-specific DNA marker (Zhang *et al*., 2016). The conserved region surrounding the translation initiation codon ATG was used to generate primers for the SCoT marker (Petrovicova *et al*., 2017). ISSRs are microsatellite-lined genomic sites. Using a single primer PCR amplification of these regions produces a variety of amplification products that could be function as a dominant multi locus marker to understand genetic variation in various organisms. Markers (ISSR) are simple to use, inexpensive and have minimum demand When compared to other dominant markers especially when knowing the genetic information of the organisms under study (Ng and Tan, 2015). Some EPS have anticoagulant properties by inhibiting thrombin, initiating against thrombin III, or lengthening the coagulation time. Furthermore, these molecules may have antithrombotic properties effect by interfering with thrombin movement via the heparin cofactor II (Li *et al*., 2012; De Jesus *et al*. 2015).

The present study was carried out in order to improve the production of EPS in *Bacillus sp.* BAB3450 by using chemical mutation (AO) and to investigate the genetic similarity between *Bacillus sp.* and their mutations using different marker systems. An empirical study was carried out to investigate the use of EPS as an anticoagulant.

MATERIALS AND METHODS

strain:

The strain *Bacillus sp*. BAB3450 (GenBank) at Microbial Genetics Department, National Research Centre.

Acridine orange mutation induction:

The acridine orange used in this study was supplied by Sigma Chemical Company in the United States. Acridine orange stock solution was prepared. AO concentrations ranging from 10 to 100 µg/ml were given out in test tubes including 2 ml of media broth, with one tube as a control. After that the tubes were inoculated with 0.5 ml of overnight grown culture, the samples were then incubated at 37°C for 18 hours before being centrifuged at 3000 g. Water washes were used repeatedly to remove the AO. The treated *Bacillus sp.* cells were grown on nutrient agar in serial dilutions and by comparing the number of colonies with the size of the control sample multiplied by the reciprocal of the dilution, the percentage of vitality in the total number of bacteria was calculated (Arshad *et al*., 2006).

Production Medium:

The (MI) medium containing; glycerol 2.0%, $(NH_4)2HPO_4$ 0.5%, $K2HPO_4$ 0.1%, Na_2SO_4 0.05%, yeast extract 0.2% and agar 3.0% was autoclaved (Linker and Jones, 1966).

Luria broth Medium (LB): was prepared after Davis *et al.* (1980)

Minimal medium: was prepared after Faires *et al.* (1999)

Anticoagulant activity:

Venous blood was prepared according to Jurd *et al.* (1995). The anticoagulant activities of the polysaccharides were determined (USA-Pharmacopoeia, 1985) and compared to heparin as a control.

Bacterial genetic diversity:

DNA Extraction:

The total genomic DNA of *Bacillus sp*. and mutants were obtained by D Neasy Mini Kit (QIAGEN).

Amplification Technique Using Inter Simple Sequence Repeat (ISSR):

ISSR-PCR reactions were carried out with the help of eleven anchored primers synthesised by Eurofins in Germany. Table (1) shows the primer names and sequences. The reaction conditions were determined and the reagents listed below were mixed in a final volume of 25µl: 1 X of green *GoTaq* buffer; 1.5mM of $MgCl_2$; 200 µM of dNTPs; 25pucM of primer; 1 U of *GoTaq*; 25ng of DNA and up to 25 µl distilled water. The following programmer was used to amplify the DNA: 94°C/5min (1 cycle); [94°C/45 sec, 45°C/50 sec, 72°C/1.5 min] (40 cycles); 72°C/7 min (1 cycle) and 4°C (infinitive). The ISSR-PCR product was resolved in a volume of 7µl using 1.3 percent agarose gel electrophoresis with ethidium bromide. As a DNA molecular weight standard, marker was used. The results were photographed using a Molecular Imager® Gel DocTM System with Image LabTM Software from Bio-Rad and visualised on a UV transilluminator.

Table (1). List of eleven primers with different names and nucleotide sequences that were used in ISSR analysis.

Primer	Sequence 5'-3'
ISSR- 1	5'-AGAGAGAGAGAGAGAGY[1]C-3'
ISSR- 2	5'-AGAGAGAGAGAGAGAGY[1]G-3'
ISSR- 3	5'-ACACACACACACACACY[1]T-3'
ISSR- 4	5'-ACACACACACACACACY[1]G-3'
ISSR- 11	5'-ACACACACACACACACY[1]A-3'
ISSR- 12	5'-ACACACACACACACACY[1]C-3'
ISSR- 13	5'-AGAGAGAGAGAGAGAGY[1]T-3'
ISSR- 14	5'-CTCCTCCTCCTCCTCTT-3'
ISSR- 16	5'-TCTCTCTCTCTCTCTCA-3'
ISSR- 18	5'-H[2]V[3]H[2]CACACACACACACAT-3'
ISSR- 19	5'-H[2]V[3]H[2]TCCTCCTCCTCCTCC-3'

[1] Y----C/T [2] H---A or C or T [3] V--- A or G

Start Codon Targeted (SCoT) Technique:

This study made use of ten SCoT primers, which are listed in Table (2). The reaction in a final volume of 25 µl containing 2 µL of DNA template; 2.5 µL primer; 0.5 µL dNTPs; 0.15 µL Taq DNA polymerase; 5 µL 5X PCR buffer; and 14.75 µL distilled water. The amplification was as follows: initial denaturation was carried out at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1.5 min, and end extension at 72°C for 7 min. The PCR products were separated in 1.3% agarose gels and photographed by Molecular Imager® Gel Doc™ System with Image Lab™ Software, Bio-Rad.

Table (2). List of ten primers and their nucleotide sequences which used in SCoT analysis.

Name	Sequence 5'-3'
SCoT -1	5'-CAACAATGGCTACCACCA-3'
SCoT -2	5'-CAACAATGGCTACCACCC-3'
SCoT -3	5'-CAACAATGGCTACCACCG-3'
SCoT -4	5'-CAACAATGGCTACCACCT-3'
SCoT -20	5'-ACCATGGCTACCACCGCG-3'
SCoT -22	5'-AACCATGGCTACCACCAC-3'
SCoT -28	5'-CCATGGCTACCACCGCCA-3'
SCoT -35	5'-CATGGCTACCACCGGCCC-3'
SCoT -33	5'-CCATGGCTACCACCGCAG-3'
SCoT -36	5'-GCAACAATGGCTACCACC-3'

Data Analysis

For estimating the similarity among all tested samples, the presence (1) or absence (0) of SCOT and ISSR bands was determined by hand. Clustering using the Unweighted Pair-Group Method and the SPSS Gene Tools-gel analysis software (ver. 18) produced the matrix of similarity (Jaccard).

RESULTS AND DISCUSSION

Effect of AO on Bactria:

Bacterial growth was gradually inhibited by gradually increasing the

concentration of AO (Fig. 1). The results in Figure (1) showed that a little growth was observed at AO concentrations of 80 and 90 µg/ml and no growth was observed at concentrations greater than 100 µg/ml. *Bacillus sp*. survival % mean for the concentrations (AO) were found to be 100%, 71.66%, 69.81%, 59.75%, 39.18%, 26.30%, 9.17%, 2.82%, 2.00%, 1.65% and 0.0%, respectively. There was a negative relationship between AO concentrations and survival percentage mean. The data shown in Figure (1) revealed that AO had a severe effect on bacterial survival at all concentrations, with complete lethality at the highest concentration100 µg/ml. On complete and minimal media (Table 3), mutants from all concentrations were selected and tested for polysaccharide production. These outcomes agreed with the results of Francis (2011) on *Xanthomonas campestris* and Arshad *et al*. (2006) on *Escherichia coli*.

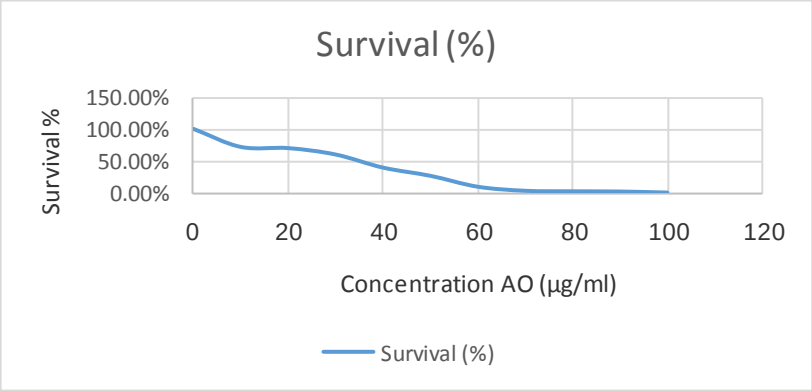

Fig. 1: *Bacillus sp*. cell survived at different AO concentrations.

Table (3) shows mutants of the wild type of strain *Bacillus sp.* (AO) that were examined for enhanced polysaccharide production; three mutants (No.2, No.3 and No.4) outperformed the parental strain and four mutants (No.5, No.6, No.7 and N.o8) lost than the wild type. Random mutagenesis was used for polysaccharide production by exposing the genus *Bacillus* to chemicals similes EMS (Haq *et al.*, 2012; Nadeem *et al*., 2010). This rise may be occurred as a result of an increase in the number of gene transcribes and DNA region amplification (Cherry, *et al*.; 2009) on *Aspergillus niger* and EL-Shaer, (2011) on *Arthrobacter*.

Table (3): Polysaccharide production of *Bacillus sp.* and their mutants.

No	Mutant code	AO (µg/ml)	Polysaccharide (g/L)
1	Wild type	0.0	5.4
2	M AO 1	50	5.9
3	M AO 2	60	6.6
4	M AO 3	70	6.1
5	M AO 4	70	4.1
6	M AO 5	70	4.5
7	M AO 6	70	4.5
8	M AO 7	70	4.3

Genetic diversity:

The development of molecular techniques and the use of genetic markers in recent decades have resulted in the development of tools, inexpensive and reproducible methods for identification of microorganisms. Molecular methods based on PCR such as ISSR and SCoT had played an important role in the advancement of biological sciences. ISSR and SCoT genotype clustering patterns were similar. Three mutants (No.2, No.3 and No.4) were discovered after screening eleven ISSR and ten SCoT primers against *Bacillus sp*. DNA were used to identify potential primers that resulted in greater number of polymorphic and repeatable fragments (Jalalizand *et al*., 2012).

ISSR-PCR identifier:

ISSR-PCR is a technique that generates multilocus markers by using microsatellite as primers in a polymerase chain reaction. ISSR markers are very polymorphic and useful in biological genetic research (Reddy *et al*., 2002). Table (4) and Figure (2) summarize the properties of fragment primers. The amplification results of *Bacillus sp.* and three mutants (No.2, No.3 and No.4) produced 116 band using eleven ISSR primers (ISSR-1, ISSR-2, ISSR-3, ISSR-4, ISSR-11, ISSR-12, ISSR-13, ISSR-14, ISSR-16, ISSR-18 and ISSR-19). ISSR-1 primer revealed 13 bands, with 3 monomorphic, 8 polymorphic and 2 unique band percentages of polymorphism (76.92%), whereas ISSR-2 primer revealed 8 bands, with 2 monomorphic, 3 polymorphic and 3 unique band with percentages of polymorphism (75.00%). ISSR-3 primer revealed 13 bands divided into 4 monomorphic, 7 polymorphic and 2 unique band with percentage of polymorphism (69.23%), while ISSR-4 primer revealed 9 bands divided into 1 monomorphic, 5 polymorphic and 3 unique bands with percentage of polymorphism (88.89%). ISSR-11 primer revealed 14 bands, 3 monomorphic, 8 polymorphic and 3 unique band, percentage of polymorphism (78.57%), while ISSR-12 primer revealed 10 bands, 6 monomorphic, 4 polymorphic and zero unique band, percentage of polymorphism (40%). ISSR-13 primer revealed 10 bands, 3 monomorphic, 7 polymorphic and zero unique band, percentage of polymorphism (70%). The ISSR-14 primer revealed six bands, five of which were monomorphic, one polymorphic, one unique with percentage of polymorphism (16.67%), while ISSR-16 primer revealed ten bands, two of which were monomorphic, one polymorphic and seven of which were unique and percentage of polymorphism (80%). Finally ISSR-18 primer revealed 9 bands, one monomorphic, seven polymorphic and one unique band, percentage of polymorphism (88.89%), whereas ISSR-19 primer revealed 14 bands, one monomorphic, seven polymorphic and six unique band with high percentage of polymorphism (92.86%). The current findings show that there was a lot of genetic variation between the wild type and its mutants, which is consistent with previous research; Hatti *et al*. (2010) on *Aspergillus flavus*, Mukadam (2010) and EL-Shaer *et al*. (2021) on *Aspergillus spp*., EL-Shaer *et al*., (2014) on Agrobacterium, Field and Wills (1998) on *Saccharomyces cerevisiae,* El Khodary *et al*. (2021) on genus *Albizia*.

Table (4). Total number of ISSR fragments, number of monomorphic, unique band, number of polymorphic and polymorphism %.

Name of Primers	Primer sequence 5'-3'	Total No. of Bands	Mono-morphic Bands	Unique Band	Poly-morphic bands	Polymorphism (P) %
ISSR- 1	5'-AGAGAGAGAGAGAGAGYC-3'	13	3	2	8	76.92
ISSR- 2	5'-AGAGAGAGAGAGAGAGYG-3'	8	2	3	3	75.00
ISSR- 3	5'-ACACACACACACACACYT-3'	13	4	2	7	69.23
ISSR- 4	5'-ACACACACACACACACYG-3'	9	1	3	5	88.89
ISSR- 11	5'-ACACACACACACACACYA-3'	14	3	3	8	78.57
ISSR- 12	5'-ACACACACACACACACYC-3'	10	6	0	4	40.00
ISSR- 13	5'-AGAGAGAGAGAGAGAGYT-3'	10	3	0	7	70.00
ISSR- 14	5'-CTCCTCCTCCTCCTCTT-3'	6	5	0	1	16.67
ISSR- 16	5'-TCTCTCTCTCTCTCTCA-3'	10	2	7	1	80.00
ISSR- 18	5'HVHCACACACACACACAT-3'	9	1	1	7	88.89
ISSR- 19	5'HVHTCCTCCTCCTCCTCC-3'	14	1	6	7	92.86
Total		**116**	**31**	**27**	**58**	**70.64**

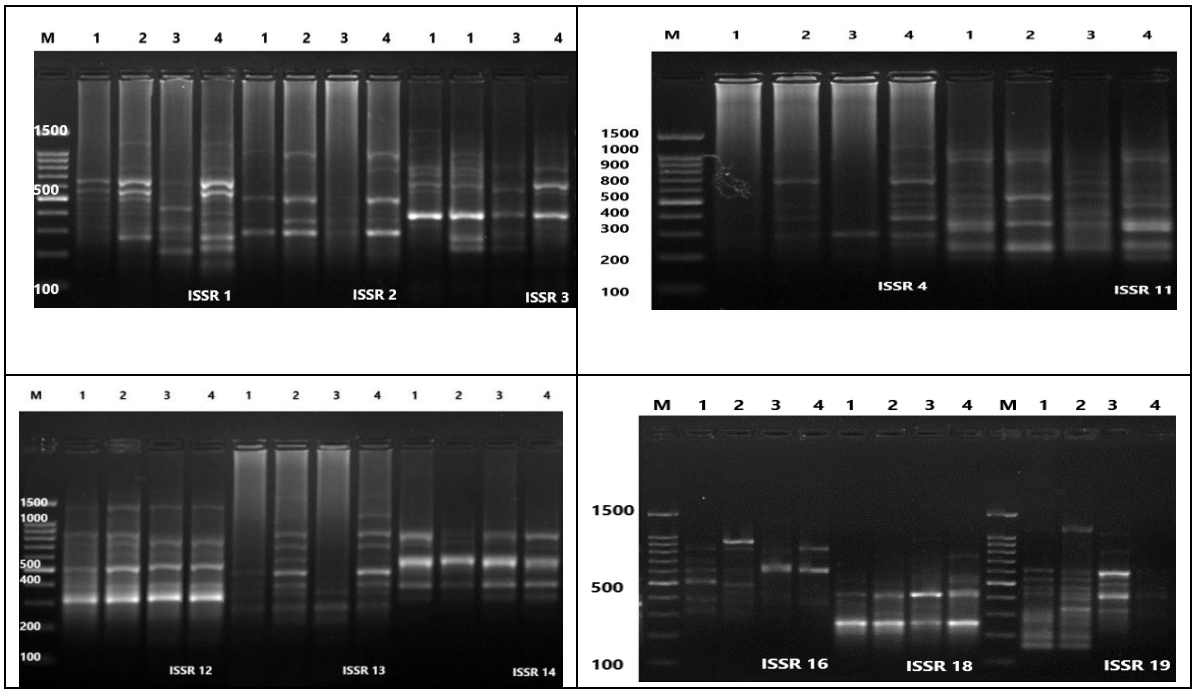

Fig. (2): ISSR-1, 2, 3, 4, 11, 12, 13, 14, 16, 18 and 19 PCR molecular analysis of *Bacillus sp.* and three mutants.

Cluster analysis and similarity between *Bacillus sp.* and three mutants based on ISSR markers:

Similarity index based on ISSR-PCR analysis was demonstrated using UPGMA computer analysis (Table 5). The highest similarity index recorded was 77% between mutant No. 2 (M AO 1) and mutant No. 4 (M AO 3), while the lowest similarity index recorded was 58%between mutant No. 3 (M AO 2) and mutant No. 4 (M AO 3). A dendrogram was used to represent the

genetic relationships between the mutants and the wild type, as shown in Figure (3). The *Bacillus sp.* and three mutants (No.2, No.3 and No.4) were divided into two clusters, with cluster one containing only mutant No. 3. The second cluster is divided into two groups, one of which includes the wild type and the other include mutants No. 2 and mutant No. 4. El-Kawokgy *et al*. (2018) mentioned that ISSR and SCoT profiles generated for *Bacillus Thuringiensis* and *Bacillus Sphaericus* can be used as a taxonomic identification and diversity marker. The present results were in-agreement with those observed by Attallah *et al*. (2014) on *Beauvaria* sp., Kumar and Agrawal (2019) and Mohamed *et al*. (2015).

Table (5). Similarity among *Bacillus sp.* and three mutants ISSR-PCR analysis.

	1 (Wild type)	2 (M AO 1)	3 (M AO 2)	4 (M AO 3)
1 (Wild type)	100			
2 (M AO 1)	66	100		
3 (M AO 2)	61	59	100	
4 (M AO 3)	68	77	58	100

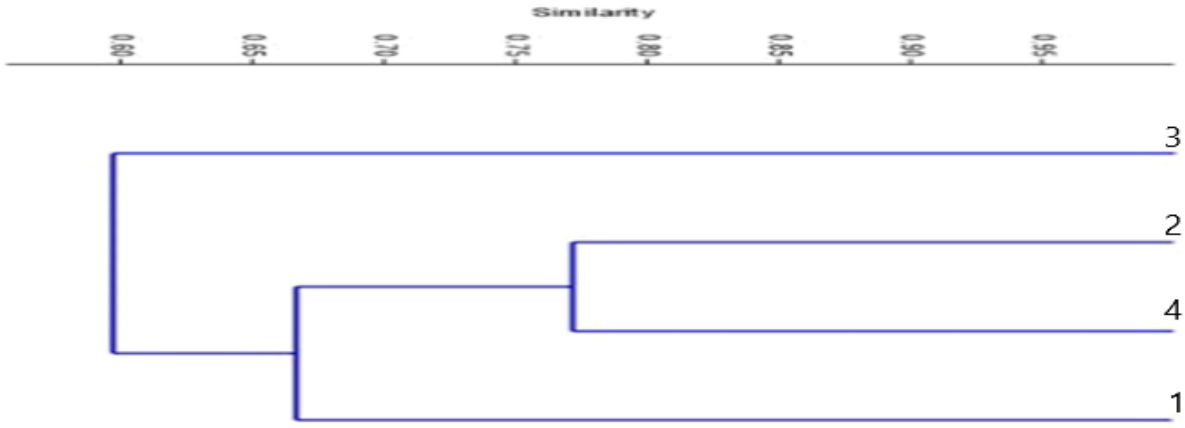

Fig. 3: UPGMA dendrogram tree indicating the genetic relationships among *Bacillus sp.* and three mutants based on ISSR markers.

SCoT identifier:

The average recorded percentage of phenotypic polymorphism was 73.72 %, ranging from 22.22 % to 90.00 %. In addition, the average of monomorphic band was 34 and the polymorphic band was 70, while that of the unique band was 44 (Table 6 & Fig. 4). The results of the *Bacillus sp.* and the three mutants (No.2, No.3 and No.4) obtained by ten SCoT primers (SCoT-1, SCoT-2, SCoT-3, SCoT-4, SCoT-20, SCoT-22, SCoT-28, SCoT-33, SCoT-35 and SCoT-36) produced 184 bands. SCoT-1primer revealed 16 bands that divided into

2 monomorphic, 8 polymorphic and 6 unique bands, percentage of polymorphism (87.50%), while SCoT-2 primer revealed 20 bands that divided into 2 monomorphic, 8 polymorphic and 10 unique bands with high percentage of polymorphism (90.00%). SCoT-3 primer revealed 18 bands divided into 2 monomorphic, 10 polymorphic and 6 unique bands, percentage of polymorphism (88.88%), while SCoT-4 primer revealed 14 bands that divided into 3 monomorphic, 6 polymorphic and 5 unique bands, percentage of polymorphism (78.57%). SCoT-20 primer revealed 17 bands that divided into 2 monomorphic, 13 polymorphic and 2 unique band, percentage of polymorphism (88.23%), while SCoT-22 primer revealed 17 bands that divided into 5 monomorphic, 8 polymorphic and 4 unique bands, percentage of polymorphism (70.58%).

SCoT-28 primer revealed 11 bands that divided into 4 monomorphic, 3 polymorphic and 4 unique bands, percentage of polymorphism (63.63%). SCoT-33 primer revealed 14 bands divided into 5 monomorphic, 4 polymorphic and 5 unique bands, percentage of polymorphism (64.28%), while SCoT-35 primer revealed 12 bands that divided into 2 monomorphic, 8 polymorphic and 2 unique bands, percentage of polymorphism (83.33%). Finally SCoT-36, primer revealed 9 bands divided into 7 monomorphic, 2 polymorphic and zero unique band percentage of polymorphism (22.22%). Luo *et al*. (2010) studied the differences in mangoes using the SCoT. The present findings revealed that there is a great deal of genetic variations between the wild type and its mutants. This is consistent with the findings of previous studies of EL-Shaer *et al*. (2014) on *Agrobacterium*, Kumar and grawal (2019), Field and Wills (1998) on *Saccharomyces cerevisiae*, El Khodary *et al.* (2021) on genus *Albizia* and EL-Shaer *et al*. (2021**)** on *Aspergillus* spp.

Table (6). Total number of SCoT fragments, number of monomorphic, unique band, number of polymorphic and polymorphism%.

Name of Primers	Primer sequence 5'-3'	Total No. of Bands	Mono-morphic Bands	Unique Bands	Poly-morphic bands	Polymorphism (P) %
SCoT-1	5'-CAACAATGGCTACCACCA-3'	16	2	6	8	87.50
SCoT-2	5'-CAACAATGGCTACCACCC-3'	20	2	10	8	90.00
SCoT-3	5'-CAACAATGGCTACCACCG-3'	18	2	6	10	88.88
SCoT-4	5'-CAACAATGGCTACCACCT-3'	14	3	5	6	78.57
SCoT-20	5'-ACCATGGCTACCACCGCG-3'	17	2	2	13	88.23
SCoT-22	5'-AACCATGGCTACCACCAC-3'	17	5	4	8	70.58
SCoT-28	5'-CCATGGCTACCACCGCCA-3'	11	4	4	3	63.63
SCoT-33	5'-CCATGGCTACCACCGCAG-3'	14	5	5	4	64.28
SCoT-35	5'-CATGGCTACCACCGGCCC-3'	12	2	2	8	83.33
SCoT-36	5'-GCAACAATGGCTACCACC-3'	9	7	0	2	22.22
Total\ Average		**148**	**34**	**44**	**70**	**73.72**

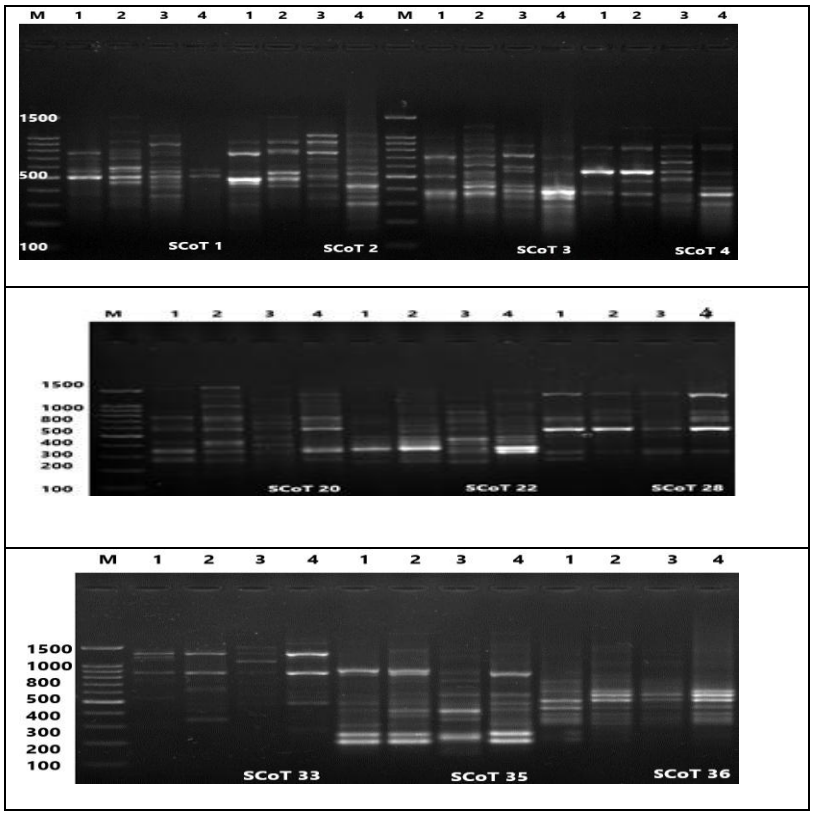

Fig. (4). SCoT-1, 2, 3, 4, 20, 22, 28, 33, 35 and 36 PCR molecular analysis of *Bacillus sp.* and the three mutants.

Cluster analysis and similarity between *Bacillus sp.* and three mutants based on SCoT markers:

The results showed that the cluster analysis divided two 2 groups at the distance (0.55). The first cluster include mutant No.3 (M AO 2) only. The second group, which in turn was divided into two subgroups at the distance (0.65). The first sub-group include mutant No.4 (M AO 3). The second group also includes two mutants No.2 (M AO 1) and 1 (Wild type) (Fig. 5). The highest similarity percentage was 67 % between mutant No.2 and mutant No. 4. While, the lowest similarity rate was 56% between wild type and mutant No.2 (Table 7). El-Kawokgy *et al*. (2018) indicated that the ISSR and SCoT profiles generated for *Bacillus thuringiensis* and *Bacillus sphaericus* can be used as taxonomic and diversity markers.

Table (7). Similarity among *Bacillus sp.* and three mutants SCoT-PCR analysis.

	1 (Wild type)	2 (M AO 1)	3 (M AO 2)	4 (M AO 3)
1 (Wild type)	100			
2 (M AO 1)	0.67	100		
3 (M AO 2)	0.56	0.58	100	
4 (M AO 3)	0.66	0.67	0.60	100.00

Fig. (5). UPGMA dendrogram tree indicating the genetic relationships among *Bacillus sp.* and three mutants based on SCoT markers.

Anticoagulant activity of (EPS) of *Bacillus sp.* and the three mutants:

The *in vitro* anticoagulant activities of (EPS) from *Bacillus sp.* and the three mutants (2 mg) were compared to heparin (2 mg) at 15 and 45 min. Four samples, wild type, mutant No.2 (M AO 1), No.3 (M AO 2), and No.4 (M AO 3) were used in comparison to heparin after 15 and 45 min. The results are shown in Figure (6). Exopolysaccharide from wild type and mutant No.2 (M AO 1) demonstrated the greatest activity *in vitro* anticoagulant (15>%) comparing to the other mutants (10>%), while heparin gave the highest value (80>%) at both times. The findings were consistent with those proposed by EL-Shaer, (2011) on *Arthrobacter*, Abdelhamid *et al*., (2020) and Zhang and Yi (2022).

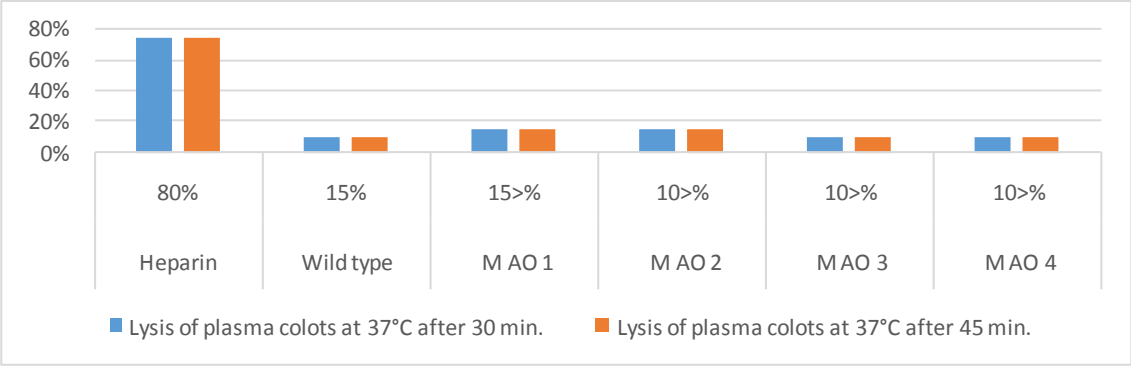

Fig. (6): *In vitro* anticoagulant activity of (EPS) of *Bacillus sp.* and the three mutants comparing with heparin.

CONCLUSION

Biology can play an important role in polysaccharide development. One promising avenue to increase EPS production is the induction of mutations that occur during gene expression. The resulting ISSR and SCoT profile can be effectively used as a genetic diversity among *Bacillus sp.* and mutations. More research is needed for fully understand the biosynthesis and to know the different pharmaceutical applications of polysaccharides.

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التأثير المطفر للأكريدين البرتقال على إنتاج السكريات في *Bacillus sp* وتطبيقاتة كمضاد للتجلط والتنوع الجيني بواسطة ISSR و SCoT

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المستخلص

يتم إنتاج العديد من المواد الهامة من قبل بعض الكائنات الحية ، مثل السكريات خارج الخلية (EPS). جنس *Bacillus* مهم في هذا المجال. نتيجة لذلك ، في هذه الدراسة ، سلالة معرفة من *Bacillus sp* BAB3450 في بنك الجينات استخدمت لإنتاج EPS بهدف زيادة الإنتاجية عن طريق إحداث الطفرات بواسطة الأكريدين البرتقالى. تم الحصول من النتائج على ثلاث طفرات كانت أكثر إنتاجية من النوع البري. من ناحية أخرى ، بإستخدام بادئات ISSR و SCoT ، تم إجراء مقارنة بين الطفرات الثلاثة والنوع البري على المستوى الجزيئي. تم استخدام أحد عشر بادئة ISSR. أظهرت النتائج أن ISSR-19 بنسبة اختلاف 92.86٪ كان الأفضل. بإستخدام عشرة بادئات SCoT ، أوضحت النتائج أن SCoT-2 كان الأفضل ، مع 90٪ نسبة إختلاف. تم عمل شجرة القرابة بإستخدام نتائج ISSR و SCoT ، تم تقسيم النوع البري و الثلاثة طفرات إلى مجموعتين رئيسيتين بناءً على تحليل العلامات بمسافة 60 إلى 75٪ و 55 إلى 65٪ على التوالي. تم استخدام التحليلات العنقودية لإنشاء مخطط شجري لسلالات *Bacillus* والطفرات ، وكشف عن تباين جيني مرتفع بين النوع البري والطفرات. أخيرًا ، السكريات خارج الخلية (EPS) لديها مجموعة واسعة من التطبيقات الصناعية في الطب الحيوي ، مثل مضادات الت جلط. اختبرنا EPS من النوع البري و الثلاثة طفرات لمضادات للتجلط مقارنة مع الهيبارين. أعطت الطفرة رقم 1 أفضل مضاد للتجلط بنسبة 15٪ بينما أعطى النوع البري والطفرة رقم 2 والطفرة رقم 3 والطفرة رقم 4 (10٪) مقارنة مع الهيبارين 80٪.