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Molecular Detection and Occurrence of Lipopeptide Biosurfactant Genes in Different *Bacillus* Related Species

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Abstract

Bacillus-related species is one of the most studied and identified bacteria with a higher ability to produce metabolite products. Among various metabolites produced by many strains of *Bacillus*-related species are lipopeptides. The current study aimed to molecularly screen *Bacillus*-related bacteria isolated from various soils in Basrah Province for the presence of surfactin, iturin C, bacillomycin, and fengycins genes using a molecular tool based on PCR technique. Forty-four isolates belong the genera *Bacillus*, *Cytobacillus*, *Priestia*, and *Peribacillus* were reported in the collected soils from various locations of the province of Basrah. The *sfp* genes were detected in 43.18% of *Bacillus*-related isolates, and the most frequently detected in *B. subtilis*. *itu*C genes were detected in 47.72% of *Bacillus*-related isolates, and the most frequently detected in *B. licheniformis*. The *bam* genes were detected in 20.45% of *Bacillus*-related isolates and the most frequently detected in *B. licheniformis*. *fen*D genes were detected in 29.54% of *Bacillus*-related isolates and the most frequently detected in *B. licheniformis*. *itu*C and *sfp* genes were the most frequently detected 47.72% and 43.18% respectively, followed by *fen*D 29.54%, whereas *bam*C 20.45% was the less frequent gene. One isolate had all the biosynthetic lipopeptides genes, 7 isolates had 3 genes, 12 isolates had 2 genes and 13 isolates had 1 gene simultaneously, while 11 of the isolates did not report the presence of any biosynthetic lipopeptides genes. *B. licheniformis* was the most frequently isolated reported presence of biosynthetic lipopeptides genes followed by *B. subtilis.*

Keywords: *Bacillus* isolates, *sfp* genes, lipopeptide biosurfactants, *itu*C genes, *bam*C genes, *fen*D genes

Резюме

Видовете, свързани с род *Bacillus* (*Bacillus*-related species, BRS), са едни от най-изучаваните и идентифицирани бактерии с по-висока способност да произвеждат метаболити. Сред различните метаболити, произвеждани от много щамове на видове, свързани с род *Bacillus* са липопептидите. Настоящото проучване има за цел да скринира щамове BRS, изолирани от различни почви в провинция Басра за наличие на гени за синтезата на сурфактин, итурин С, бациломицин и фенгицин, използвайки молекулярно-биологични подходи (PCR техника). Четиридесет и четири изолата, принадлежащи към родовете *Bacillus, Cytobacillus, Priestia* и *Peribacillus*, бяха докладвани в събраните почви от различни места в провинция Басра. Гените sfp са открити в 43.18% от изолатите, свързани с *Bacillus*, като най-често са открити в *B. subtilis*. Гените ituC са открити в 47.72% от изолатите, свързани с *Bacillus*, като най-често са открити в *B. licheniformis*. Гените bam са открити в 20.45% от изолатите, свързани с *Bacillus*. Те се откриват най-често в *B. licheniformis*. Гените fenD са открити в 29.54% от изолатите, свързани с *Bacillus* и най-често се откриват в *B. licheniformis*. Гените ituC и sfp са най-често откриваните - съответно 47.72% и 43.18%, следвани от fenD с 29.54%, докато bamC с 20.45% е по-рядко срещаният ген. Един изолат е имал всички гени за биосинтетични липопептиди, 7 изолата са имали 3 гена, 12 изолата са имали 2 гена и 13 изолата са имали 1 ген едновременно, докато 11 от изолатите не показват наличие на никакви гени за биосинтетични липопептиди. *B. licheniformis* е най-често изолираният вид, при който се установява наличие на гени за биосинтетични липопептиди, следван от *B. subtilis*.

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Introduction

Bacillus-related species are the most studied and identified bacteria of Gram-positive bacteria due to their capacity to produce endospores and secrete a large number of metabolites products with various potent commercial applications, which can be used in different industrial fields (Mora *et al.,* 2015). *Bacillus*-related species could be grown in the laboratory with an easy procedure using conventional culture media with a higher ability to produce metabolite products. Among various metabolites produced by many strains of *Bacillus*-related species are biosurfactants (Stincone *et al.,* 2020). Biosurfactants are becoming an important biotechnology product and can be used in various applications, which include bioremediation of organic pollutants in the environment, the medical and pharmaceutical industries, enhanced oil recovery, food processing industries, agriculture, and cosmetics (Elazzazy *et al.,* 2015; Alyousif *et al.,* 2022).

The biosurfactants produced by many strains of *Bacillus*-related species are lipopeptides (LPs) that fall into three main families: surfactins, iturins (including e.g., iturin, mycosubtilin, bacillomycin), and fengycins (plipastatins) (Stincone *et al.,* 2020). Non-ribosomal peptide synthetases (NRPSs) catalyze a complex enzymatic mechanism of biosynthesis that results in the secretion of lipopeptide biosurfactants at the end of the exponential growth phase (Fira *et al.,* 2018).

The bacterial isolates of *Bacillus* closely related species which carry genes for iturin A, surfactin, and subtilisin A lipopeptide biosurfactants showed antimicrobial activity against pathogenic fungi, pathogenic bacteria, and bacteria that cause food spoilage in industry, as *Listeria monocytogenes* and *Bacillus cereus* (Perez *et al.,* 2017). LPs are chemically defined as cyclic oligopeptides with D-amino acid residues connected to β-hydroxy fatty acids as a mixture of homologs and isoforms that differ in the fatty acid chain's length and the amino acid sequence's composition. The fatty acid chain can be branched or linear, with variations in its length (usually C6–C18) and degree of oxidation (Płaza *et al.,* 2015).

It has been shown that some *Bacillus*-related bacterial species to produce different LPs, such as *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens, Bacillus velezensis, Bacillus amyloliquefaciens,* and *Paenibacillus polymyxa* (Cochrane and Vederas, 2014; Stincone *et al.,* 2020). In *Bacillus*-related bacteria, a number of activities, including sporulation, the production of extracellular degradative enzymes, and the synthesis of lipopeptides are activated in response to nutritional stress (Mandic-Mulec *et al.,* 2016; Perez *et al.,* 2017).

The study of molecular genetics and mechanisms of biosurfactant gene regulation provides important information about the biosynthesis of various bacterial biosurfactants of industrial, medical, and environmental importance (Płaza *et al.,* 2015). The molecular characterization of *Bacillus*-related bacteria was performed as a practical technique for identifying isolates that produce lipopeptides in various environments. The PCR methodology has proven to be a rapid means of identifying and determining bacterial isolates that have the potential to produce biosurfactants (Srinivasan *et al.,* 2015; Foysal and Lisa, 2018). The PCR technique is used to characterize genes involved in the synthesis or regulation of biosurfactant biosynthesis, such as srfA3, *sfp, rhlA*, and *rhl*B (Mulligan *et al.,* 2014; Płaza *et al.,* 2015).

Therefore, the PCR technique is an optimal tool for the detection of enzymes encoding genes involved in biosurfactant syntheses such as surfactin, iturin, and fengycin. This approach enhances the screening and the characterization of environmental *Bacillus*-related species which are co-producing biosurfactants (Płaza *et al.,* 2015). The aim of the current work was performed with a collection of soil samples from Basrah Province to isolate and identify *Bacillus*-related bacteria by sequencing and the molecular screening of isolates using of molecular tool based on the PCR approach for determining the presence of lipopeptide biosurfactants including surfactin and iturin C, bacillomycin and fengycins among *Bacillus*-related bacteria that were isolated from several Basrah Province soils.

Materials and Methods

Bacterial isolation

The bacteria utilized in the current study were isolated in a previous study (Alyousif, 2022), where ten soil samples were collected from different locations throughout Basrah province between August 2020 and October 2020. Four soil grams of each sample were mixed with 96 ml of sterile distilled water, and the mixture was agitated for two minutes in a 250 ml Erlenmeyer flask. The samples were heated to 60°C for 60 minutes in a water bath (Chilcott and Wigley, 1993). Sterile distilled water was used to dilute the suspensions. Each sample was spread on a nutrient agar, the samples were incubated for 24 hours at 35°C. To obtain pure culture, the colonies with different morphological features

were separated and sub-cultured onto fresh nutrient agar.

Identification and characterization of Bacillus-related bacteria

The chromosomal DNA of bacterial species was isolated and the isolates were identified in a previous study (Alyousif, 2022). The universal primers that were used in the study to identify the isolates were adopted from (Miyoshi *et al.,* 2005) by amplifying the 16S rDNA gene. In order to determine the 16S rDNA gene sequence of the bacterial isolates, the PCR products of the 16S rDNA gene were sent to Macrogen Company (South Korea). The sequences were processed using the mega-X program and matched with nucleotide sequence databases of the NCBI using BLAST tools.

Surfactin gene detection

The surfctin (*sfp*) gene was amplified from the isolated DNA of *Bacillus*-related bacteria using primers adopted from (Hsieh *et al.,* 2004) as shown in Table (1). The PCR assays of *sfp* gene were made in a volume of 25 µl, the mixture was vortexed. The PCR condition for amplifying the *sfp* gene was an initial denaturation of 95°C for 5 min, followed by 35 cycles involving 95°C for 1 min, primer annealing at 52°C for 30 s, extension temperature at 72°C for 1 min, and final extension at 72°C for 5 min. The PCR products of *sfp* gene are about 675 bp size was detected by 1% subjected to agarose gel electrophoresis analysis.

Iturin C gene detection

The Iturin C (*ituC*) gene was amplified from the isolated DNA of Bacillus-related bacteria using primers adopted from (Chung *et al.,* 2008) as shown in Table 1.

The PCR assays of the *itu*C gene were made in a volume of 25 µl, the mixture was vortexed. The PCR condition for amplifying the *itu*C gene was an initial denaturation of 95°C for 5 min, followed by 35 cycles involving 95°C for 1 min, primer annealing at 55°C for 1 min, extension temperature at 72°C for 1 min, and final extension at 72°C for 5 min. The PCR products of the *itu*C gene are about 594 bp size and were detected by 1% subjected to agarose gel electrophoresis analysis.

Bacillomycin C gene detection

The Bacillomycin C (*bam*C) gene was amplified from the isolated DNA of *Bacillus*-related bacteria using primers adopted from (Chung *et al.,* 2008) as shown in Table 1. The PCR assays of the *bam*C gene were made in a volume of 25 µl, the mixture was vortexed. The PCR condition for amplifying the *bam*C gene was an initial denaturation of 95°C for 5 min, followed by 35 cycles involving 95°C for 1 min, primer annealing at 55°C for 1 min, extension temperature at 72°C for 1 min, and final extension at 72°C for 5 min. The PCR products of the *bam*C gene are about 957 bp size was detected by 1% subjected to agarose gel electrophoresis analysis.

Fengycin D gene detection

The fengycin D (*fen*D) gene was amplified from the isolated DNA of *Bacillus*-related bacteria using primers adopted from (Mora *et al.,* 2011) as shown in Table 1. The PCR assays of the *fen*D gene were made at a volume of 25 µl, the mixture was vortexed. The PCR condition for amplifying the *fen*D gene was an initial denaturation of 95°C for 5 min, followed by 35 cycles involving 95°C for 1 min, primer annealing at 58°C for 30 s, extension temperature at 72°C for 1 min, and final extension at 72°C for 5 min. The PCR products of the *fen*D gene are about 269 bp size was detected by 1% subjected to agarose gel electrophoresis analysis.

Results

Identification and characterization of Bacillus-related bacteria

The isolates were molecularly identified using PCR technique and 16S rDNA gene sequencing as shown in Table 2. Forty-four isolates belong the

Table 1. The primers used for screening the lipopeptide genes

Lipopeptides	Primers name	Sequences
Surfactin	sfp-f	5'-ATGAAGATTTACGGAATTTA-3'
	sfp-r	5' TTATAAAAGCTCTTCGTACG-3'
Iturin C	ItuC-f	5' -CCCCCTCGGTCAAGTGAATA-3'
	ItuC-r	5' -TTGGTTAAGCCCTGATGCTC-3'
Fengycin D	Fend-f	5'- GGCCCGTTCTCTAAATCCAT-3'
	Fend-r	5'-GTCATGCTGACGAGAGCAAA-3'
Bacillomycin C	BamC-f	5'-AGTAAATGAACGCGCCAATC-3'
	BamC-r	5'-CCCTCTCCTGCCACATAGAG-3'

Table 2. Identification and characterization of *Bacillus*-related bacteria by 16S rDNA gene sequencing

Site	Code	Species
A	A1	*B. subtilis* strain SXKF16-3
	A2	*Cytobacillus firmus* strain NFB28
	A3	*B. licheniformis* strain SIITMB5
	A4	*B. licheniformis* strain ESR26
	A5	*B. subtilis* strain B4 16S
	A6	*B. subtilis* strain TPS4
B	B1	*Priestia flexa* strain NJNPD41
	B2	*B. tequilensis* strain YJ-S4
	B3	*Priestia megaterium* strain DK2
	B4	*B. safensis* strain kp9
	B5	*B. pumilus* strain LX11
	B6	*B. licheniformis* strain PG5
	B7	*B. safensis* strain P9
C	C1	*C. oceanisediminis* strain C26
	C2	*B. licheniformis* strain QT201
	C3	*B. subtilis* strain YEBN5
	C4	*B. cereus* strain 2.3AL8
D	D1	*C. firmus* strain BF3-5
	D2	*B. cereus* strain AA6
E	E1	*B. sonorensis* strain SRCM101395
	E2	*B. licheniformis* strain WAS3-5
	E3	*B. subtilis*
F	F1	*B. licheniformis* strain KKR2017
	F2	*B. licheniformis* strain WSR-KSU302
	F3	*B. infantis* strain SIITMB9
	F4	*B. cereus* isolate HKS 2-2
	F5	*Peribacillus simplex* strain EH12
G	G1	*B. subtilis* strain ge25
	G2	*C. oceanisediminis* strain 224-LR35
	G3	*C. firmus* strain NPBR2
	G4	*B. licheniformis* strain SUM-KSU304
	G5	*B. vallismortis* strain T25-9
	G6	*B. albus* strain FA80
H	H1	*B. licheniformis* strain KKR2017
	H2	*B. subtilis* strain B29
	H3	*C. oceanisediminis* strain GA51
	H4	*B. subtilis* strain WZ-2
	H5	*Priestia megaterium* strain DK2
I	I1	*B. licheniformis* isolate S8
	I2	*B. velezensis* strain ONU 553
	I3	*B. subtilis* strain MK736112.1
J	J1	*C. firmus* strain MR-39
	J2	*B. subtilis* strain TUST018
	J3	*B. subtilis* strain B29

genera *Bacillus*, *Cytobacillus*, *Priestia*, and *Peribacillus* were reported in the collected soils from various locations of the province of Basrah. The two most widely spread species in the ecosystems as reported in the current study were *B. subtilis* and *B. licheniformis*.

Surfactin gene detection

The results of PCR screening that obtained, the gene responsible for generating the surfactin biosurfactant were detected molecularly in several species of *Bacillus* and *Cytobacillus* genera as shown in Fig. 1. and Table 3. The PCR amplifica-

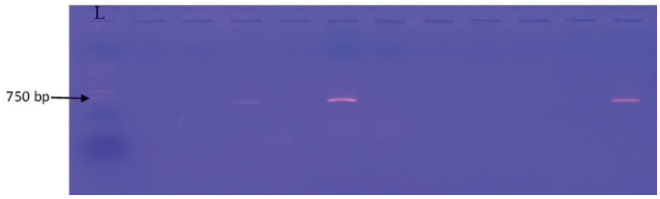

Fig. 1. Agarose gel electrophoresis for PCR products of *sfp* gene (675 bp) for *Bacillus*-related bacteria. Lane L: 1000 bp DNA ladder

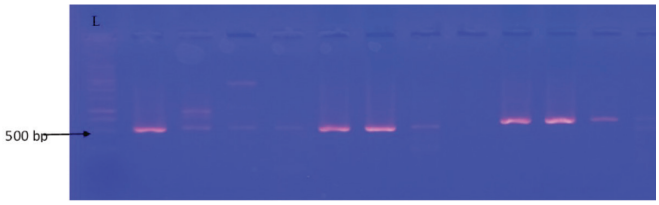

Fig. 2. Agarose gel electrophoresis for PCR products of *itu*C gene (594 bp) for *Bacillus*-related bacteria. lane L: 1000 bp DNA ladder

tion of *sfp* gene in *Bacillus*-related bacteria isolates was observed in nineteen isolates.

The *sfp* genes were detected in 43.18% of *Bacillus*-related isolates, and the most frequently detected in 9 isolates of *B. subtilis* followed by 3 isolates of *B. licheniformis*, 2 isolates of *B.cereus,* and 1 isolate of each *B. tequilensis, B. safensis, C. oceanisediminis and C.firmus*. *sfp* gene is detected in several bacterial isolates from several bacterial species of *Bacillus*-related species.

ituC gene detection

The results of PCR screening that obtained, the gene responsible for generating the iturin C biosurfactant were detected molecularly in several species of *Bacillus*, *Cytobacillus,* and *Priestia* genera as shown in Fig. 2 and Table 4.

The *itu*C genes were detected in 47.72% of *Bacillus*-related isolates, and the most frequently detected in *B. licheniformis* (7 isolates) followed by *B. subtilis* (3 isolates), *B. cereus* (3 isolates), *C. firmus* (3 isolates) and 1 isolate of *P. flexa, B. tequilensis, B. infantis, P. megaterium, B. velezensis* species. *itu*C gene is the most common gene among *Bacillus*-related species compared to other studied genes in the present study.

bamC gene detection

The results of PCR screening that obtained, the gene responsible for generating the Bacillomycin C biosurfactant were detected molecularly in some species of the *Bacillus* genus as shown in Fig. 3 and Table 5.

Table 3. Detection of *Sfp* gene in *Bacillus*-related bacteria

No.	Isolates code	Species
1	A1	*B. subtilis* strain SXKF16-3
2	A5	*B. subtilis* strain B4 16S
3	A6	*B. subtilis* strain TPS4
4	B2	*B. tequilensis* strain YJ-S4
5	B7	*B. safensis* strain P9
6	C2	*B. licheniformis* strain QT201
7	C4	*B. cereus* strain 2.3AL8
8	E2	*B. licheniformis* strain WAS3-5
9	F2	*B. licheniformis* strain WSR-KSU302
10	F4	*B. cereus* isolate HKS 2-2
11	G1	.
12	G2	*Cytobacillus oceanisediminis* strain 224-LR35
13	G6	*B. albus* strain FA80
14	H2	*B. subtilis* strain B29
15	H4	*B. subtilis* strain WZ-2
16	I3	*B. subtilis* strain MK736112.1
17	J1	*C. firmus* strain MR-39
18	J2	*B. subtilis* strain TUST018
19	J3	*B. subtilis* strain B29

Table 4. Detection of *itu*C gene in *Bacillus*-related bacteria

No.	Isolates code	Species
1	A1	*B. subtilis* strain SXKF16-3
2	A2	*C. firmus* strain NFB28
3	A3	*B. licheniformis* strain SIITMB5
4	A4	*B. licheniformis* strain ESR26
5	B1	*Priestia flexa* strain NJNPD41
6	B2	*B. tequilensis* strain YJ-S4
7	C2	*B. licheniformis* strain QT201
8	C4	*B. cereus* strain 2.3AL8
9	D2	*B. cereus* strain AA6
10	E2	*B. licheniformis* strain WAS3-5
11	F2	*B. licheniformis* strain WSR-KSU302
12	F3	*B. infantis* strain SIITMB9
13	F4	*B. cereus* isolate HKS 2-2
14	G3	*C. firmus* strain NPBR2
15	H1	*B. licheniformis* strain KKR2017
16	H5	*P. megaterium* strain DK2
17	I1	*B. licheniformis* isolate S8
18	I2	*B. velezensis* strain ONU 553
19	I3	*B. subtilis* strain MK736112.1
20	J1	*C. firmus* strain MR-39
21	J3	*B. subtilis* strain B29

Fig. 3. Agarose gel electrophoresis for PCR products of *bam*C gene (957 bp) for *Bacillus*-related bacteria. lane L: 1000 bp DNA ladder

Table 5. Detection of *bam*C gene in *Bacillus*-related bacteria

No.	Isolates code	Species
1	A4	*B. licheniformis* strain ESR26
2	C2	*B. licheniformis* strain QT201
3	E2	*B. licheniformis* strain WAS3-5
4	F2	*B. licheniformis* strain WSR-KSU302
5	F3	*B. infantis* strain SIITMB9
6	G4	*B. licheniformis* strain SUM-KSU304
7	G5	*B. vallismortis* strain T25-9
8	H1	*B. licheniformis* strain KKR2017
9	H2	*B. subtilis* strain B29

The *bam*C genes were detected in 20.45% of *Bacillus* related isolates and the most frequently detected in *B. licheniformis* (6 isolates) followed by 1 isolate of *B. infantis, B. vallismortis and B. subtilis. bam*C gene is the less common gene among *B.* related species compared to other studied genes in the present study.

fenD gene detection

The results of PCR screening obtained, the gene responsible for generating the fengycin D biosurfactants were detected molecularly in some species of *Bacillus* and *Cytobacillus* genera as shown in Fig. 4 and Table 6.

Fig. 4. Agarose gel electrophoresis for PCR products of *Fen*D gene (269 bp) for *Bacillus*-related bacteria. lane L: 1000 bp DNA ladder

The *fen*D genes were detected in 29.54% of *Bacillus*-related isolates and the most frequently detected in *B. licheniformis* (4 isolates) and *B. subtilis* (4 isolates) followed by 1 isolate of *B. safensis, B. vallismortis, C. oceanisediminis, B. velezensis* and *C. firmus.* The prevalence of the *fen*D gene in *Bacillus*-related species is not widespread according to the result of the present study.

Table 6. Detection of *Fen*D gene in *Bacillus*-related bacteria

No	Isolates code	Species
1	B7	*B. safensis* strain P9
2	E2	*B. licheniformis* strain WAS3-5
3	G4	*B. licheniformis* strain SUM-KSU304
4	G5	*B. vallismortis* strain T25-9
5	H1	*B. licheniformis* strain KKR2017
6	H2	*B. subtilis* strain B29
7	H3	*CytoB. oceanisediminis* strain GA51
8	I1	*B. licheniformis* isolate S8
9	I2	*B. velezensis* strain ONU 553
10	I3	*B. subtilis* strain MK736112.1
11	J1	*CytoB. firmus* strain MR-39
12	J2	*B. subtilis* strain TUST018
13	J3	*B. subtilis* strain B29

Occurrence of lipopeptide genes

The most frequent occurrence of lipopeptide genes among *Bacillus*-related isolates was *ituC* (47.72%) and *sfp* (43.18%), followed by *fenD* (29.54%). *BamC* was the least common gene (20.45%) as shown in Fig. 5. A 2.27% (1 isolate) of the isolates had all the biosynthetic lipopeptides genes, 15.90% (7 isolates) of the isolates had 3 biosynthetic lipopeptides genes, 27.27% (12 isolates) of the isolates had 2 biosynthetic lipopeptides genes and 29.54% (13 isolates) of the isolates had 1 biosynthetic lipopeptides genes simultaneously, while 25% (11 isolates) of the isolates did not report the presence of any biosynthetic lipopeptides genes.

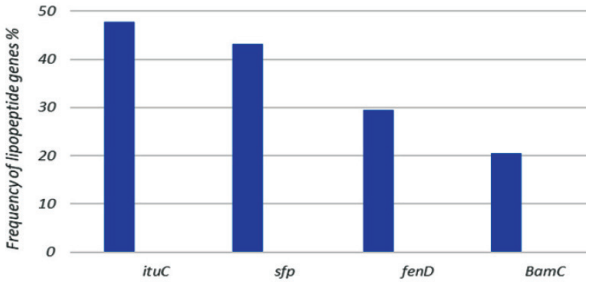

Fig. 5. Occurrence and frequency of lipopeptides genes in *Bacillus*-related species

Discussion

The main source of *Bacillus*-related bacteria is soil. Studies on the genes responsible for the biosynthesis of lipopeptides in *Bacillus*-related bacteria may help identify novel isolates with promising biotechnological applications. *Bacillus*-related bacteria are utilized to produce a very wide range of significant substances, including amino acids, biosurfactants, and antibiotics (Płaza *et al.,* 2015).

Numerous strains of *Bacillus*-related bacteria are capable of producing biosurfactants belonging to the lipopeptide group including four main families: the surfactins, the iturins, the fengycins, and bacillomycin (Jacques, 2011; Alyousif *et al.,* 2020). *Bacillus*-related bacteria identification was determined using morphological characteristics and molecular methods based on 16s rDNA sequencing (Al-Dhabaan, 2019). Screening of *Bacillus*-related bacteria obtained from soil samples covering a wide range of various sites in Basrah province.

The sfp gene is a crucial component of peptide synthesis systems and controls the expression of genes involved in surfactin production (Swaathy *et al.,* 2014). Bacillus-related species' sfp gene encodes surfactin synthetase, which is necessary for surfactin's nonribosomal production (Jacques, 2011). In the present study, *sfp* gene was observed in *B. licheniformis, B. cereus*, *B. subtilis*, *B. tequilensis, B. safensis, B. albus, CytoB. oceanisediminis* and *CytoB. firmus.* The *sfp* gene has been reported in previous studies in several species of *B.*-related bacteria, such as *B. licheniformis, B. cereus*, *B. subtilis* (Płaza *et al.,* 2015; Swaathy *et al.,* 2014 (.

Iturins are a significant class of lipopeptide biosurfactants produced by members of the *B. subtilis* group that have been extensively studied for their antimicrobial properties (Aktuganov *et al.,* 2014; Dunlap *et al.,* 2019). Iturin inhibits a wide range of plant fungal diseases, therefore it is regarded as a molecule with strong biocontrol capabilities with potential utility in agriculture and food storage (Mulligan *et al.,* 2014). In the present study, *Iturin C* gene was observed in *B. licheniformis, B. cereus*, *B. subtilis*, *B. tequilensis, B. infantis, B. velezensis, Priestia flexa, Priestia megaterium* and *CytoB. firmus.* The Iturin C gene has been reported in previous studies in many species of *B.*-related bacteria, such as *B. licheniformis, B. cereus*, *B. subtilis*)Płaza *et al.,* 2015(.

Bacillomycin C is a member of the iturin family, which also includes mycosubtilin, bacillomycin F, bacillomycin A, C, D, and E. Bacillomycin a biological control agent, does not have the same harmful effects as chemically manufactured fungicides (Lin *et al.,* 2022). The Bacillomycin D operon has four open reading frames (BamD, BamA, BamB, and BamC) and is 37.8 kb long. Its structural organization is similar to that of the Iturin A and Mycosubtilin operons (Moyne *et al.,* 2004). It is a cyclic lipopeptide that is an antifungal agent generated by strains of *B. subtilis,* which exhibits an inhibiting of the aflatoxin-producing fungus *Aspergillus flavus* (Lin *et al.,* 2022). The Bacillomycin gene was reported in the current study in *B. licheniformis, B. subtilis, B. infantis,* and *B. vallismortis. B. vallismortis* is a species that is closely related to *B. subtilis* and may be easily differentiated from it by variations in its DNA sequences (Duan *et al.,* 2022). *B. vallismortis*, which has produced Bacillomycin, as a result, it can be a biocontrol agent for pathogens in plants (Thepbandit *et al.,* 2023).

The fengycin is a cyclic lipopeptide produced mainly by *B. subtilis* (Sur *et al.,* 2018). It demonstrates antibacterial activity against a broad range of plant-pathogenic fungi and foodborne pathogenic bacteria, including Gram-positive, Gram-negative, and fungal pathogens. It is targeting and damaging the outer membranes of microorganisms. Furthermore, they maintain their stability toward a range of enzymes, chemical reagents, and severe environmental conditions (Lin *et al.,* 2020; Zhu *et al.,* 2023). In the current study, fengycin gene was reported in *B. licheniformis, B. subtilis*, *B. safensis, B. vallismortis, B. velezensis, Cytobacillus oceanisediminis,* and *Cytobacillus firmus.* As a result, these isolates can fengycin a substance that can be used in the biological control of many pathogens that infect crops.

According to the findings, many *Bacillus*-related species have genes that code for lipopeptide biosurfactants, where these bacteria can produce lipopeptide biosurfactants if appropriate cultural conditions are provided for isolates, which can be exploited in many fields, most notably inhibiting plant-pathogenic fungi, in addition to their use in the medical fields in inhibiting pathogenic bacteria.

Conclusion

Members of *Bacillus*-related species are reservoirs of a wide range of important lipopeptide biosurfactants. The results indicated that most bacterial isolates of the current study contain genes encoding lipopeptide biosurfactants, and *Bacillus*-related isolates vary in the number of encoding genes that were detected, ranging from 4 genes to 1 gene. *itu*C and *sfp* genes were the most frequently detected, followed by *fen*D and *bam*C genes. One isolate reported having all the biosynthetic lipopeptides genes, 7 isolates had 3 biosynthetic lipopeptides genes, 12 isolates had 2 biosynthetic lipopeptides genes, 13 isolates had 1 biosynthetic lipopeptides genes simultaneously, while 11 isolates did not report the presence of any biosynthetic lipopeptides genes. *B. licheniformis* was the most frequently isolated reported presence of biosynthetic lipopeptides

genes followed by *B. subtilis*. The current study showed that *B. licheniformis* strain WAS3-5 is a promising isolate as it contains all the lipopeptide genes and can be used in various applications if the appropriate conditions are provided.

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References

Aktuganov, G., J. Jokela, H. Kivela, E. K. Alexander, A. Melentjev, N. Galimzianova, L. Kuzmina, P. Kouvonen, J-P., Himanen, P. Susi, T. Korpela (2014). Isolation and identification of cyclic lipopeptides from *Paenibacillus ehimensis*, strain IB-X-b. *J. Chrom.* B **973**: 9-16. http://dx.doi.org/10.1016/j.jchromb.2014.09.042.

Al-Dhabaan, F. (2019). Morphological, biochemical and molecular identification of petroleum hydrocarbons biodegradation bacteria isolated from oil polluted soil in Dhahran, Saudi Arabia. *Saudi J. Biol. Sci.* **26**: 1247-1252. DOI: 10.1016/j.sjbs.2018.05.029.

Alyousif, N. A., Y. Y. Y. AL-Luaibi, W. Hussein (2020). Distribution and molecular characterization of biosurfactant-producing bacteria. *Biodiversitas* **21**: 4034-4040. DOI: 10.13057/biodiv/d210914.

Alyousif, N. A. (2022). Distribution, occurrence and molecular characterization of *Bacillus* related species isolated from different soil in Basrah Province, Iraq. *Biodiversitas* **23**: 679-686. DOI: 10.13057/biodiv/d230209.

Alyousif, N. A., W. H. Al-Tamimi, M. A. A. Al-sahib (2022). Evaluation of the effect of various nutritional and environmental factors on biosurfactant production by *Staphylococcus epidermidis*. *Biodiversitas* **23**: 3533-3538. DOI: 10.13057/biodiv/d230729.

Chilcott, C. N., P. J. Wigley (1993). Isolation and toxicity of *Bacillus thuringiensis* from soil and insect habitats in New Zealand. *J. Invertebr. Pathol.* **61**: 244-247. DOI: 10.1006/jipa.1993.1047.

Cochrane, S. A., J. C. Vederas (2016). Lipopeptides from *Bacillus* and *Paenibacillus* spp.: a gold mine of antibiotic candidates. *Med. Res. Rev.* **36**: 4-31. http://dx.doi.org/10.1002/med.21321.

Duan, Y., R. Chen, R. Zhang, W. Jiang, X. Chen, C. Yin, Z. Mao (2022). Isolation and identification of *Bacillus vallismortis* HSB-2 and its biocontrol potential against apple replant disease. *Biol. Control* **170**: 104921⁦ https://doi.org/10.1016/j.biocontrol.2022.104921.

Dunlap, C. A., M. J. Bowman, A. P. Rooney (2019). Iturinic lipopeptide diversity in the *Bacillus subtilis* Species Group - important antifungals for plant disease biocontrol applications. *Front. Microbiol*. **10**: 1794. doi: 10.3389/fmicb.2019.01794.

Elazzazy, A. M., T. S. Abdelmoneim, O. A. Almaghrabi (2015). Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia. *Saudi J. Biol. Sci*. **22**: 466-475. DOI: 10.1016/j.sjbs.2014.11.018.

Fira, D., I. Dimkić, T. Berić, J. Lozo, S. Stanković (2018). Biological control of plant pathogens by *Bacillus* species. *J. Biotechnol.* **285**: 44–55. https://doi.org/10.1016/j. jbiotec.2018.07.044.

Foysal, M. J., A. K., Lisa (2018). Isolation and characterization of *Bacillus* sp. strain BC01 from soil displaying potent antagonistic activity against plant and fish pathogenic fungi and bacteria. *J. Genet. Eng. Biotechnol*. **16**: 387-392⁦ https://doi.org/10.1016/j.jgeb.2018.01.005.

Hsieh, F-C., M-C. Li, T-C. Lin, S-S. Kao (2004). Rapid detection and characterization of surfactin-producing *Bacillus subtilis* and closely related species based on PCR. *Curr. Microbiol*. **49**: 186–191. doi: 10.1007/s00284-004-4314-7.

Jacques, P. (2011) Surfactin and other lipopeptides from *Bacillus* spp. In Biosurfactants: From Genes to Applications Soberon-Chavez, G. (Ed.), Microbiology Monographs vol. 20, Berlin Heidelberg: Springer, pp. 57–93. https://doi.org/10.1007/978-3-642-14490-5_3.

Lin, F., X. Zhu, J. Sun, F. Meng, Z. Lu, Y. Lu (2022). Bacillomycin D-C16 inhibits growth of *Fusarium verticillioides* and production of fumonisin B1 in maize kernels. *Pestic. Biochem. Phys.* **181**: 105015⁦ doi: 10.1016/j.pestbp.2021.105015.

Lin, L-Z., Q-W. Zheng, T. Wei, Z-Q. Zhang, C-F. Zhao, H. Zhong, QXu, Q-Y., Lin, J-F. Guo (2020). Isolation and characterization of fengycins produced by *Bacillus amyloliquefaciens* JFL21 and its broad-spectrum antimicrobial potential against multidrug-resistant foodborne pathogens. *Front. Microbiol*. **11**: 579621. doi: 10.3389/fmicb.2020.579621.

Mandic-Mulec, I., P. Stefanic, J. D. van Elsas (2016). Ecology of *Bacillaceae*. In: Eichenberger, P., A. Driks (Eds.) The Bacterial Spore: From Molecules to Systems. *ASM Press, Washington*, DC. DOI: 10.1128/9781555819323.ch3.

Miyoshi, T., T. Iwatsuki, T. Naganuma (2005). Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2-micrometer pore-size filters. *Appl. Environ. Microbiol*. **71**: 1084-1088. DOI: 10.1128/AEM.71.2.1084-1088.2005.

Mora, I., J. Cabrefiga, E. Montesinos (2011). Antimicrobial peptide genes in *Bacillus* strains from plant environments. *Int. Microbiol*. **14**: 213-223. doi: 10.2436/20.1501.01.151.

Mora, I., J. Cabrefiga, E. Montesinos (2015). Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated *Bacillus* against phytopathogenic bacteria. *PLoS ONE*. **10**: e0127738. doi: 10.1371/journal.pone.0127738.

Moyne, A. L., T. E. Cleveland, S. Tuzun (2004). Molecular characterization and analysis of the operon encoding the antifungal lipopeptide bacillomycin D. *FEMS Microbiol. Let*. **234**: 43-49⁦ https://doi.org/10.1111/j.1574-6968.2004.tb09511.x.

Mulligan, C. N., S. K. Sharma, A. Mudhoo (2014). Biosurfactants. Research Trends and Applications. Boca Raton, London, New York: CRC Press Taylor and Francis Group.

Perez, K. J., J. D. S. Viana, F. C. Lopes, J. Q. Pereira, D. M. Dos Santos, J. S. Oliveira, R. V. Velho, S. M. Crispim, J. R. Nicoli, A. Brandelli, R. M. D. Nardi (2017). *Bacillus* spp. isolated from Puba as a source of biosurfactants and antimicrobial lipopeptides. *Front. Microbiol.* **8**: 61. doi: 10.3389/fmicb.2017.00061.

Płaza, G., J. Chojniak, K. Rudnicka, K. Paraszkiewicz, P. Ber-

nat (2015). Detection of biosurfactants in *Bacillus* species: genes and products identification. *J. Appl. Microbiol.* **119**: 1023-1034. doi: 10.1111/jam.12893.

Srinivasan, R., U. Karaoz, M. Volegova, J. MacKichan, M. Kato-Maeda, S. Miller, R. Nadarajan, E. L. Brodie, S. V. Lynch (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS One* **10**: e0117617. DOI: 10.1371/journal.pone.0117617.

Stincone, P., F. F. Veras, J. Q. Pereira, F. Q. Mayer, A. P. M. Varela, A. Brandelli (2020). Diversity of cyclic antimicrobial lipopeptides from *Bacillus* P34 revealed by functional annotation and comparative genome analysis. *Microbiol. Res.* **238**: 126515. doi: 10.1016/j.micres.2020.126515.

Sur, S., T. D. Romo, A. Grossfield (2018). Selectivity and mechanism of fengycin, an antimicrobial lipopeptide, from molecular dynamics. *J. Phys. Chem. B* **122**: 2219-2226. doi: 10.1021/acs.jpcb.7b11889.

Swaathy, S., V. Kavitha, A. S. Pravin, G. Sekaran, A. B. Mandal, A. Gnanamani (2014). Phylogenetic framework and biosurfactant gene expression analysis of marine *Bacillus* spp. of Eastern Coastal Plain of Tamil Nadu. *Intern. J. Bacteriol.* **2014**: 860491. doi.org/10.1155/2014/86049.

Thepbandit, W., A. Srisuwan, S. Siriwong, S. Nawong, D. Athinuwat (2023). *Bacillus vallismortis* TU-Orga21 blocks rice blast through both direct effect and stimulation of plant defense. *Front. Plant Sci.* **14**: 1103487. DOI 10.3389/fpls.2023.1103487.

Zhu, H., S.Wu, S.Tang, J. Xu, Y. He, Z. Ren, E. Liu (2023). Isolation, identification and characterization of biopotential cyclic lipopeptides from *Bacillus subtilis* strain JN005 and its antifungal activity against rice pathogen Magnaporthe oryzae. *Biological Control.* **182**: 105241. https://doi.org/10.1016/j.biocontrol.2023.105241