Bioactive pyrrole-pyrazine derivative from a novel Bacillus species and review of the literature

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The rising antibiotic resistance is urging researchers to explore for new forms of antibiotics, notably from soil microorganisms such as Bacillus species. This study aimed to screen Bacillus strains from soil samples in Sudan for antifungal activity and to review relevant compounds from members of the genus in the literature. Out of 10 isolates from soil in Sudan, the strain JS6 (DSM 28831) was found active against representative zygomycete fungi and consequently subjected to thorough identification and chemical analyses of secondary metabolites. Confirmation of the initially identified Bacillus spp. was done using 16S rDNA gene sequence analysis which indicated a novel species (accession MF099872) that is closely related to Bacillus siamensis, Bacillus amyloliquefaciens, and Bacillus nakamurai. The crude culture-free filtrate and both chloroform and ethyl acetate extracts authenticated the initial antifungal activity of this strain, which exceeded that of amphotericin B, a standard antifungal agent. GC-MS results of the extracts revealed 32 compounds which included long-chain fatty acids, fatty acid methyl esters, alkaloids, and alcoholic compounds. Seven biologically active compounds were identified from Bacillus spp. strain JS6 and are equally found in the literature originating from plant or microbial sources. In the literature, these compounds show various activities such as antifungal, antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavour, haemolytic, alpha reductase inhibitor, and other antimicrobial activities. The analysis identified a major unique antifungal peak (rt, 23.142; area, 25.36%) as 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrrolo[1,2-a:1′,2′-d]pyrazine, a heterocyclic aromatic organic compound, that has been previously detected in Lactobacillus casei.

Key words: Bacillus species, chromatography, TLC, GC-MS, soil, aromatic organic compounds.

INTRODUCTION

Members of the genus Bacillus are Gram-positive aerobic bacteria that are widely distributed in nature, notably soil. They are known to produce large amounts of secondary metabolites with different biological and biotechnological
activities including antimicrobials (Boottanun et al., 2017). Since the late 19th Century, Bacillus species have been recognized as a significant source of antibiotics (Schaffer, 1969).

Bacillus spp. express a range of secondary metabolite gene clusters encoding polyketide synthases and non-ribosomal peptide synthetases responsible for a notable number of polyketides and lipopeptides. Such compounds have been, in part, exploited for medical and agricultural purposes (Aleti et al., 2015). These metabolites have been a subject for wide arrays of scientific studies including chemical, biological activities, and industrial pharmaceuticals (Aleti et al., 2015; Mondol et al., 2013; Sansinenea and Ortiz, 2011). Screening of extracts from Bacillus spp. uncovered a considerable structural variety of many natural compounds with broad biological activities. These activities include antimicrobial, antiviral, immunosuppressive, antitumor activities, and antioxidants (Youcef-Ali et al., 2014).

Microbial sources have been shown to be powerful natural products of many kinds (Zeilha, 2017). Several studies have focused on plant compounds (Erkan et al., 2008). However, only a few reports have been conducted on the antioxidant power of microbial extracts (Moktan et al., 2008). Production of such compounds allows the bacterium to survive in its natural ecosystem (Sansinenea and Ortiz, 2011). Antifungal peptides produced by Bacillus spp. include mycobactins (Majumdar and Bose, 1958), surfactins (Kluge et al., 1988), mycosubtilins (Peypoux et al., 1976), and fungistatins (Islam et al., 2012). Bacillus spp. can produce a wide range of other metabolites, including chitinases and other cell wall-degrading enzymes (Frandberg and Schnurer, 1994), volatiles (Sadfi et al., 2001), and compounds that elicit plant resistance mechanisms (Islam et al., 2012). Volatile metabolites produced from Bacillus spp. have been reported to inhibit mycelia growth of Fusarium oxysporum, with the highest effect on the reduction of Fusarium wilt of onion (Sharifi Tehrani and Ramezani, 2003). Ryu et al. (2003) reported on the promotion of growth and induction of systemic resistance (ISR) response in Arabidopsis thaliana against Erwinia carotovora subsp. carotovora by volatile substances (VS) (acetylpyruvate and acetoin, same as the present purified compounds) from Bacillus amyloliquefaciens isolate IN937a and Bacillus subtilis isolate GB03. Therefore, VS-producing bacteria can be used as biocontrol agents for protection against microbial plant diseases (Islam et al., 2012). Genomic studies have revealed that the genome of B. amyloliquefaciens holds many gene clusters involved in the synthesis of antifungal and antibacterial acting secondary metabolites. Five gene clusters, srf, bmy, fen, nrs, dlb, covering altogether 137 kb, direct non-ribosomal synthesis of the cyclic lipopeptides surfactin, bacillomycin, fengycin, an unknown peptide, and the iron siderophore bacillibactin have been identified (Chen et al., 2009).

The present study was carried out to screen potential bioactive compounds from a Bacillus strain JS6, to review the literature and compare these with relevant ones in the literature.

MATERIALS AND METHODS

Isolation of bacilli from soil and their identification

Soil samples collected from different locations in Sudan were spread onto Tryptic soya agar (TSA; Oxoid Inc., UK) and incubated at 37°C for one week. Ten colonies were isolated from the soil samples and were identified to be members of the genus Bacillus. The isolates were at first identified using phenotypic criteria: microscopy, growth properties, physiological and biochemical tests (Tumbull, 1999), and subsequently considered for antifungal screening.

Confirmation of the initially identified Bacillus spp. was done using 16S rDNA gene sequence analysis. The sequencing was done by Macrogen Inc. (Seoul 08511, Korea). The acquired DNA sequences (1495 bp) were initially inspected and adjusted by Chromas (version 2.6.6 (2018), Techne lynys Pty Ltd., South Brisbane, Queensland, Australia). The sequence was then aligned alongside all obtainable 16S rRNA gene data of valid species by using the BLAST: Basic Local Alignment Search Tool (nih.gov) (Zhang et al., 2000) and the EZbiocloud 16S database (www.ezbiocloud.net) to show the phylogenetic relationship. The final phylogenetic tree was constructed by the MEGA7 program (Kumar et al., 2016), using the neighbor-joining method with bootstrap values based on 100 replications.

Antibiotic production

Isolated bacilli were screened for potential antibiotic production. Each bacillus was cultured as one line and fungi were streaked on a perpendicular line on TSA plates and incubated at 37°C. The tested fungi were Basidiobolus zygomyctetes, namely: Basidiobolus haptosporus-like strain 49-4, strain V81 (DSM06014), and strain Doza.

Incubated plates were checked daily for zones of inhibition. One isolate, Bacillus spp. strain JS6, was found potentially active and selected for further analysis.

Potential bioactive Bacillus spp. strain JS6, which showed zones of inhibition, was purified, subcultured in TSA broth (250-500 mL), incubated at 37°C, and checked for purity. A few drops from the broth containing bacteria and envisaged antimicrobials were placed on a fresh TSA plate containing bacteria and fungi to see and confirm the presence of inhibition again. The crude antibiotic solution which contained live bacilli and media ingredients in the 250 to 500 mL flask was subjected to separation via filtration. The pure filter solution was ready for chemical work.

Paper disk assay

Paper disk assay was performed as per CLSI Guidelines (CLSI, 2018). 500 μL of inoculums was spread on the surface of nutrient agar medium. Sterile discs (6 mm diameter) made of Whatman paper No. 1 were dipped into the test extract and were put onto the agar surface after complete drying. Commercially available discs were served as positive control. Plates were then incubated at 30°C for 24 h. After incubation, plates were observed for zones of inhibition.
Antibiotic extraction and thin layer chromatography

The culture broth (500 L) was centrifuged at 5,000 rpm for 10 min to separate the supernatant and mycelial biomass. Mycelium and culture supernatants were extracted with ethyl acetate and chloroform, respectively. The extracts were dried and resuspended in a small volume of methanol and kept for the next separation steps. To visualize the number of compounds present in the extracts, thin layer chromatography (TLC) was performed. Aluminium plates precoated with silica gel and two mobile phases [ethyl acetate: methanol (1:1) and Petroleum ether: chloroform (1:1)]. Chromatograms were visualized by ninhydrin reagent and then observed under UV light.

Gas chromatography mass spectrometer (GC-MS)

The antifungal compounds were identified by using GC-MS technique (GC-MS, Shimadzu QP2010 Ultra, Japan) following standard method (Adams, 2005). GC-MS was equipped with a capillary column (30 m × 0.25 mm i.d.; 0.25 mm) and a HP 5975B mass selective detector and 70 eV energy was used for electron ionization detection at 50°C for 3 min, then steadily increased to 250°C at 3°C/min rate and held at this temperature for 4 min. Injector and MS transfer line temperature was held at 220 and 250°C and helium was used as carrier gas (1 mL/min flow rate). The compounds were identified on the basis of comparison of the retention time and mass spectra with those in the NIST98 GC-MS library.

16S rDNA sequence analysis

16S rDNA nucleotide sequence data (1495 bp; Genbank accession no. MF099872) from Bacillus spp. JS6 was run on BLAST system with nearly all Bacillus spp. found in Genbank database. The sequence was further analyzed by MEGA6 software (Tamura et al., 2013), which assigned it to Bacillus sp. (Figure 2). All positions containing gaps and missing data were eliminated from the final analysis.

The strain was found to have a closer resemblance to Bacillus siamensis KCTC 13613, 99.86%; B. amyloliquefaciens strain DSM 7, 99.59%; Bacillus nakamurai NRRL B-41091, 99.59%; B. subtilis NCIB 3610, 99.59%; and Bacillus nematocida B-16, 99.52%.

RESULTS

Isolation and identification of bacilli from soil

Ten isolates were initially identified as belonging to the genus Bacillus. The strain JS6, which was found active against tested fungal species, was subjected to detailed identification and stored for future research. A few morphological characteristics were found sufficient to designate the isolate as a Bacillus sp. (Figure 1). The strain JS6 showed typical bacillus properties, namely, short thick Gram-positive rods, which was demonstrated in a 5-day-old aerobic culture on TSA medium at 37°C. The strain was deposited in the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Inhoffenstraße 7B 38124 Braunschweig, Germany (DSM 28831) for future description or use.

Thin layer chromatography

The present study adopts solvent extraction, thin layer
chromatography (TLC) separation, and GC-MS chromatography to purify and identify the active antifungal compounds produced by strain JS6. Ethyl acetate and chloroform extraction was performed on purified cell-free culture solution. The TLC plate of the chloroform extract showed a better result than ethyl acetate extract, but in both extract fractions A, B and C were distinct (Figure 3). Some tailings can also be seen, and hence more solvent systems with polarity adjusting should be used for further studies.

Figure 2. A phylogenetic tree showing the relationship of Bacillus sp. strain JS6 (red bar) to closely related validly described members of the genus Bacillus (species name|strain number|accession number|% similarity with JS6). The analysis was based on the neighbor-joining method using MEGA7 software (Kumar et al., 2016). The bar represents 2 nucleotide substitutions per alignment position.
Inhibitory activity of the chloroform and ethyl acetate extracts

Fractions A, B, and C of the chloroform extract and ethyl acetate extraction were found to have inhibitory activity against many molds. Figure 4 shows that the wider zones of inhibition by chloroform and ethyl acetate have been compared to amphotericin B and the aqueous extract. No significant inhibition was revealed by the aqueous extract. The activity that is comparable to amphotericin B but the aqueous extract showed less activity.

GC-MS chromatogram

The GC-MS chromatogram of strain JS6 in general showed narrow, resolved peaks; a noisy baseline could indicate the existence of a trace amount of compounds in the extracts. The GC-MS analysis identified a total of 32 compounds from the extract of both ethyl acetate (Figure 5) and chloroform (Figure 6). The extract of ethyl acetate revealed 19 compounds (Table 1), and 13 compounds from the chloroform extract (Table 2), but any of these compounds could be responsible for the antifungal activity that detected in this study. Interestingly, these compounds found in the chloroform extract differ from the compounds found in the ethyl acetate extract due to the solvent’s polarity difference. The antifungal activity of both extracts is significant due to the presence of a variety of interesting compounds.

DISCUSSION

The discovery of novel medicinal drugs is a top task of scientific, economic, and biomedical importance. Traditionally, the bulk of the new drugs have been generated from natural products (secondary metabolites) and compounds originated from natural products are likely sources of fascinating bioactive secondary
Figure 4. Inhibition of fungal growth by *Bacillus* sp. JS6 extracts. Note the wider zone of inhibition by chloroform and ethyl acetate that are comparable to amphotericin B, but the aqueous extract showed less activity.

Figure 5. GC-MS chromatogram of the compounds in the ethyl acetate bacterial crude extract.

metabolites (Zhang et al., 2020). During the previous two decades, pharmaceutical research in the field of natural products has dropped, partly because of an emphasis on high-throughput screening of synthetic libraries. At present, there is a considerable drop in new drug approvals and this could lead to an imminent loss of
Table 1. Compounds identified in the ethyl acetate bacterial crude extract by GC-MS.

| Peak | Rt    | Area% | Name                                                                 | Molecular formula | Molecular weight (g/mol) |
|------|-------|-------|----------------------------------------------------------------------|-------------------|--------------------------|
| 1    | 16.563| 1.25  | Butylated hydroxytoluene                                             | C₁₅H₂₄O           | 220.4                    |
| 3    | 20.982| 3.67  | n-Nonadecanol-1                                                       | C₁₉H₄₀O           | 284.5                    |
| 2    | 17.839| 4.33  | 9-Eicosene                                                            | C₂₀H₄₀             | 280.5                    |
| 4    | 21.538| 6.25  | L-Proline, N-valeryl-, hexadecyl ester                               | C₂₀H₄₅NO₃         | 423.7                    |
| 5    | 21.880| 10.19 | L-Proline, N-valeryl-, tetradecyl ester                              | C₂₄H₆₅NO₃         | 355.5                    |
| 6    | 22.707| 2.01  | Hexadecanoic acid methyl ester                                       | C₁₇H₃₄O           | 270.5                    |
| 7    | 22.770| 3.23  | L-Leucine, N-cyclopropylcarbonyl-, undecyl ester                     | C₂₁H₄₉NO₃         | 353.5                    |
| 8    | 22.975| 6.98  | Bromocriptine                                                        | C₇₃H₉₉BrN₅O₅     | 654.6                    |
| 9    | 23.019| 3.51  | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | C₁₈H₂₈O₃         | 292.4                    |
| 10   | 23.092| 8.13  | L-Proline N-valeryl-, octadecyl ester                                | C₂₈H₅₃NO₃         | 451.7                    |
| 11   | 23.147| 11.08 | 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1′,2′-d]pyrazine | C₁₉H₂₂N₂O₂        | 250.3                    |
| 12   | 23.464| 2.61  | 9-Tricosene                                                           | C₂₃H₄₈             | 322.6                    |
| 13   | 24.625| 5.38  | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester                       | C₁₉H₃₄O₂           | 294.5                    |
| 14   | 24.674| 3.89  | 9-Octadecanoic acid, methyl ester, (E)-                              | C₁₉H₃₄O₂           | 296.5                    |
| 15   | 25.561| 1.51  | 1-Heptacosanol                                                       | C₂₁H₅₆O           | 396.7                    |
| 16   | 25.731| 1.63  | Heptacosyl acetate                                                   | C₂₀H₅₈O₂           | 438.8                    |
| 17   | 27.296| 13.79 | Ergotaman-3′,6′,18-trione, 9,10-dihydro-12′-hydroxy-2′,5′-bis(1-methylethyl)-, (5′α,10′α) | C₃₁H₄₁N₂O₅     | 563.7                    |
| 18   | 27.691| 9.39  | Ergotaman-3′,6′,18-trione, 12′-hydroxy-2′-(1-methylethyl)-5′-(2-methylpropyl)-, (5′α) | C₃₂H₄₁N₂O₅     | 575.7                    |
| 19   | 27.8680| 1.16 | Phenol, 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl- | C₃₀H₉₃O₈P         | 1049.4                   |
The strain JS9 of this study produced the unique compound 5,10-diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine. This compound showed antifungal activity against zygomycete fungi. This compound is documented in the Springer Nature References (PubChem 2004). It has been first detected in Lactobacillus casei (Li et al., 2012a) and found to have antifungal activity. This result supports the current findings both in chemical structure and antimicrobial properties. To our knowledge, no other information are available regarding this unique compound.

A chain of 2-pyrazolines 5-9 has been manufactured from α,β-unsaturated ketones 2-4. Some of the newly identified pyrazolone and pyrazole derivatives were found to have antibacterial and antifungal activity (Hassan, 2013). Several compounds including 4-(2,5-dimethylpyrrol-1-yl)/4-pyrrol-1-yl benzoic acid hydrazide analogs have been synthesized and were found to have in vitro antibacterial, antifungal, and antitubercular activity at non-cytotoxic concentrations (Joshi et al., 2013).

**Biologically active compounds identified from Bacillus sp. strain JS6 and are equally detected from other sources**

In this search, seven major compounds have been detected in the strain JS6. These compounds have already been detected from different sources, and all have biological activities. Names of these compounds, their natures, activities, sources, and references are shown in Table 3. The structures of these compounds are as shown in Figure 7. Beevi et al. (2014) have investigated the antibacterial activity and chemical characterization of metabolites of *B. subtilis* isolated from Sea Surface Microlayer. GC-MS analysis revealed the presence of 9-Ecosene with 15.0% with a potential antibacterial activity (Akpuaka et al., 2013). Hema et al. (2011) carried out the GC/MS analysis of metabolites of *B. subtilis* isolated from Sea Surface Microlayer. GC-MS analysis revealed the presence of 9-Ecosene with 15.0% with a potential antibacterial activity (Akpuaka et al., 2013).

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Table 2. Compounds identified in the chloroform bacterial crude extract by GC-MS.

| Peak | Rt  | Area% | Name                                                                 | Molecular formula | Molecular weight (g/mol) |
|------|-----|-------|----------------------------------------------------------------------|-------------------|--------------------------|
| 1    | 20.056 | 3.74  | 3-Methyl-1,4-diaza[6]bicyclo[4.3.0]nonan-2,5-dione, N-acetyl-         | C_{10}H_{16}N_{2}O_{3} | 210.2                    |
| 2    | 20.426 | 2.06  | 3-Pyrrolidin-2-yl-propionic acid                                      | C_{7}H_{13}NO_{2}  | 143.2                    |
| 3    | 20.737 | 3.34  | n-Nonadecanol-1                                                       | C_{19}H_{39}O      | 284.5                    |
| 4    | 21.832 | 1.34  | L-Proline, N-valeryl-, hexadecyl ester                               | C_{20}H_{45}NO_{3} | 423.7                    |
| 5    | 21.536 | 7.31  | L-Proline, N-valeryl-, tetradecyl ester                              | C_{19}H_{43}NO_{3} | 395.6                    |
| 6    | 21.881 | 17.65 | Hexadecanoic acid methyl ester                                       | C_{17}H_{34}O_{2}  | 270.5                    |
| 7    | 22.769 | 2.94  | L-Leucine, N-cyclopropylcarbonyl-, undecyl ester                     | C_{12}H_{26}BrN_{2}O_{2} | 654.6                |
| 8    | 22.976 | 5.34  | Bromocriptine                                                         | C_{18}H_{29}O_{5}  | 329.4                    |
| 9    | 23.020 | 1.93  | Benzenepropionic acid, 3,5-bis(1,1-dimethyl-1)-4-hydroxy-, methyl ester | C_{26}H_{47}NO_{3} | 409.6                    |
| 10   | 23.095 | 8.64  | L-Leucine, N-cyclopropylcarbonyl-, pentadecyl ester                  | C_{18}H_{43}NO_{2}  | 250.3                    |
| 11   | 23.142 | 25.36 | 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine | C_{25}H_{49}N_{5}O_{5} | 563.7                |
| 12   | 27.288 | 11.07 | Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2',5'-bis(1-methyl-1)-, (5'α,10'α)- | C_{25}H_{49}N_{5}O_{5} | 575.7                |
| 13   | 27.687 | 9.24  | Ergotaman-3',6',18-trione, 12'-hydroxy-2',1'-methyl-1)-, (5'α)-       | C_{25}H_{49}N_{5}O_{5} | 575.7                |
significant producers of diverse groups of peptides
determination of bioactive components of *Murraya
goenigii*, the curry tree. The curry tree, sometimes
called sweet neem, is a tropical tree (Rutaceae) which is native in most Asian countries. Their results
revealed the presence of 9-octadecanoic acid, methyl ester (E)- in this plant. Additionally, Asghar et al. (2011) have done GC-MS analysis of petroleum ether extract (oil) and bioassay of crude extract of *Iris germanica*, which is a species of flowering plant in the family Iridaceae commonly known as bearded iris or the German bearded iris. The petroleum ether extract (oil) of this plant has resulted in the identification of eleven compounds 9-octadecanoic acid methyl ester (E)- was identified with an anti-carcinogenic activity (Yeong et al., 1989).

Li et al. (2012b) distinguished 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrrolo[1,2-a:1',2'-d]pyrazine as an antifungal in the identification of antifungal compounds produced by *Lactobacillus* AST18. The antioxidant activity, total phenolics, and GC-MS study of *Vitex negundo* was investigated (Kumar et al., 2010). The study revealed the activity of 9, 12-octadecanoic acid (Z,Z), methyl ester (E)- and hexadecanoic acid methyl ester shown in Table 3. Bashir et al. (2012) studied the chemical composition and antifungal, phytotoxic, brine shrimp cytotoxicity, insecticidal, and antibacterial activities of the essential oils of *Azadirachta indica* (Neem) (Akpuaka et al., 2013).

| Compound name | Compound nature | Activity | Source of the compound other than strain JS6 and the reference |
|---------------|-----------------|----------|---------------------------------------------------------------|
| 9-Eicosene    | long-chain fatty acid | Anti-microbial and cytotoxic properties | The carob (*Ceratonia siliqua*), tree (Hsouna et al. 2011), *Bacillus subtilis* (Beevi et al., 2014) |
| 9-Octadecanoic acid, methyl ester (E)- | Stearic acid Ester | Anti-inflammatory, Preventive, Hepatoprotective, Antihistaminic, Antiandrogenic, Ant coronary and Insectifuge. | The curry tree (*Murraya koenigii*) (Hema et al., 2011); *Iris germanica* plant (Asghar et al., 2011) |
| n-Nonadecanol-1 | Alcoholic compound | Anti-microbial and cytotoxic properties | *Crotalaria incana* herb (Alemu et al., 2015) |
| Hexadecanoic acid methyl ester | Palmitic acid ester | Antifungal, Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavour, haemolytic, 5-Alpha reductase inhibitor, potent antimicrobial activity. | The carob (*Ceratonia siliqua*) tree (Hsouna et al., 2011); The curry tree (*Murraya koenigii*) (Hema et al., 2011) |
| 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrrolo[1,2-a:1',2'-d]pyrazine | Heterocyclic aromatic organic compound | Antifungal | *Lactobacillus casei* AST18 (Li et al., 2012a) |
| Benzenepropanoic acid 3 5-bis(1 1-dimethylethyl)-4-hydroxy- methyl ester | Benzoic acid compound | Antifungal, Antioxidant activities | Essential oils of *Acacia modesta* (Bashir et al., 2012) |
| 9,12-Octadecanoic acid (Z,Z), methyl ester (E)- | Linoleic acid ester | Anticancer, Antimicrobial, Antioxidant and Hypercholesterolemic | *Azadirachta indica* (Neem) (Akpuaka et al., 2013) |

Compounds identified from *Bacillus* spp. with antifungal activity

Members of the Bacillaceae family, including *Bacillus*, *Brevibacillus*, *Paenibacillus*,
Aneurinibacillus, and Halobacillus species are with antibacterial, antifungal, and antiviral activities (Zhao et al., 2018). A range of bacteria produce lipopeptides, but Bacillus and Paenibacillus spp. produced several potent antimicrobial lipopeptides. These lipopeptides have been renowned for years as a prospective source of antibiotics such as polymyxins, octapeptins, polypeptins, iturins, surfactins, fengycins, fusaricidins, tridecaptins, and kurstakins (Cochrane and Vederas, 2016). Iturins, which is a special class of pore-forming lipopeptides, have been extracted from culture media of B. subtilis and was found to have strong antifungal activity against many yeasts and fungi (Maget-Dana and Peypoux, 1994).

The antifungal antibiotics fusaricidins A, B, C, and D, which are yielded by Bacillus polymyxa KT-8, were found more effective than bacillopeptins in their antimicrobial activity (Kaneda and Kajimura, 2002). Bacillus licheniformis 09IDYM23 produces leodoglucoside C glycolipids which are considered good candidates for the development of new fungicides (Tareq et al., 2015). Bacillus spp., among other microbes associated with sponges, were recognized as noticeable producers of antimicrobial compounds (Indraningrat et al., 2016). Bacillus brevis produces an antimicrobial peptide named tyrothricin. Tyrothricin showed activity against bacteria, fungi, and some viruses (Lang and Staiger, 2016). Lipopeptides of Bacillus spp. were found to have a range of biological activities, including interactions with biofilms and anti-fungal, anti-inflammatory, anti-tumor, anti-virus, and antiplatelet properties (Zhao et al., 2017). Mass spectrometry and HPTLC bioautography analysis of purified compounds from Bacillus isolates indicated the presence of lipopeptides, thus confirming their biocontrol function (Fira et al., 2018).

**Conclusion**

The present study was an attempt to search for antifungal compounds from an alternative natural source. Seven biologically active compounds were identified from our Bacillus sp. strain JS6 and are equally detected from other sources recovered from the literature. These compounds exhibit various activities such as antifungal, antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavour, haemolytic, alpha reductase inhibitor, and other antimicrobial activities.

The present study revealed the presence of a distinctive antifungal compound, a pyrrole-pyrazine...
derivative; peak: rt, 23.142 with an area 25.36%, as 5,10-dioeloxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a;1',2'-d]pyrazine. This is a heterocyclic aromatic organic compound, which has been detected in L. casei before. Optimizing culture conditions may influence the production of secondary metabolites from Bacillus spp.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interest.

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