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Antimicrobial, plant growth-promoting and genomic properties of the peanut endophyte *Bacillus velezensis* LDO2

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ABSTRACT

Peanut suffer from a number of fungal and bacterial pathogens, while plant endophytes were considered excellent candidates as biocontrol agents. In this study, the peanut endophytic bacterium LDO2 was evaluated for the potential of peanut pathogens inhibition and growth-promotion, and the genetic mechanisms were explored by genome mining. Strain LDO2 significantly inhibited the growth of peanut pathogenic fungi and pathogenic bacteria, and specifically, it showed pronounced inhibition on mycelia growth of *Aspergillus flavus* mycelia and caused mycelial deformity. Gene clusters responsible for antifungal metabolites (fengycin, surfactin, bacilysin) and antibacterial metabolites (butirosin, bacillaene, difficidin, macrolactin, surfactin, bacilysin) were identified. Strain LDO2 also exhibited several growth-promoting related features including phosphate solubilization, siderophore production and growth promotion of peanut root. Genes associated with plant growth promotion were also identified and analyzed, as well as genes related to secreted proteins. These findings suggested that this peanut endophyte could be a potential biocontrol agent in peanut production and a source of antimicrobial compounds for further exploitation.

1. Introduction

Peanut (*Arachis hypogaea* L.) is one of the most important food and oilseed crops worldwide (Sobolev et al., 2013), and China is one of the major producers of peanut in the world (Kong et al., 2010). Unfortunately, peanut is susceptible to a variety of diseases caused by fungi or bacteria, leading to yield loss, quality decline and mycotoxin contamination (Vargas Gil et al., 2008; Jiang et al., 2017; Liu et al., 2017; Sobolev et al., 2018). Among these peanut diseases, fungi responsible for the most important ones, such as leaf blight (*Alternaria tenuissima*), damping-off (*Aspergillus* sp., *Fusarium oxysporum*, *Rhizoctonia solani, Rhizopus* sp.), crown rot (*A. niger*), root rot (*F. moniliforme*) and pod rot (*R. solani*) (Rojo et al., 2007; Haggag and Timmusk, 2008; Vargas Gil et al., 2008), these fungi can infect peanut leaves, roots, fruits, seeds as well as seedlings. Reports showed that the increasingly severe of fungal diseases decreased peanut yield and quality, combined yield losses due to incidence of the diseases can be as high as 50% (Rojo et al., 2007; Pal et al., 2014; Liu et al., 2017). While the most devastating bacterial disease of peanut is bacterial wilt which was mainly caused by *Ralstonia solanacearum* (Jiang et al., 2017). In China, peanut bacterial wilt is prevalent in most of the 13 main peanut producing provinces, reaching nearly 16% of the total planting area and up to 50–100% yield losses in extreme cases (Jiang et al., 2017). To prevent these diseases, different control strategies such as fungicide control, biological control and resistant cultivar breeding were applied over the past few decades (Nigam et al., 2009; Liu et al., 2017), so far microbiological control has been considered to be one of the most promising and environment-friendly approach for the prevention and control of plant diseases (Dey et al., 2004; Ongena and Jacques, 2008; Kong et al., 2010).

Plant endophytes were reported to possess the capability of inhibiting host plant pathogens, accelerating seedling emergence and promoting plant growth and yield, thus were considered more suitable as biocontrol agents (Ryan et al., 2008; Eljounaidi et al., 2016; Santoyo et al., 2016). Here we reported an endophytic bacterium LDO2, which was isolated from peanut root. To further evaluate the biocontrol potential of strain LDO2 on peanut, antimicrobial activity against peanut pathogens and peanut growth promotion were evaluated. In addition, to understand the genetic mechanisms of antimicrobial activity and plant growth promotion, we sequenced and analyzed the complete genome of strain LDO2.

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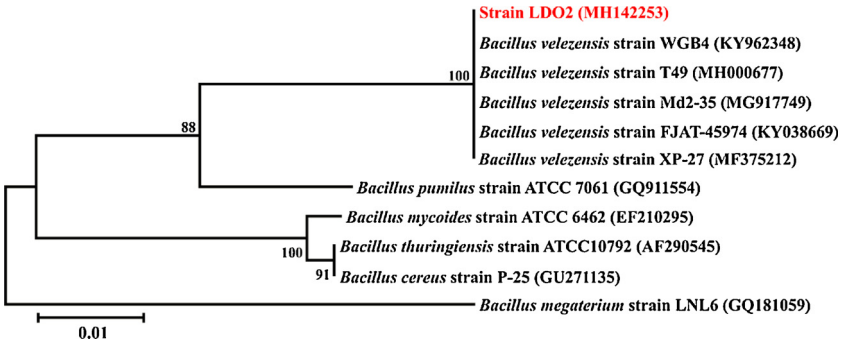

Fig. 1. Neighbor-joining tree of strain LDO2 based on 16 s rDNA sequences.

Table 1
Antimicrobial activities of endophytic bacterium LDO2 against peanut pathogens.

Pathogen	Peanut disease	Width of inhibition zone (mm)	Inhibition ratio of radial growth (%)
A. tenuissima	Leaf blight	5.8 ± 0.1	78.97%
A. flavus	*Aspergillus*, Damping-off	6.5 ± 0.2	80.77%
A. niger	Crown rot, Damping-off	6.1 ± 0.4	79.74%
F. oxysporum	Damping-off, *Fusarium* wilt	6.6 ± 0.3	81.03%
F. moniliforme	Root rot	6.7 ± 0.5	81.28%
R. solani	Damping-off, Pod rot	5.9 ± 0.2	79.23%
Rhizopus sp.	Damping-off, *Rhizopus*	4.5 ± 0.4	75.64%
R. solanacearum	Bacterial wilt	6.0 ± 0.3	/

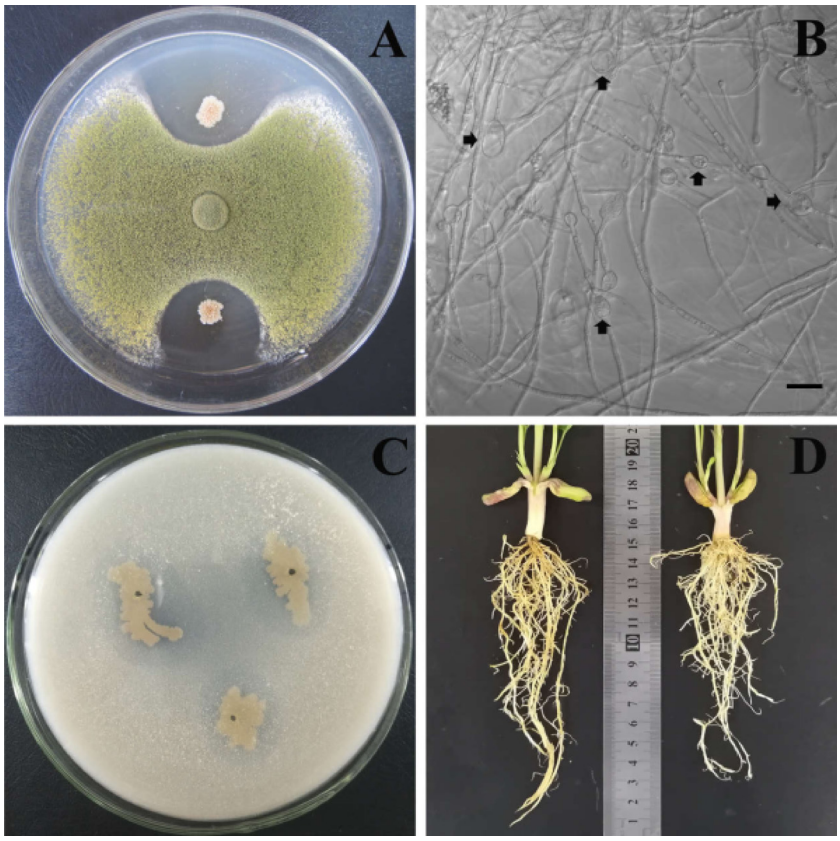

Fig. 2. Pathogens inhibiting and growth-promoting features of *B. velezensis* LDO2.
A: Mycelial growth of *A. flavus* was inhibited by strain LDO2 on PDA plate; B: Mycelial deformity (swelled hyphae) of *A. flavus* was emerging under the action of LDO2 broth, right arrow indicates swelled at the top of mycelium and up arrow indicates swelled in the middle of mycelium, bar indicates 100 μm; C: Solubilization of tri-calcium phosphate by LDO2. The phosphate-solubilizing halos were obviously observed around the colony of strain LDO2; D: Root growth bioassay with cultivar HY15. Compared to un-inoculated control (right), a significant increase in root length was observed in treated peanut plants (left) ($P < 0.05$).

2. Materials and methods

2.1. Endophytic bacterium isolation and identification

Endophytic bacterium LDO2 was isolated from peanut root according to the method described by Gao et al. (2017) with some modifications. Beriefly, peanut roots were surface sterilized by immersion in 75% ethanol for 3 min, 3% sodium hypochlorite for 6 min, and 75% ethanol for 30 s, then rinsed six times with sterile distilled water. The effect of surface sterilization was checked by spreading the final rinse water (200 μL) onto nutrient agar (NA) plates and culturing at 35 °C for 48 h. Sterilized roots were further mashed aseptically with 5 mL PBS buffer, and the obtained sample was diluted and plated onto NA plates for culture 48 h. Different colonies were

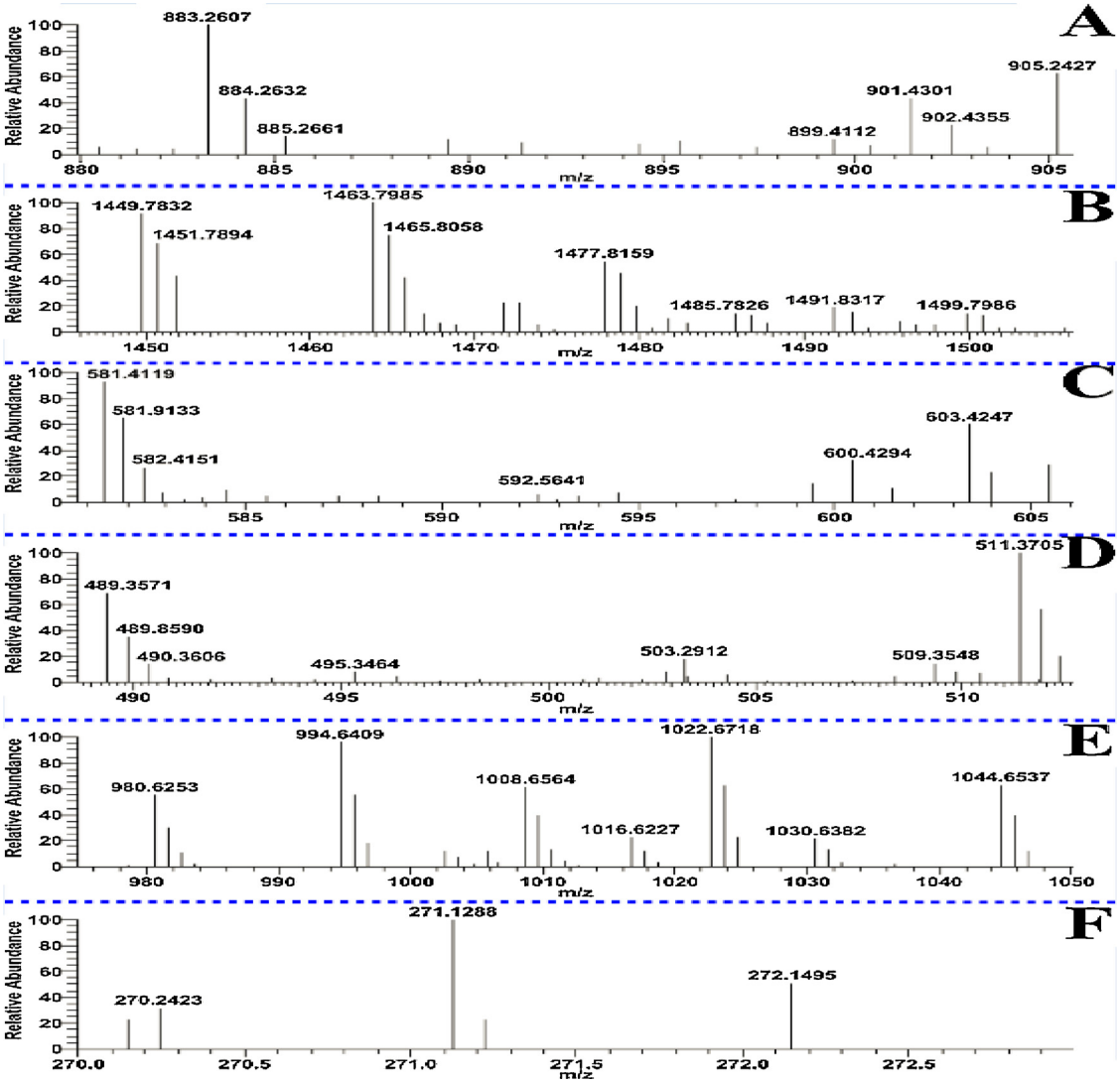

Fig. 3. UHPLC-ESI-MS analysis of secondary metabolites synthesized by strain LDO2.
A, Ions of *m/z* 883.2607 and 905.2426 correspond to the siderophore bacillibactin $[M+H]^+$) and bacillibactin $[M + Na]^+$; B, Ions of *m/z* values 1449.7832, 1463.7985, 1477.8159 correspond to $C_{13\text{-}15}$ fenygcin B $[M+H]^+$, and ions of *m/z* values 1471.7631, 1485.7826,1499.7986 correspond to $C_{13\text{-}15}$ fenygcin B $[M + Na]^+$, repectively; C, Ions of *m/z* 583.4119 and 603.4247 correspond to bacillaene A $[M+H]^+$ and bacillaene A $[M + Na]^+$; D, Ions of *m/z* 489.3571 and 511.3705 correspond to 7-o-malonyl macrolactin A $[M+H]^+$ and 7-o-malonyl macrolactin A $[M + Na]^+$; E, Ions of *m/z* values 994.6409, 1008.6564, 1022.6718 correspond to $C_{12\text{-}14}$ surfactin A $[M+H]^+$, and ions of *m/z* values 1016.6227, 1030.6382, 1044.6537 correspond to $C_{12\text{-}14}$ surfactin A $[M + Na]^+$, repectively. F, Ions of *m/z* value 271.1288 correspond to bacilysin $[M+H]^+$.

selected and streaked on NA plates to check the purity.

To identified the endophytic bacterium, morphological, physiological and molecular characterization of strain LDO2 were analyzed (Guardado-Valdivia et al., 2018). Briefly, morphological characteristics of bacterial cells were examined using light microscopy, morphological characteristics of bacterial colonies were directly observed, and physiological characteristics of strain LDO2 including carbon source utilization (glucose, sucrose, fructose, mannose, ribose, maltose, chitin and cellulose), glucose fermentation, nitrate reduction, oxidase reactions, indole production, gelatin lignification and starch hydrolysis were determined by standard methods (Dong et al., 2001). Molecular characterization was performed by 16S rDNA sequencing according to the method of Guardado-Valdivia et al. (2018).

2.2. Antimicrobial activity against peanut pathogens

Antifungal activity of strain LDO2 against peanut pathogenic fungi was evaluated using plate co-culture assay. Briefly, each fungus was cultured on PDA plate (90 mm in diameter) for 4–6 d, then a mycelia plug (6 mm in diameter) was inoculated at the center of PDA plate, and the bacterium was inoculated on the same plate with 25 mm apart from the center. Plate with only fungus inoculation served as control. The plate was cultured at 28 °C until the fungus in control plate had grown to the whole plate. Each experiment was performed three times. The presence of inhibition zone between strain LDO2 and fungus indicated antifungal activity, and the width of inhibition zone was measured from the colony edge of fungal to the colony edge of bacterium, and inhibition ratio of fungal growth was determined by the following equation:

$$\text{Inhibition ratio} = \frac{\text{Diameter of control fungus-Diameter of treated fungus}}{\text{Diameter of control fungu s}- 6}.$$

Antibacterial activity of strain LDO2 against peanut pathogenic bacteria was tested by cylinder-plate assay. Briefly, the pathogenic bacterium was inoculated into 50 mL of nutrient broth for 24 h at 30 °C, then the broth was added to nutrient agar at 45 °C with the final

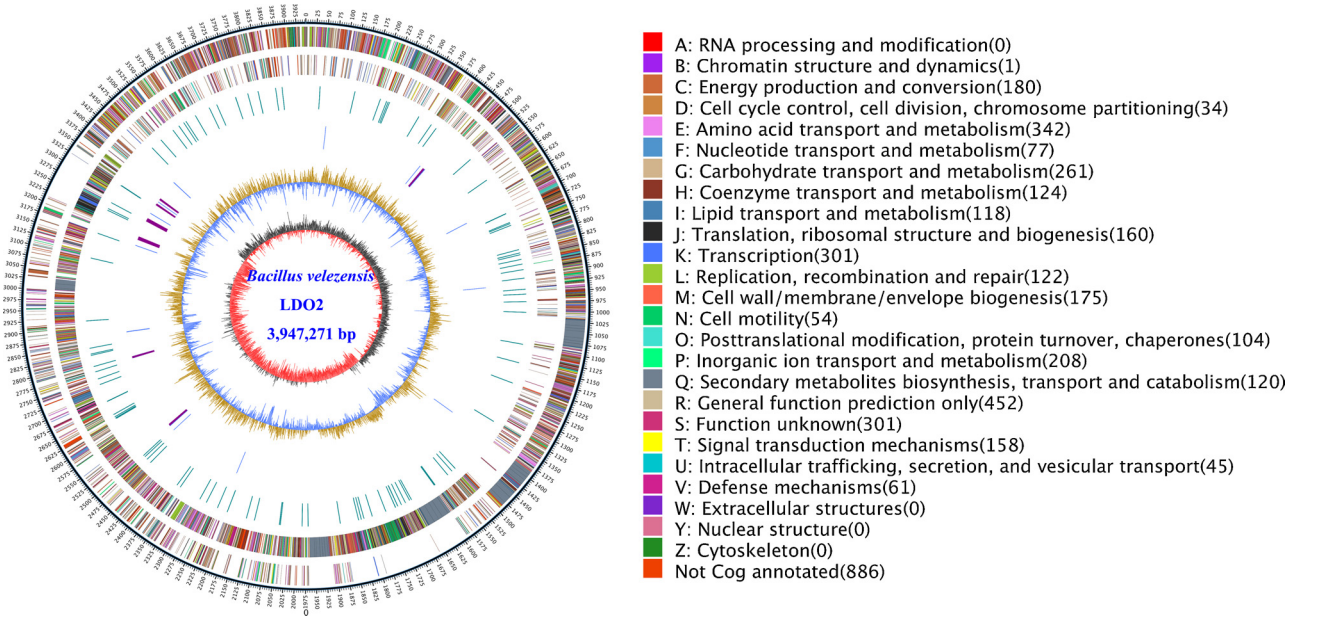

Fig. 4. Circular genome map of endophytic *B. velezensis* LDO2.
The circular genome map was created using Circos v0.66 with COG function annotation. From outside to inside, circle 1, the size of complete genome, each scale represents 5 kb; circle 2 and 3, the predicted protein-coding genes on the + and - strands, different colors represent different COG function classification; circle 4, repetitive sequence; circle 5, tRNA (light blue) and rRNA (purple); circle 6, G + C content, with > 46.50% G + C in lightbank, with ≤ 46.50% G + C in sky blue; the inner circle, G + C skew, with G% < C% in red, with G% > C% in gray.

Table 2
Effect of endophytic bacterium LDO2 on peanut seedlings growth.

Treatments	Number	Peanut seedling height (cm)	Peanut seedling dry weight (g)	Root length (cm)	Root dry weight (g)
un-inoculated control	n = 9	35.7 ± 2.6	2.23 ± 0.23	12.1 ± 1.5	0.43 ± 0.08
LDO2 broth treatment	n = 9	40.3 ± 3.1*	2.59 ± 0.15*	15.2 ± 2.3*	0.51 ± 0.10*

Values are the mean ± S.E.
* Statistically different from the un-inoculated control ($P < 0.05$).

Table 3
Antimicrobial secondary metabolite clusters in *B. velezensis* LDO2 genome.

Cluster	Cluster Type	Location	Most similar known cluster (% of genes show similarity)	Suppress object	Reference
dhb	Bacteriocin-NRPS	302192–368980	Bacillibactin biosynthetic gene cluster (100%)	Siderophore	Miethke et al. (2006)
dif	TransATPKS	999412–1099865	Difficidin biosynthetic gene cluster (100%)	Bacteria	Wu et al. (2015)
fen	NRPS-TransATPKS	1366379-1504180	Fengycin biosynthetic gene cluster (100%)	Fungi	Ongena and Jacques (2008)
bae	NRPS-TransATPKS	1568812–1671486	Bacillaene biosynthetic gene cluster (100%)	Bacteria	Chen et al. (2006)
mln	TransATPKS	1897595–1983500	Macrolactin biosynthetic gene cluster (100%)	Bacteria	Schneider et al. (2007)
btr	Otherks	2404556–2445800	Butirosin biosynthetic gene cluster (7%)	Bacteria	Llewellyn et al. (2007)
srf	NRPS	2982249–3047656	Surfactin biosynthetic gene cluster (82%)	Bacteria, Fungi	Jiang et al., (2016)
bac	Other	3669251–3710669	Bacilysin biosynthetic gene cluster (100%)	Bacteria, Fungi	Phister et al. (2004)

concentration of 10^6 cfu/mL, mixed rapidly and poured into Petri dishes (90 mm in diameter). After the agar cooled down, a sterilized cylinder was placed to the center of Petri dish, then 200 uL broth (10^8 cfu/mL) of strain LDO2 were added into the cylinder. Sterile water served as control. The plates were firstly kept at 4 °C for 2 h, and then cultured at 30 °C for 24 h. The presence of inhibition zone around the cylinder indicated antibacterial activity, and the diameter of inhibition zone was measured. Each experiment was performed three times.

2.3. UHPLC-ESI-MS analysis of secondary metabolites

Strain LDO2 was firstly inoculated into a 250 mL flask containing 100 mL of Landy medium, and cultured at 35 °C and 175 r/min for 48 h. The fermentation broth was centrifuged at 4 °C and 12,000*g* for 15 min, and the supernatant was collected and filtrated through a membrane (0.22 μm) for further analysis.

An ultrahigh-performance liquid chromatography coupled to Q-Exactive Orbitrap mass spectrometry (UHPLC-ESI-MS)(Thermo, Germany) was employed for the detection of secondary metabolites produced by strain LDO2 (Chen et al., 2018). The UHPLC conditions were as follows: C_{18} column (100 × 2.1 mm, 1.7 μm), eluent A was H_2O/0.1% formic acid, eluent B was CH_3CN/0.1% formic acid, flow rate was 300 μL/min, elution gradient was 95%A/5%B to 5%A/95%B, 60 min. The Q-Exactive Orbitrap MS was processed using Xcalibur 3.0.63 under the following conditions: HESI temperature of 350 °C, capillary temperature of 350 °C, sheath gas rate of 30 L/min, auxiliary gas rate of 5 L/min, electrospray voltage of 3.5KV for positive full scan mode, mass resolution of 70000, mass range of 200–2000 *m/z*, nebulizer gas of Nitrogen, nebulizer gas rate of 3 L/min.

Table 4
Genes related to plant growth promotion in the LDO2 genome.

Gene	Gene product	Function
dhb cluster	Bacillibactin	Siderophore synthesis
alsS	Acetolactate synthase	3-hydroxy-2-butanone synthesis
alsD	Acetolactate decarboxylase	
ilvB	Acetolactate synthase large subunit	
ilvH	Acetolactate synthase small subunit	
bdhA	2, 3-butanediol dehydrogenase	2, 3-butanediol synthesis
treA	Trehalose-6-phosphate hydrolase	Trehalose synthesis
treP	Trehalose permease component	
treR	Tre operon transcriptional repressor	
phy	3-Phytase	Phytase synthesis
ysnE	N-acetyltransferase	IAA synthesis
dhaS	Indol 3-acet-aldehyde dehydrogenase	
yhcX	Nitrilase	
speE	Spermidine synthase	Spermidine synthesis
speA	Arginine decarboxylase	
speB	Agmatinase	
potA	Spermidine import ATP-binding protein	
mdtJ	Spermidine export protein	
moaABCDE	Molybdenum cofactor synthesis protein	Nitrogen utilization
nasD	Nitrite reductase large subunit	Nitrogen utilization
nasE	Nitrite reductase small subunit	
nrgB	Nitrogen regulatory protein	
nrgA	Ammonium transporter	
nark	Nitrate transporter	
narG-J	Nitrate reductase	
narP	Nitrate/nitrite response regulator	
ktrA-D	Potassium uptake protein *A-D*	Potassium utilization
mgtE	Mg^{2+} transporter	Magnesium utilization
corA	Mg^{2+}/Co^{2+} transport protein	

2.4. Plant growth-promoting related features

Plant growth-promoting related features of endophytic bacterium LDO2 were assayed following standard procedures. For phosphate solubilization, strain LDO2 was incubated on inorganic phosphorus medium containing tricalcium phosphate, and cultured at 35 °C for 7 d to observe whether the phosphate-solubilizing halo was appeared. The capacity of strain LDO2 to produce siderophore was detected by UHPLC-ESI-MS (Miethke et al., 2006).

Pot experiments were employed for evaluation the growth-promoting effect of strain LDO2 according to the procedure of Taurian et al. (2010) with some modifications. Briefly, peanut seeds (cultivar HY15) were surface sterilized by immersion in 75% ethanol for 3 min, 3% sodium hypochlorite for 6 min, and 75% ethanol for 30 s, then rinsed six times with sterile distilled water. Surface sterilized peanut seeds were germinated in dark at 28 °C in sterilized Petri dishes with one layer of filter paper and moist cotton. Pre-germinated peanut seeds with 2 cm radicle were transferred to sterilized plastic cups (5 cm diameter, 0.16 L) filled with sterilized garden soil. Each cup was inoculated with 5 mL of 48 h Landy medium broth of strain LDO2 (10^9 cfu/mL), with un-inoculated Landy medium as control. Peanut seedlings were grown for 2 weeks after inoculation under controlled environment in a sunlight greenhouse, and peanut seedling height and dry weight, root length and dry weight was measured and compared with control. Each experiment was repeated at least three times.

2.5. Genome sequencing, assembly and annotation

Strain LDO2 was cultured in nutrient broth at 35 °C for 24 h, then genomic DNA was extracted using a bacterial genomic DNA extraction kit. Then a 20 kb insert SMRTbell DNA library was constructed and sequenced on the single molecule real-time (SMRT) sequencing platform using the PacBio RS II sequencer (Faino et al., 2015). After quality filtering, 180,480 qualified reads and 1,745,578,618 bp high-quality data, corresponding to 329 folds of genomic coverage, were obtained and de novo assembled into a single contig by Canu v1.5 (Koren et al., 2017).

Gene predictions were performed by Prodigal v2.50) (E-value < 10^{-5}) (Hyatt et al., 2010), rRNA genes and non-coding RNA genes were predicted by Infernal v1.1 (Nawrocki et al., 2015), and tRNA genes were predicted by tRNAscan-SE v1.3.1 (Lowe and Eddy, 1997). Secreted protein coding genes were predicted by SignalP v4.0 and Tmhmm v2.0c together (Krogh et al., 2001; Tjalsma et al., 2004; Petersen et al., 2011). Functional annotations of predicted genes were based on BlastP similarity searches (E-value < 10^{-5}) against different databases including Clusters of Orthologous Groups database (COG, http://www.ncbi.nlm.nih.gov/COG/), Kyoto Encyclopedia of Genes and Genomes database (KEGG, http://www.kegg.jp/kegg/), NCBI Non-Redundant Protein database (Nr, http://www.ncbi.nlm.nih.gov/), the UniProtKB/Swiss-Prot Knowledgebase (Swiss-Prot, http://www.uniprot.org/uniprot), and the UniProtKB/TrEMBL Knowledgebase (TrEMBL, http://www.uniprot.org/uniprot/), while HMMER v3.0 and Blast2GO v2.5 was used for annotation based on the Pfam protein domain database (Pfam, http://pfam.xfam.org/) and the Gene Ontology database (GO, http://www.geneontology.org/). Secondary metabolite gene clusters were predicted by antiSMASH v4.1.0 (Blin et al., 2017).

2.6. Statistical analysis

Means and standard deviations were calculated and statistically analyzed by an analysis of variance (ANOVA) at the 5% level using SPSS v13.0.

3. Results and discussion

3.1. Strain identification

Morphological observation showed cells of strain LDO2 were rod-shaped, Gram-positive, with spores and without capsules, colonies growing on nutrient agar were opaque, rough, and creamy white colonies with irregular edge. Physiologically, strain LDO2 can utilize glucose, sucrose, fructose, mannose, ribose and maltose, but cannot utilize ethanol, chitin and cellulose. Meanwhile, strain LDO2 were positive for glucose fermentation, casein hydrolysis, gelatin liquefaction, starch hydrolysis, but negative for oxidase reaction and indole production. Furthermore, the 16 s rDNA sequence of strain LDO2 (GenBank accession number MH142253) showed 100% homology to that of *Bacillus velezensis* strain ZF2 (GenBank accession number MH394318) and *B. velezensis* strain WK1 (GenBank accession number MF471767). And the Neighbor-joining tree also showed strain LDO2 formed a cluster closely related to *B. velezensis* strains (Fig. 1). Therefore, strain LDO2 should be a member of species *B. velezensis*.

3.2. Antimicrobial activity of strain LDO2

Plate co-culture assay showed *B. velezensis* LDO2 significantly inhibited the growth of *A. tenuissima, A. flavus, A. niger, F. oxysporum, F. moniliforme, R. solani, Rhizopus* sp., with 78.97%, 80.77%, 79.74%, 81.03%, 81.28%, 79.23%, 75.64% of inhibition ratio, respectively (Table 1). Specifically, strain LDO2 significantly inhibited mycelial growth of *A. flavus* (Fig. 2A), and caused the emergence of mycelial deformity (swelled hyphae) (Fig. 2B). In addition, cylinder-plate assay showed that an obvious halo was present around the cylinder treated with LDO2 broth, indicated that strain LDO2 also had obvious inhibiting effect on *R. solanacearum*. These results indicated that strain LDO2 had significant antimicrobial activity to peanut fungal and bacterial pathogens.

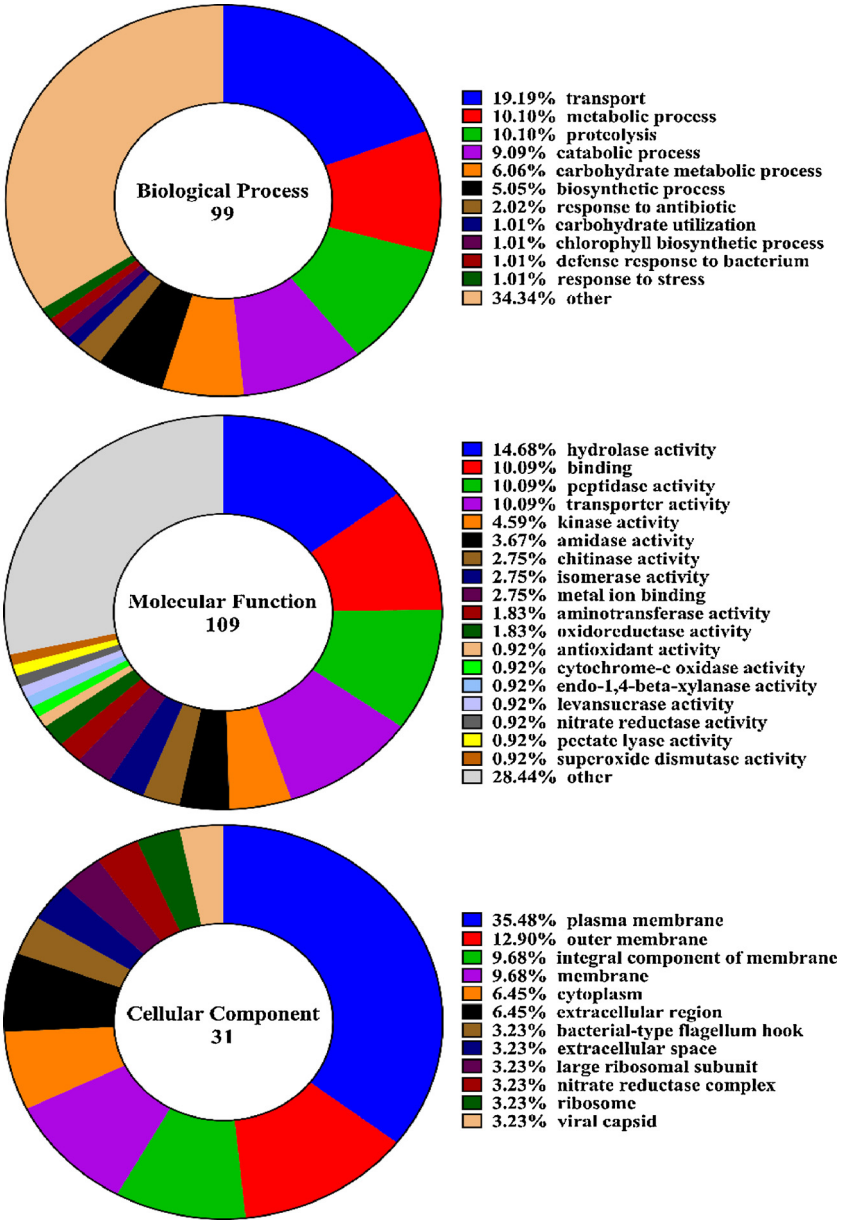

Fig. 5. Functional analysis of secreted protein of strain LDO2 by GO terms enrichment.

3.3. Plant growth-promoting related features

This peanut endophyte exhibited several growth-promoting related features including phosphate solubilization, siderophore production and growth promotion of peanut root. In detail, the phosphate-solubilizing halos were obviously observed around the colony of strain LDO2 (Fig. 2C). Bacillibactin, one of the strongest siderophore, was detected in LDO2 broth by UHPLC-ESI-MS, ions of *m/z* values 883.2607 and 905.2427 corresponded to bacillibactin $[M+H]^+$) and bacillibactin $[M + Na]^+$, respectively (Fig. 3A). Furthermore, greenhouse tests showed a significant increase in peanut seedling height, seedling dry weight, root length and root dry weight was obtained in the treated peanut plants compared with un-inoculated control plants ($P < 0.05$) (Fig. 2D), indicating that strain LDO2 could promote the growth of peanut seedlings.

3.4. Genomic features of strain LDO2

Strain LDO2 had a 3,947,271 bp circular chromosome with an average G + C content of 46.50% (GenBank accession number CP029034). A total of 3774 protein-coding genes, 27 rRNA genes, 86 tRNA genes and 135 secreted protein coding genes were predicted in the genome (Fig. 4). Among the 3774 protein-coding genes, 3765 (99.76%) ones were annotated with predicted function. Furthermore, 2888 genes were classified into 20 COG categories, and most of them were associated with functions such as amino acid transport and metabolism, carbohydrate transport and metabolism, transcription, inorganic ion transport and metabolism, and secondary metabolites biosynthesis, transport and catabolism (Fig. 4). To date, few complete genome sequences of peanut endophytic bacteria have been published.

3.5. Genetic basis for pathogens inhibition

Through genome mining, 32 putative gene clusters involved in the biosynthesis of secondary metabolite were identified in the LDO2 genome by antiSMASH v4.1.0 (Fig. S1), and 8 of which were identified to associate with exact antimicrobial secondary metabolites, including four polyketide antibiotics (butirosin, bacillaene, difficidin,

macrolactin), two cyclic lipopeptides (fengycin, surfactin), one siderophore (bacillibactin) and one dipeptide (bacilysin) (Table 2). Except the siderophore bacillibactin, butirosin, bacillaene, difficidin, and macrolactin were reported to possess excellent antibacterial activity (Chen et al., 2006; Wu et al., 2015), fengycin was well-recognized for strong antifungal activity against filamentous fungi (Ongena and Jacques, 2008), while surfactin and bacilysin were proved to have antibacterial and antifungal activity (Phister et al., 2004; Ongena and Jacques, 2008; Jiang et al., 2016). More importantly, one antifungal metabolite (fengycin), two multifunctional (antifungal and antibacterial) metabolites (surfactin, bacilysin) and two antibacterial metabolites (bacillaene, macrolactin) were successfully detected through UHPLC-ESI-MS, confirming their successful production of strain LDO2 (Fig. 3). More valuably, the coproduction of these antimicrobial metabolites may generate synergistic effect against pathogens. These metabolites constituted the basis for pathogens inhibition of this endophytic bacterium.

3.6. Genetic basis for plant growth promotion

A series of genes/gene clusters associated with plant growth promotion were identified in the LDO2 genome, including siderophore synthesis, production of growth-promoting VOCs and growth-promoting hormones, and nutrition utilization (Table 4). The siderophore bacillibactin favors for plant growth and contribute to protect plant against pathogenic infections by complexing iron and making iron less available to pathogens (Arguelles-Arias et al., 2009). The *dhb* gene cluster encoding the synthetase of bacillibactin, is present in the LDO2 genome (Tables 2 and 3). The two well-known VOCs, 3-hydroxy-2-butanone and 2, 3-butendiol, are reported to promote plant growth and induce systemic resistance against plant pathogens (Fincheira and Quiroz, 2018). And genes responsible for the synthesis of 3-hydroxy-2-butanone including acetolactate synthase (*alsS*), acetolactate decarboxylase (*alsD*), acetolactate synthase large subunit (*ilvB*) and acetolactate synthase small subunit (*ilvH*), are found in the genome (Table 4). Meanwhile, gene encoding 2, 3-butanediol dehydrogenase (*bdhA*), which catalyzes 3-hydroxy-2-butanone to 2, 3-butanediol, is also found (Table 4). Besides, genes involved in the production of growth-promoting hormones (indole acetic acid (IAA), phytase and trehalose) are both found in the genome (Table 3). Trehalose can prevent plant cell desiccation and water loss, and its accumulation was proved to improve the viability of peanut rhizobia strains and the resistance of peanut against various environmental stresses (Dardanelli et al., 2000). In addition, genes responsible for the synthesis of spermidine including spermidine synthase (*speE*), arginine decarboxylase (*speA*), agmatinase (*speB*), spermidine import ATP-binding protein (*potA*) and spermidine export protein (*mdtJ*), are found (Table 4). Spermidine was shown to play important roles in plant growth, shoot differentiation, and abiotic stress responses (Vasudevan et al., 2008; Zhou et al., 2016). And more genes/gene clusters related to plant growth promotion are summarized in Table 4.

3.7. Genes related to secreted proteins

Endophytic bacteria colonize the internal tissues of host plant, form a stable mutualistic relationships with the host and share some metabolisms as well as metabolites (Ryan et al., 2008). Notably, secreted proteins are one of them, which is known to play several very important roles such as nutrients uptake, cell communication, detoxification or competitors killing. In the LDO2 genome, 135 genes encoding secreted proteins, 3.57% of the total protein-coding genes, were identified. And a total of 86 genes were annotated with 239 GO terms, 99, 109, 31 GO terms corresponded to the categories biological process, molecular function and cellular component (Fig. 5). Detailed, the top 3 terms for biological process were transport (19.19%), metabolic process (10.10%) and proteolysis (10.10%), the top 3 terms for molecular function were hydrolase activity (14.68%), binding (10.09%) and peptidase activity/transporter activity (10.09%), while plasma membrane was the most common one for cellular component (Fig. 5). Furthermore, among the 135 genes encoding secreted proteins, 42 genes (31.11%) encoded various secretases including hydrolases, oxidoreductases, transferases, lyases and isomerases. While the hydrolase (64.29%) was the most common ones in these secretases, such as acylhydrolase, amidase, arabinosidase, carboxypeptidase, esterase, lactamase, peptidase, phytase and xylanase. All of the secreted proteins as well as secretases provide a solid foundation and better understand for this endophytic bacterium in transport, metabolism and nutrient utilization.

4. Conclusion

The peanut endophyte *B. velezensis* LDO2 possessed the powerful capabilities to synthesize kinds of antimicrobial metabolites, showing strong antagonistic activities against peanut pathogenic fungi and bacteria, and it also exhibited several growth-promoting related features. These findings suggested that this peanut endophyte could be a potential biocontrol agent in peanut production and a source of antimicrobial compounds for further exploitation. In addition, the complete genome of strain LDO2 provided effective ways to understand the genetic mechanisms underlying pathogens inhibition and growth promotion, and basic support to develop this endophyte as a microbial inoculum in future. To our knowledge, few complete genome sequences of peanut endophytic bacteria have been published.

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References

Arguelles-Arias, A., Ongena, M., Halimi, B., Lara, Y., Brans, A., Joris, B., Fickers, P., 2009. *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. Microb. Cell Fact. 8, 63.

Blin, K., Wolf, T., Chevrette, M.G., Lu, X., Schwalen, C.J., Kautsar, S.A., Suarez Duran, H.G., De Los Santos, E.L., Kim, H.U., Nave, M., 2017. antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res. 45, W36–W41.

Chen, X.H., Vater, J., Piel, J., Franke, P., Scholz, R., Schneider, K., Koumoutsi, A., Hitzeroth, G., Grammel, N., Strittmatter, A.W., Gottschalk, G., Sussmuth, R.D., Borriss, R., 2006. Structural and functional characterization of three polyketide synthase gene clusters in *Bacillus amyloliquefaciens* FZB42. J. Bacteriol. 188, 4024–4036.

Chen, L., Heng, J.Y., Qin, S.Y., Bian, K., 2018. A comprehensive understanding of the biocontrol potential of *Bacillus velezensis* LM2303 against *Fusarium* head blight. PLoS One 13, e0198560.

Dardanelli, M.S., González, P.S., Bueno, M.A., Ghittoni, N.E., 2000. Synthesis, accumulation and hydrolysis of trehalose during growth of peanut rhizobia in hyperosmotic media. J. Basic Microbiol. 40, 149–156.

Dey, R., Pal, K.K., Bhatt, D.M., Chauhan, S.M., 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol. Res. 159, 371–394.

Dong, X.Z., Cai, M.Y., Lu, Y.Y., Xie, J.Y., Liu, X.L., 2001. Identification methods of common bacteria. In: Dong, X.Z., Cai, M.Y. (Eds.), Handbook of Common Bacteria Systematic Identify. Science Press, Beijing, pp. 349–398 (in Chinese).

Eljounaidi, K., Lee, S.K., Bae, H., 2016. Bacterial endophytes as potential biocontrol agents of vascular wilt diseases-review and future prospects. Biol. Control 103, 62–68.

Faino, L., Seidl, M.F., Datema, E., van den Berg, G.C., Janssen, A., Wittenberg, A.H., Thomma, B.P., 2015. Single-molecule real-time sequencing combined with optical mapping yields completely finished fungal genome. MBio 6, e00936–00915.

Fincheira, P., Quiroz, A., 2018. Microbial volatiles as plant growth inducers. Microbiol. Res. 208, 63–75.

Gao, Z., Zhang, B., Liu, H., Han, J., Zhang, Y., 2017. Identification of endophytic *Bacillus velezensis* ZSY-1 strain and antifungal activity of its volatile compounds against *Alternaria solani* and *Botrytis cinerea*. Biol. Control 105, 27–39.

Guardado-Valdivia, L., Tovar-Pérez, E., Chacón-López, A., López-García, U., Gutiérrez-Martínez, P., Stoll, A., Aguilera, S., 2018. Identification and characterization of a new *Bacillus atrophaeus* strain B5 as biocontrol agent of postharvest anthracnose disease in soursop (*Annona muricata*) and avocado (*Persea americana*). Microbiol. Res. 210, 26–32.

Haggag, W.M., Timmusk, S., 2008. Colonization of peanut roots by biofilm-forming *Paenibacillus polymyxa* initiates biocontrol against crown rot disease. J. Appl. Microbiol. 104, 961–969.

Hyatt, D., Chen, G.L., Locascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J., 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinf. 11, 119.

Jiang, J., Gao, L., Bie, X., Lu, Z., Liu, H., Zhang, C., Lu, F., Zhao, H., 2016. Identification of novel surfactin derivatives from NRPS modification of *Bacillus subtilis* and its antifungal activity against *Fusarium moniliforme*. BMC Microbiol. 16, 31.

Jiang, G., Wei, Z., Xu, J., Chen, H., Zhang, Y., She, X., Macho, A.P., Ding, W., Liao, B.S., 2017. Bacterial wilt in China: history, current status, and future perspectives. Front. Plant Sci. 8, 1549.

Kong, Q., Shan, S., Liu, Q., Wang, X., Yu, F., 2010. Biocontrol of *Aspergillus flavus* on peanut kernels by use of a strain of marine *Bacillus megaterium*. Int. J. Food Microbiol. 139, 31–35.

Koren, S., Walenz, B.P., Berlin, K., Miller, J.R., Bergman, N.H., Phillippy, A.M., 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res. 27, 722–736.

Krogh, A., Larsson, B., von Heijne, G., Sonnhammer, E.L., 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. 305, 567–580.

Liu, J., Li, X., Jia, Z., Zhang, T., Wang, X., 2017. Effect of benzoic acid on soil microbial communities associated with soilborne peanut diseases. Appl. Soil Ecol. 110, 34–42.

Llewellyn, N.M., Li, Y., Spencer, J.B., 2007. Biosynthesis of butirosin: transfer and deprotection of the unique amino acid side chain. Chem. Biol. 14, 379–386.

Lowe, T.M., Eddy, S.R., 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25, 955–964.

Miethke, M., Klotz, O., Linne, U., May, J.J., Beckering, C.L., Marahiel, M.A., 2006. Ferribacillibactin uptake and hydrolysis in Bacillus subtilis. Mol. Microbiol. 61, 1413–1427.

Nawrocki, E.P., Burge, S.W., Bateman, A., Daub, J., Eberhardt, R.Y., Eddy, S.R., Floden, E.W., Gardner, P.P., Jones, T.A., Tate, J., Finn, R.D., 2015. Rfam 12.0: updates to the RNA families database. Nucleic Acids Res. 43, D130–137.

Nigam, S.N., Waliyar, F., Aruna, R., Reddy, S.V., Kumar, P.L., Craufurd, P.Q., Diallo, A.T., Ntare, B.R., Upadhyaya, H.D., 2009. Breeding peanut for resistance to Aflatoxin contamination at ICRISAT. Peanut Sci. 36, 42–49.

Ongena, M., Jacques, P., 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 16, 115–125.

Pal, K.K., Dey, R., Tilak, K., 2014. Fungal diseases of groundnut: control and future challenges. In: Goyal, A., Manoharachary, C. (Eds.), Future Challenges in Crop Protection Against Fungal Pathogens. Springer, New York, New York, pp. 1–29.

Petersen, T.N., Brunak, S., von Heijne, G., Nielsen, H., 2011. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat. Methods 8, 785–786.

Phister, T.G., O'Sullivan, D.J., McKay, L.L., 2004. Identification of bacilysin, chlorotetaine, and iturin A produced by *Bacillus sp.* strain CS93 isolated from pozol, a Mexican fermented maize dough. Appl. Environ. Microbiol. 70, 631–634.

Rojo, F.G., Reynoso, M.M., Ferez, M., Chulze, S.N., Torres, A.M., 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. Crop Protect 26, 549–555.

Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J., Dowling, D.N., 2008. Bacterial endophytes: recent developments and applications. FEMS Microbiol. Lett. 278, 1–9.

Santoyo, G., Moreno-Hagelsieb, G., Orozco-Mosqueda Mdel, C., Glick, B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183, 92–99.

Schneider, K., Chen, X.H., Vater, J., Franke, P., Nicholson, G., Borriss, R., Sussmuth, R.D., 2007. Macrolactin is the polyketide biosynthesis product of the pks2 cluster of *Bacillus amyloliquefaciens* FZB42. J. Nat. Prod. 70, 1417–1423.

Sobolev, V.S., Orner, V.A., Arias, R.S., 2013. Distribution of bacterial endophytes in peanut seeds obtained from axenic and control plant material under field conditions. Plant Soil 371, 367–376.

Sobolev, V., Arias, R., Goodman, K., Walk, T., Orner, V., Faustinelli, P., Massa, A., 2018. Suppression of Aflatoxin production in *Aspergillus* species by selected peanut (*Arachis hypogaea*) stilbenoids. J. Agric. Food. Chem. 66, 118–126.

Taurian, T., Anzuay, M.S., Angelini, J.G., Tonelli, M.L., Ludueña, L., Pena, D., Ibáñez, F., Fabra, A., 2010. Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329, 421–431.

Tjalsma, H., Antelmann, H., Jongbloed, J.D.H., Braun, P.G., Darmon, E., Dorenbos, R., Dubois, J.-Y.F., Westers, H., Zanen, G., Quax, W.J., Kuipers, O.P., Bron, S., Hecker, M., van Dijl, J.M., 2004. Proteomics of protein secretion by *Bacillus subtilis*: separating the "secrets" of the secretome. Microbiol. Mol. Biol. Rev. 68, 207–233.

Vargas Gil, S., Meriles, J.M., Haro, R., Casini, C., March, G.J., 2008. Crop rotation and tillage systems as a proactive strategy in the control of peanut fungal soilborne diseases. BioControl 53, 685–698.

Vasudevan, A., Selvaraj, N., Ganapathi, A., Kasthurirengan, S., Ramesh Anbazhagan, V., Manickavasagam, M., Choi, C.W., 2008. Leucine and spermidine enhance shoot differentiation in cucumber (*Cucumis sativus* L.). In Vitro Cell. Dev. Biol. Plant 44, 300.

Wu, L., Wu, H., Chen, L., Yu, X., Borriss, R., Gao, X., 2015. Difficidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice pathogens. Sci. Rep. 5, 12975.

Zhou, C., Ma, Z., Zhu, L., Xiao, X., Xie, Y., Zhu, J., Wang, J., 2016. Rhizobacterial strain *Bacillus megaterium* BOFC15 induces cellular polyamine changes that improve plant growth and drought resistance. Int. J. Mol. Sci. 17, 976.