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Evaluating the potential of *Bacillus licheniformis* YZCUO202005 isolated from lichens in maize growth promotion and biocontrol

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ABSTRACT

Lichens exist in an organismal organization of mycobiont, photobiont, and non-photoautotrophic bacteria. These organisms contribute to the growth of lichens even in poor nutrition substrates. However, studies on the isolation and application of non-photoautotrophic bacteria in plant growth and biocontrol are scanty. Therefore, a study was conducted to isolate and evaluate the potential of non-photoautotrophic bacteria from lichen tissues in maize plant growth promotion and biocontrol of plant pathogens (fungi and bacteria). Five bacterial strains were isolated and tested for their ability to produce indole-3-Acetic Acid (IAA). One bacterium named YZCUO202005 produced IAA, siderophores and biofilms, solubilized phosphate and potassium and exhibited extracellular enzymes (cellulases, proteases, amylase, and β −1,3-Glucanase). Based on the 16S rRNA sequence analysis results, YZCUO202005 was identified as *Bacillus licheniformis*. The strain inhibited the growth of five pathogenic fungi with an inhibition percent of between 58.7% and 71.7% and two pathogenic bacteria. Under greenhouse conditions, YZCUO202005 was tested for its abilities to enhance maize seed germination, and vegetative growth. Compared with the control treatment, the strain significantly enhanced the growth of stem length (i.e. 18 ± 0.64 cm, 78 ± 0.92 cm), leaf length (i.e. 10 ± 0.36 cm, 57 ± 1.42 cm), leaf chlorophyll levels (i.e., 13 ± 0.40, 40 ± 0.43 SPAD), and root length (i.e, 9.8 ± 2.25 cm, 22.5 ± 6.59 cm). Our results demonstrated that *B. licheniformis* YZCUO202005 from lichens has the potential to promote plant growth and reduce fungal and bacterial pathogens' growth. Furthermore, the results suggest that lichens are naturally rich sources of plant growth promotion and biocontrol agents that would be used in agriculture.

1. Introduction

Lichens are ecologically diversified, widely distributed, and are used in various applications including food production and biotechnology [1,2]. Lichen is one of the unique life forms on earth that exists in an organismal association consisting of mycobiont (fungi), photobiont (green algae and/or cyanobacteria), and non-photoautotrophic bacteria [3]. Each of these microorganisms provides for the growth and survival of lichen including in most extreme stresses [4,5].

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Lichens harbor diverse bacterial strains that produce compounds with the potential to be used in biotechnology such as *Bacillus*, *Paenbacillus*, *Pseudomonas*, *Nostoc* and *Burkholderia* [6]. Other strains isolated from lichens have been reported to have relevance in drug discovery because of their ability to produce bioactive molecules that act as antitumor, antimicrobials, and inhibitor proteins [7, 8]. On the other hand, previous studies have also shown that lichen-associated bacterial communities exhibit growth-promoting and biocontrol properties such as bio-compounds, phytohormones, mineral elements (nitrogen, phosphate, potassium, siderophores, etc) and ensuring their availability and acquisition by plants [9,10].

Based on the above background, lichens could be regarded as a source of plant growth promotion and biocontrol bacterial agents. However, many studies have not emphasized on the isolation of non-photoautotrophic bacteria with plant growth promotion from lichens [11,12] despite the contribution these bacteria offer to the growth of lichens. Therefore, the isolation and determination of non-photoautotrophic bacterial strains would be a milestone in the discovery and exploration of plant growth promotion and biocontrol agents in agriculture.

Although there are a dozen cereal crops used for food, maize is an important human food and calories source globally [13]. Despite being an important crop, maize plants are so sensitive to environmental stresses [14]. Moreover, seed dormancy delays germination resulting in non-uniform germination and seedling development [15]. To overcome this challenge, studies have reported the ability of the beneficial bacteria to enhance seed germination and promote the vegetative growth of maize plants in various conditions [16]. However, the information about the application of lichen-associated bacterial strains in maize production is scanty. Therefore, based on the food and economic importance of maize globally and considering its nutrition requirements to germinate, grow and develop into a full plant and reproduce, a study was conducted to determine the influence of lichen associated bacteria on maize growth and development.

This study aimed to isolate lichen-associated non-photoautotrophic bacteria with high potential to promote plant growth from *Collema undulatum* lichens taking advantage of the ability of this lichen to grow in poor nutrition substates. Specifically, the research isolated and evaluated lichen-associated bacteria with the potential to (1) exhibit plant-growth-promoting and biocontrol traits; (2) promote maize seed germination, and vegetative growth. The study will help in furthering our understanding on the positive interactions between plants and beneficial bacteria from lichens that would be incorporated into sustainable agriculture.

2. Materials and methods

2.1. Collection of Collema undulatum lichens and bacteria isolation

Collema undulatum lichens were collected from the uncultivated fields around the College of Agriculture of Yangtze University (112.22 E, 30.34 N) during the 2019–2020 period. The samples were packed in sterile plastic bags and transferred to the laboratory. To isolate the non-photoautotrophic bacteria, lichen thalli were washed with running tap water to remove soil particles and other debris. Isolation was done on the sterilized laminar floor to control the entrance of foreign microorganisms. Lichen thalli were washed with sterilized water two times. To ensure external disinfection, lichen thalli were dipped in 2.5% NaClO two times for 30 s each then rinsed with sterilized distilled water 5 times for 30 s. Subsequently, lichen thalli were macerated into homogenized pieces using a mortar followed by serial dilution of up to 10^{-5} in microcentrifuge tubes. Thereafter, 20 μL of ground lichen thalli solution was inoculated into the LB agar plates. All inoculated Petri dishes were incubated at 28 °C for 5 days. The bacteria colony characteristics such as color, texture, and shape, were observed and single colonies were collected and restreaked on the LB agar plates for 3 days.

2.2. Qualitative determination of indole-3-acetic acid

All the five lichen-associated bacterial strains under study were determined for IAA synthesis and production. The method described by Ndeddy Aka and Babalola [17] was followed with some modifications. The 20 μL of freshly prepared bacterial culture was inoculated in 50 mL LB liquid media amended with ʟ -tryptophan (0.2 g/L) and incubated at 28 °C for 48 h. Then 1 mL of bacteria culture was collected and centrifuged at 10,000 rpm for 10 min. Thereafter, 1 mL of supernatant was collected and added to 0.5 mL of Salkowasky reagent (50 mL of 35% perchloric acid in 1 mL of 0.5 M $FeCl_3$). The mixture was left to stand in the dark at 25 °C for 30 min. LB liquid media without bacterial inoculation served as a control. The development of the pink color confirmed the production of IAA. Among the five bacterial strains, one strain with abilities to produce IAA was selected for further study taking into considerations the crucial role that IAA plays in enhancing plant and microbial interactions and plant growth and development [18–20]. The experiment was repeated three times to validate the findings.

2.3. Quantitative determination of indo-3-acetic acid (IAA)

To determine IAA production quantitatively, a modified Salkowski colorimetric technique was used [21]. In brief, reagent S2 was made by adding 4.5 g $FeCl_3$ dissolved in 413 mL distilled water, then slowly adding 587 mL of 98% H_2SO_4. The bacterial strain was initially cultured in LB liquid medium supplemented with 2.5 mM ʟ -tryptophan (Trp) (0.2 g/L) in a 200 rpm/min rotary shaker (LH-111B model, Zhengzhou Laboao Istrument Equipment Co., Zhenghou, China) at 28 °C. Then 2 mL of centrifuged supernatant of bacteria were collected every 24 h for 10 days, added to 2 mL of S2 reagent and mixed thoroughly. The mixture was incubated in the dark at 25 °C for 30 min. The absorbance was measured by using spectrophotometer (GZ-83059-15, Cole-Parmer Instrument, MAPADA Instruments, Shanghai, China) at OD_{530}. IAA standard concentrations for the standard curve were set up with the following values: 0, 50, 100, 150, and 200 mg/L. The experiment was done in triplicates.

2.4. Molecular identification and phylogenetic analysis

Genomic DNA of isolated bacterial strain was extracted using a Bio-tek Bacterial DNA kit (Omega Bio-tek, Inc. Georgia, USA), according to the manufacturer's instructions. The 16S rRNAs gene fragments were later amplified using universal primer pair with 27F and 1492R [22]. The total PCR reaction was 40 μL including 2 μL of DNA template, 20 μL of 2 × Taq PCR super mix, 2 μL of each primer, and 14 μL of ddH_2O. The amplification process had thermal cycling conditions of 98 °C for 3 min, 34 cycles of denaturation at 98 °C for 30 s, annealing temperature at 55 °C for 30 s, and synthesis process at 72°Cfor 1 min, an extension of 72 °C for 5 min, and the infinity cooling temperature of 10 °C. To determine the presence of the NAC gene, specific primers based on all the known NAC genes at the time of design were chosen from the highly conserved amino acid sequence (Table 1). The total PCR reaction was 40 μL including 2 μL of DNA template, 20 μL of 2 × Taq PCR super mix, 16 μL of sterilized double distilled water, and 1 μl of each primer pair sequence. The amplification process had thermal cycling conditions of 98 °C for 3 min, 34 cycles of denaturation at 98 °C for 30 s, annealing temperature of between 52 °C- 58 °C for 30 s, synthesis process at 72 °C for 1 min, an extension of 72 °C for 5 min, and the infinity cooling temperature of 16 °C. To determine the presence of the *nif* gene, specific primers based on the known *nif* genes at the time of design were chosen from the highly conserved amino acid sequence (Table 1). The total PCR reaction was 40 μL including 2 μL of DNA template, 20 μL of 2 × Taq PCR super mix, 16 μL of sterilized double distilled water, and 1 μl of each primer pair sequence. The amplification process had thermal cycling conditions of 98 °C for 3 min, 34 cycles of denaturation at 98 °C for 30 s, annealing temperature of between 55 °C-58 °C for 30 s, synthesis process at 72 °C for 1 min, an extension of 72 °C for 5 min, and the infinity cooling temperature of 16 °C.

2.5. Effect of YZCUO202005 on plant nutrients availability

2.5.1. Nitrogen fixation

To examine the potential of isolated bacteria strain to fix atmospheric nitrogen, the NFb medium was used [24]. The medium contains (g L^{-1}): malic acid, 5.0, K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $CaCl_2$. $2H_2O$, 0.02; micronutrient solution ($CuSO_4$. $7H_2O$, 0.12, H_3BO_3, 1.40; Na_2MoO_4. H_2O, 1.0; $MnSO_4$. H_2O, 1.175. Complete volume to 1000 mL with distilled water), 2 mL bromothymol blue (5 g/L in 0.2 N KOH), 2 mL; FeEDTA (solution 16.4 g/L), 4 mL; vitamine solution (biotin, 10 mg; pyridoxal-HCl, 20 mg), 1 mL; KOH, 4.5 g; and 15 g agar to make a solid medium. All the inoculated culture media plates were incubated at 28 °C for 14 days for potential bacteria growth. The experiment was done thrice in triplicates to validate the results.

2.5.2. Phosphate solubilization

The isolated bacterial strain was tested for its activity on tricalcium phosphate to release inorganic phosphate using the National Botanical Research Institute Phosphate (NBRIP, Rana Pratap Marg, P.B. 436, Lucknow 226001, India) growth medium containg the following ingredients per liter: glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $MgCl_2$. $6H_2O$, 5 g; $MgSO_4$. $7H_2O$, 0.25 g; KCl, 0.2 g, and $(NH_4)_2$ SO_4, 0.1 g [25]. Initially, the bacteria cells were inoculated in LB liquid medium and incubated for 24 h. Then the 1.5 mL of bacteria culture was collected and centrifuged at 10,000 rpm at 25 °C for 10 min to remove LB liquid media. The bacterial cells were diluted with deionized water to a spectrophotometer concentration of $OD_{600} = 1.0$. Then 10 μL of bacterial suspension were spotted at the center of the NBRIP agar plates. The plates were incubated at 28 °C for 8 days. The experiment was done thrice in triplicates to validate the results.

Table 1
Primers used in this study.

Gene name	Primer Name	Primer Sequences (5′-3′)
NAC	Nac1-F	F: ATGAATTTAAGACGGCTGAAAT
	Nac1-R	R: CTAGCTGACCAGGGTGAGT
	Nac5-F	F: CATATTGCCCAGCCTGCGT
	Nac5-R	R: ATGTGAAAGGTTGAGCGATAA
	Nac8-Ft	F: ATGCGCGCGTTATTCTGCG
	Nac8-Rt	C: ATCCATGCCTGGGTGGAA
	NcF	F: GGCGGTGCTCTATGAGCGTT
	NcR	R: TTGCCGCGGCGGATAAAGAT
	NA-F	F: AACCTATCAGCGGCGGCTTC
	NA-R	R: TGTTGGCTAAGGGCCGGTTG
	NCCF	F: CCGTACAAAGCGGGGCGTTA
	NCCR	R: AGCTCACCAATTGCCACTGC
NifH	Pol1	F: 5′-CATATTGCCCAGCCTGCGT-3′
	Pol5	R: 5-ATGTGAAAGGTTGAGCGATAA-3
	NnF	F: TGGCGGTGCATAACGTTGGT
	NnR	R: CGGGCAGTTCAGATTTCCCGT

All the PCR products were analyzed by 1% agarose gel electrophoresis to formalize the DNA fragment length. The PCR products were sent to Tsingke Biotechnology Co., Ltd. (Wuhan, China) for DNA sequencing by the Sanger sequencing method. The sequences were submitted to Genebank and performed similarity analysis on the EZBio-Cloud database (https://www.ezbiocloud.net/). Some reference sequences were retrieved for further analysis. Phylogenetic analysis was conducted using the Neighbor-joining methods of the MEGA X [23]. Phylogenetic tree was evaluated by using the bootstrap method with 1000 repeats.

2.5.3. Potassium solubilization

To determine whether the strain could solubilize potassium, a modified Aleksandrovsk agar medium assay containing per liter glucose, 5.0 g, magnesium sulphate, 0.5 g; ferric chloride, 0.005; calcium carbonate, 0.1 g; calcium phosphate 2 g; supplemented with insoluble feldspar stone powder (Tianhuibao, Hebei Tianhuibao Technology Co. Ltd, Hebei, China) was employed. Aleksandrovsk agar medium plates amended with acid-base indicator phenol red dye [26] were made with wells in the center where 50 μL of bacteria suspension was inoculated. All the agar plates were incubated at 28 °C for 5 days and observed for the formation of clear halos around the wells.

2.5.4. Siderophore production

The CAS essay method for the detection of siderophores was employed for the determination of siderophore production with some modifications [27]. A 21.9 mg quantity of HDTMA was dissolved in 25 mL water while stirring constantly over low heat. In a separate container 1.5 mL of 1 mM $FeCl_3$.$6H_2O$ (in 10 mM HCl) was mixed with 7.5 mL of 2 mM CAS. This solution was slowly added to the HDTMA solution while stirring and the mixture was transferred to a 100 mL volumetric flask. Then 9.76 g of MES was dissolved in 50 mL of water to be used as a buffer. The pH of the solution was adjusted to 7.0 with KOH, and then the buffer solution was added to the volumetric flask. Thereafter, 15 g of agar were added to make a solid medium made with wells at the center where 50 μL bacterial suspension was added. Uninoculated CAS agar plates served as control. All the agar plates were incubated at 28 °C for 5 days. The test was done three times in triplicates. The formation of a yellow to orange visible halo around the bacteria colony was used as a sign of siderophore production.

2.6. Determination of extracellular enzymes activities

2.6.1. Cellulase enzymes activity

The basic mineral liquid medium with an addition of 0.2% Sodium Carboxymethyl Cellulose (CMC) (SINOCMC, Qingdao, China) was used [28]. The content of the CMC-agar medium included (L^{-1}) CMC, 10 g; KH_2PO_4, 4 g; Na_2HPO_4, 4 g; tryptone, 2 g; $MgSO_4$. $7H_2O$, 0.2 g; $CaCl_2$, 0.001; $FeSO_4$. $7H_2O$, 0.001 g; agar, 15 g; pH 7. In brief, bacteria cultures were grown on CMC liquid media for 24 h. Carboxymethyl Cellulose agar plates were made with wells on the center by cork borers. Then, 50 μL of bacterial cell culture was pipetted into the wells and incubated at 28 °C for 72 h. Thereafter, CMC agar plates were removed from the incubator, dyed with 1% Congo red dye and left to stand for 30 min before being washed with 5% sodium chloride for 5 min. The study was done in triplicates to validate the results. The formation of a round yellow halo was taken as evidence of the release of cellulase enzyme by the isolate.

2.6.2. Protease enzymes activity

Protease activity was detected on a potato dextrose agar (PDA) medium supplemented with double sterilized 5% fresh skimmed milk [29]. Wells were made on the center of the PDA plates using the sterile cork borers. Following this, 50 μL of the suspended isolated bacteria strain were added to the wells. Uninoculated agar medium plates served as control. All agar plates were incubated at 28 °C for 48 h and then observed for the formation of visible clear zones around the wells. The experiment was done three times to validate the results.

2.6.3. Amylase production

Amylase production was determined by the soluble starch agar medium containing 10 g peptone, 5 g yeast extract, 2 g soluble starch, and 20 g agar in 1 L distilled water, pH 7.0. After 48 h of incubation at 28 °C, the ability of the isolate to hydrolyze the amylase was determined by the appearance of a halo zone around the colonies and confirmed by the Lugol's iodine solution for 15 min and 70% ethanol. The uninoculated plates were used as a control [30].

2.6.4. β-1,3-Glucanase production

β −1,3-glucanase production was determined as previously described [31]. The bacterial isolate was inoculated for 5 days on the medium containing 0.03% yeast extract, 0.03% casamino acid, 0.03% D-glucoe, 0.05% K_2HPO_4, and 1.8% agar (*w/v*) which was later supplemented with 0.005% (*w/v*) aniline blue to confirm the formation of clear halo around the bacterial colony. The experiment was done in triplicates to validate the results.

2.7. Determination of biofilm formation

Determination of biofilm formation by the isolated lichen-associated bacteria strain was done by the crystal violet method [32,33]. A 5 mL of overnight bacteria strain culture was transferred into the 10 mL tubes for centrifugation at 10,000 rpm for 10 min. Bacteria cell pellets were collected and the supernatant was discarded. Collected bacteria cell pellets were diluted with the LB liquid media to a final concentration $OD_{600} = 0.1$, 0.5, and 1.0. Sterilized glass tubes were prepared into which 5 mL of bacteria culture were added and left to stand for 72 h at 28 °C. Glass tubes with an uninoculated media served as control. Bacteria cells and the medium from each glass tube were discarded and washed 3 times with sterilized distilled water. Thereafter, 5 mL of 1% crystal violet was added to each of the glass tubes and left to stand for 30 min at ambient temperature. Glass tubes were later washed with water 3 times to remove crystal violet stains and observe the presence of biofilms around the glass tubes.

2.8. Pot experiment for maize seed germination, vegetative growth, and adventitious root formation

The pot experiments were conducted to test the influence of the bacteria strain on the growth performance of maize plants. Hybrid maize (Jing Ke Nuo 2000, China) was used as the test variety. The experiment was conducted according to Tang et al. [16], with some modifications. The experiment was conducted in a greenhouse at the School of Agriculture of Yangtze University. The experimental site had an average temperature of 30 °C. Each plastic pots (bottom 18 cm × top 28 cm × height × 19 cm) were filled with 3 kg autoclaved soil (peat moss 10–15%, perlite 35–40%, coco peat 45–50%, and zeolite 6–8%, containing NH_4^+ ~90 mg/L, NO_3^- ~205 mg/L, P_2O_5 ~350 mg/L and K_2O ~100 mg/L). The treatments were the plastic pots treated with bacteria dissolved in water and pots treated with water only as a control. The treatments were laid out in a complete randomized design with 3 replicates.

Maize seeds were washed using sterilized water before sowing to remove any fungicidal coatings. Then the seed was surface disinfected with 0.2% NaClO for 2 min and rinsed 5 times with sterilized distilled water. The soil in each pot was kept moistened with distilled water before sowing. Four seeds were sown directly into the planting holes at a soil depth of 3 cm and covered with soil from the surface. At 7 days after the emergency of the seedlings, the number of germinated seeds were noted and the germination percentage was calculated. At the same time (7 days), thinning was done to 2 plants per pot to minimize competition. Soil moisture was maintained at field capacity using distilled water. Bacterial cells suspended in sterilized distilled water with a spectrophotometer reading of $OD_{600} = 1$ were inoculated into the soil 2 cm away from the maize stalk every 2 weeks. Measurements of various parameters were taken at 2 weeks, 4weeks 6weeks, and 8weeks after the emergence of the seedlings. The measuring tape was used to measure stem length and leaf length, a digital vernier caliper was used to measure stem diameter and leaf breadth while a SPAD meter (SPAD-502Plus, Konika Minota, Inc. Japan) was used to measure the amount of leafy chlorophyll levels. In 10th week, when the plant was tasseling, plant roots were removed from the soil and partially washed to remove the soil. Root length data were collected.

2.9. Antimicrobial activity of isolated bacterial strain

Pathogenic fungi and their host sources used during the assessment for antifungal abilities were *Fusarium oxysporum f.sp. vasinfectum,* (Cotton), *Fusarium graminearum,* (Wheat), *Nigrospora sphaerica,* (Sesame), *Rhizoctonia solani* (Rice), *Sclerotium rolfsii* (*Bletilla striata*). The pathogenic bacteria used were *Agrobacterium tumefaciens* and *Ralstonia solanacearum* isolated from peach roots and tobacco leaves respectively. All pathogenic fungi and bacteria were obtained from the Plant Pathology Laboratory of the College of Agriculture of Yangtze University.

A dual culture method was employed to assess the antifungal ability of the isolated lichen-associated bacteria isolate [34]. PDA media was plated out in petri dishes with three wells of 2.5 cm from the center of the dish. Bacteria cells were grown in a PDA liquid medium on the shaking incubator at 28 °C for 24 h. A loop of fungal mycelium was inoculated on the center of the agar medium plates followed by inoculation of 100 μL of prepared bacteria suspension onto the two of the PDA medium wells and finally the control (ddH_2O) onto the remaining well of the agar medium. The agar plates were incubated at 28 °C for 14 days. After 14 days the radii of fungal colonies were measured, and the inhibition ratio was calculated (equation (1)).

$$(R - r) \ / \ R \times 100, \tag{1}$$

where 'R' is the growth distance of the hyphal plug facing each hole in control plates, and 'r' is that in the treatment plates. The experiment was repeated three times in triplicates.

Antibacterial activity of the isolated bacterial strain was tested by cylinder-plate assay [35] with some modifications. Briefly, both the isolated bacterial strain and pathogenic bacteria were cultured in 50 mL of LB liquid on a rotary shaker at 130 rpm for 48 h. Thereafter, about 100 μL of the pathogenic bacteria fermentation broth was added to 100 mL of LB agar medium at around 40 °C, mixed rapidly and poured into Petri dishes (75 mm in diameter). After the LB agar medium cooled down, holes (5 mm in diameter) were made in the center of the LB solid plates with a sterile cork borer, and then 100 μL of bacterial culture of the isolated bacterial strain was added into each hole. Equal volume of LB liquid medium served as control. The plates were incubated at 28 °C for 48 h. The presence of inhibition zone around the hole indicated antibacterial activity, and the diameter of the inhibition zone was measured. Each experiment was performed three times.

2.10. Statistical analysis

The statistical analyses were performed using GraphPad Prism version 8 software by *t*-test ($P < 0.05$). The variation was recorded as mean ± SE (****:$P < 0.0001$, ***: $P < 0.001$, **$P < 0.01$, *:$P < 0.05$).

3. Results

3.1. Isolation and morphological characteristics of lichen associated bacteria strain

A total of 5 lichen-associated bacteria strains were isolated from lichen on the LB agar media. One bacterial strain named YZCUO202005 was selected based on IAA production for further analysis. The morphological and microscopic observation of the YZCUO202005 bacterial strain was found to be whitish, aerobic, spore-forming, motile, and rod-shaped. The biochemical results show that the bacterium is gram-positive and strongly catalytic. These characteristics showed that the bacterial isolate belongs to the genus

Bacillus.

3.2. Determination of indole-3-acetic acid

Among the 5 isolated bacterial strains, it was found that one test tube turned a pinkish color increasing in intensity for 30 min. This is a clear indication that this isolate could synthesize and produce IAA. No color changes were observed in the control. Another study was conducted to test the influence of L -tryptophan on IAA production. When L -tryptophan was added to the culture media, the results showed that a deep reddish color was produced in the tubes.

The potential quantity that the bacterial strain can produce was determined after every 24 h. Data was collected and compared with that of the preceding day. The IAA production of the isolate was further quantified by the calculated standard curve equation Y = 0.004716x-0.004317 (Fig. 2a). Our results found that IAA production was gradually increasing from day 0 with a maximum of 450 mg/L on the 9th day. On the 10th day of incubation, the IAA produced was stable as the previous day and starts decreasing in the next subsequent days (Fig. 2b).

3.3. Molecular identification of isolated bacterial strain

The sequence similarity analysis was conducted using EZBio-Cloud databases showing 99.17% similarity with *Bacillus licheniformis* strains. A neighbor-joining (NJ) analysis was conducted with an evolutionary model of K2 under 1000 bootstrap replicates by using MEGA X. The phylogenetic tree showed that the strain YZCUO202005 clustered with *Bacillus. licheniformis* strain ATCC 14580^T, CICC 10037 and SX-1 (Fig. 1). The 16S rRNA gene sequence (1440bp) of strain YZCUO202005 was deposited to NCBI GenBank under the accession number: MW672343.

3.4. Exhibition of plant nutrition elements

In addition to the production of IAA, the bacteria isolate was tested for its ability to produce other growth promotion and biocontrol traits. The bacteria isolate was found to grow on both liquid and solid minimal nitrogen medium (Fig. 3a). However, the growth on the solid medium was very slow and takes a minimum of 10 days. Because the bacteria were able to grow on a minimal nitrogen medium, efforts were made to identify the availability of the *nif* and NAC genes in its genomic DNA. PCR method using specific primers for nitrogen fixation and nitrogen assimilation control genes were used. However, no *nif* or NAC genes were identified.

On phosphate solubilization, there was a formation of a visible halo zone around the *B. licheniformis* YZCUO202005 on NBRIP media supplemented with tri-calcium phosphate (Fig. 3b). This indicates the ability of the isolated bacteria strain to solubilize

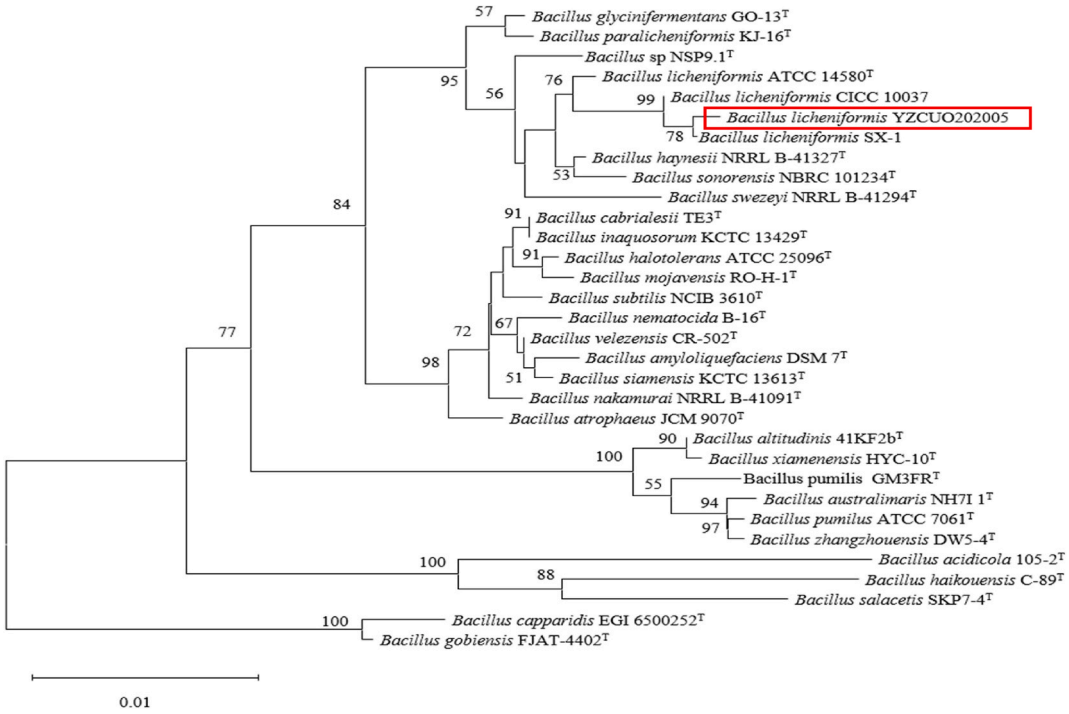

Fig. 1. Neighbor-joining tree derived from aligning most similar 16S rRNA sequences in related taxa for phylogenetic analysis of *B. licheniformis* YZCUO202005 using MEGA X.

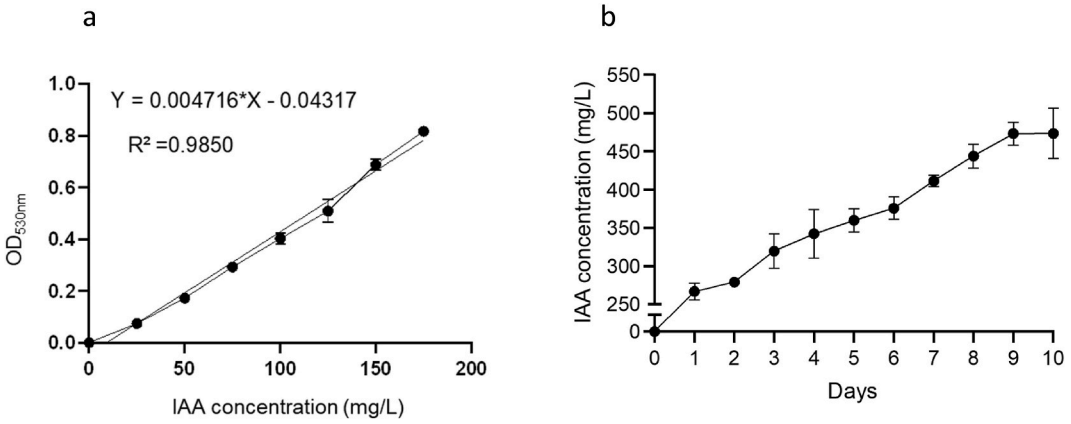

Fig. 2. (A) The IAA standard curve (b) and IAA production curve by lichen-associated YZCUO202005 bacteria. Bars indicate the standard error of the calculated means.

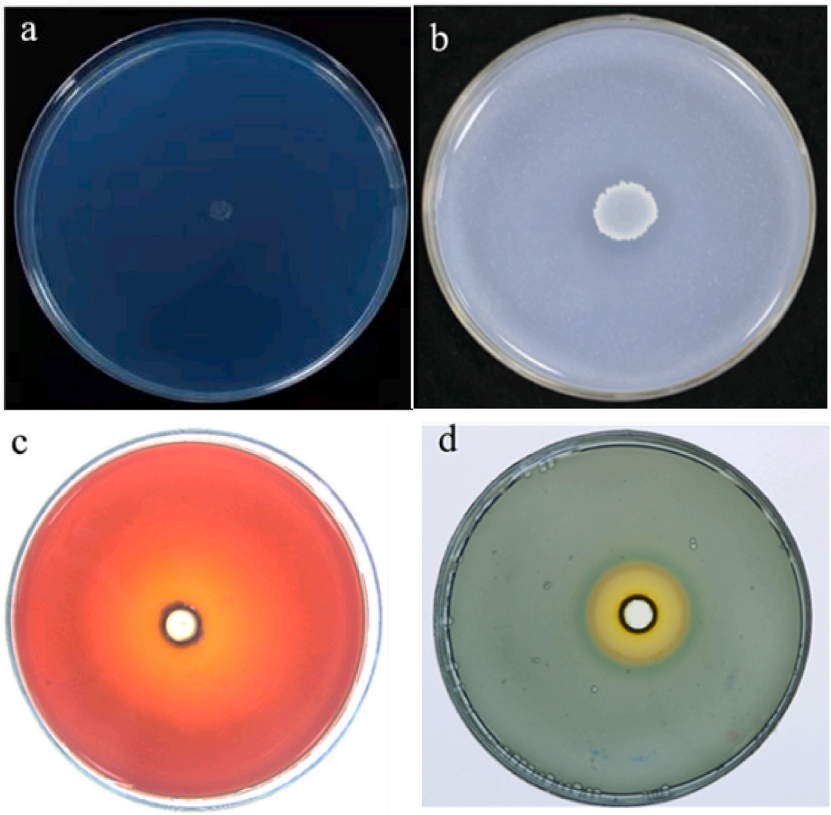

Fig. 3. The in vitro evaluation of plant growth promotion traits (a) nitrogen fixation (b) phosphate solubilization (c) potassium solubilization (d) siderophore production.

unavailable phosphate into a useable form. On the assessment of *B. licheniformis* YZCUO202005 to solubilize potassium and siderophore, the results were shown that the bacteria strain was able to form a clear orange halo on a modified Aleksandrovsk agar medium and yellowish zone on CAS agar plates respectively. Meanwhile, no color changes were observed in the control plates (Fig. 3c and d respectively).

3.5. In vitro exhibition of extracellular enzymes

Production of cellulase by the YZCUO202005 strain was measured by its ability to degrade CMC on the agar media. At 7days after

incubation, there was the formation of a large visible halo around the lichen-associated YZCUO202005 strain that confirmed the availability of cellulose-degrading enzyme produced by the bacteria in the presence of sodium carboxymethylcellulose (Fig. 4a). In the assessment of the *B. licheniformis* YZCUO202005 to degrade protein, the results showed that the bacterial strain had high activity to degrade proteins on the PDA that was supplemented with skimmed milk. The formation of a clear and visible halo zone around the center of the PDA agar plate indicated protease enzyme activity (Fig. 4b) compared with the water inoculated agar plate where there was no clear visible circle around the center.

B. licheniformis YZCUO202005 was also tested for its ability to hydrolyze the amylase. The amylase detection plate was stained with iodine solution after 48 h of culture, and a clear hydrolysis circle appeared around the colony, indicating that *B. licheniformis* YZCUO202005 produced amylase (Fig. 4c). β −1,3-Glucanase production was determined on agar medium. A positive reaction was read after 5days of incubation at 28 °C. There was a formation of a halo zone surrounding *B. licheniformis* YZCUO202005 indicating a positive result for β-1,3-Glucanase production (Fig. 4d). Since non-inoculated plates were used as control, there was no formation of the clear zone areas on these agar plates.

3.6. Biofilm production

Beneficial bacterial strains with ability to produce biofilms are crucial in promoting plant growth and biocontrol of plant pathogens. Our results show large and strong compact biofilms formed around the glass tubes where the bacterial strain was inoculated. The results confirm the ability of *B. licheniformis* YZCUO202005 to form biofilms. Amongst the concentrations, it was observed that the small biofilms were present in the lowest bacterial cell concentration ($OD_{600=}0.1$) and the big biofilms in the highest bacterial cell concentrations ($OD_{600=}1$). Based on these results, it is suggested that *B. licheniformis* YZCUO202005 strain biofilm formation is highly influenced by the bacterial cell concentration levels, a phenomenon that needs to be considered when deciding the bacterial concentration to inoculate in plants.

3.7. Pot experiment for maize growth and development

3.7.1. Influence of B. licheniformis YZCUO202005 on maize seed germination

It was observed that seeds from *B. licheniformis* YZCUO202005 treated pots germinated quickly with an average of 4 days after sowing and the emergence was uniform, and vigorous. On the other hand, the uninoculated seeds germinated late, and the emergence was very slow, weak, and ununiform (the earliest was on the 6th day). By the 14th day after germination, the interaction between the

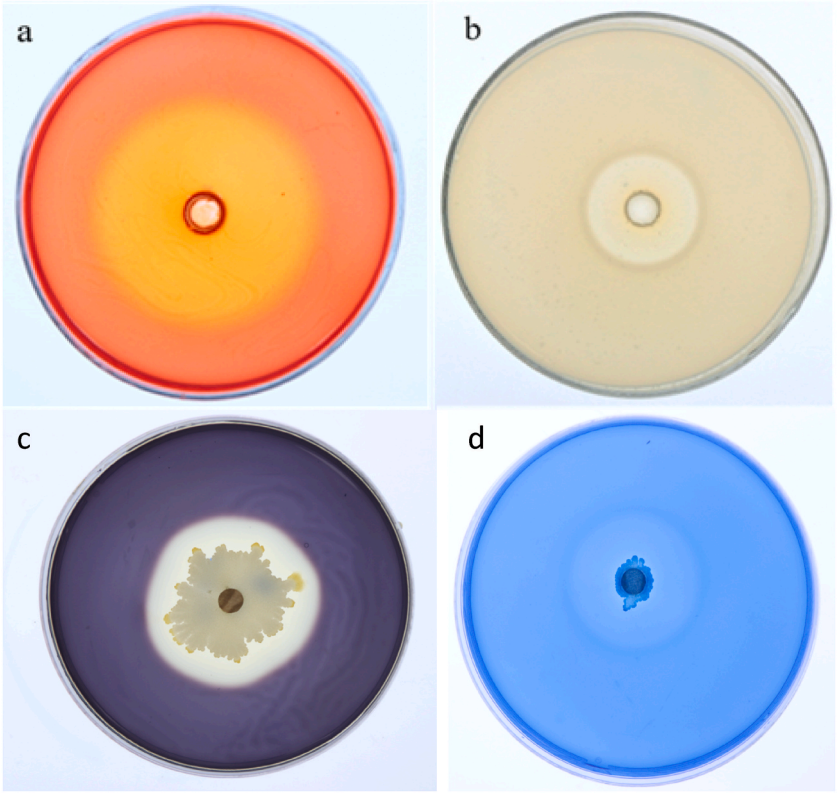

Fig. 4. The ability of *B. licheniformis* YZCUO202005 to produce (a) cellulase (b) protease (c) amylase (d) β-1,3-Glucanase enzymes.

YZCUO202005 strain and maize seedlings significantly improves the shoot length of maize seedlings. The results clearly show the influence of *B. licheniformis* YZCUO202005 on maize seed germination and early seedling growth.

3.7.2. Influence of B. licheniformis YZCUO202005 on maize growth and development

The plant growth and development of maize plants were measured at different time points (2, 4 6, 8, and 10 weeks after germination). Only at 2 weeks, YZCUO202005 strain inoculated plants showed a remarkable difference in growth in almost all growth parameters (Stem length stem diameter, leaf length, leaf breadth, and root length) as compared to the water-treated plants. At week number 8, the inoculated stem length was more than 4 times the stem length of the control treatment (78 ± 0.92 cm 18 ± 0.64 cm respectively) (Fig. 5a). The average stem diameter for YZCUO202005 strain inoculated plants were 10 times more than the control treatments (60 ± 1.05 mm and 6 ± 0.24 mm respectively) (Fig. 5b). Furthermore, throughout the vegetative stage, it was visually noted that there were significant differences in terms of leaf length, breadth, and green color between the *B. licheniformis* YZCUO202005 inoculated plants and the uninoculated plants. By 8th week, the average leaf length for *B. licheniformis* YZCUO202005 inoculated maize plants was 5.7 times more than the average length of the control plants (57 ± 1.42 cm and 10 ± 0.36 cm respectively) (Fig. 5c). The average leaf breadth of the inoculated plants was 3.4 times the average breadth of the uninoculated plants (68 ± 5.19 mm and 20 ± 0.72 mm respectively) (Fig. 5d). Like other leaf parameters, chlorophyll levels were also enhanced in the inoculated plants. The highest significance differences were noted during the 4th week where the average chlorophyll levels for *B. licheniformis* YZCUO202005 inoculated plants were 3 times more than the uninoculated plants with an average SPAD value of 40 ± 0.43 and 13 ± 0.40 SPAD respectively (Fig. 5e). In influencing the root development, elongation and growth, it was noted that *B. licheniformis* YZCUO202005 inoculated maize plants had more adventurous roots that were very long compared to that of the uninoculated plants. The *B. licheniformis* YZCUO202005 treatment root length was 2.3 times more than the uninoculated maize plants (225 ± 6.59 mm and 98 ± 2.25 mm respectively) (Fig. 5f). This significant difference indicates that *B. licheniformis* YZCUO202005 influences the elongation and development of adventurous roots in maize plants. Overall, the results demonstrated that *B. licheniformis* YZCUO202005 plays a significant role in positively influencing all growth parameters of the maize plants. This might be contributed by the availability of growth promotion traits attributed by the YZCUO202005 strain. On the other hand, the uninoculated maize plants experienced nutrition stress which hinders normal growth.

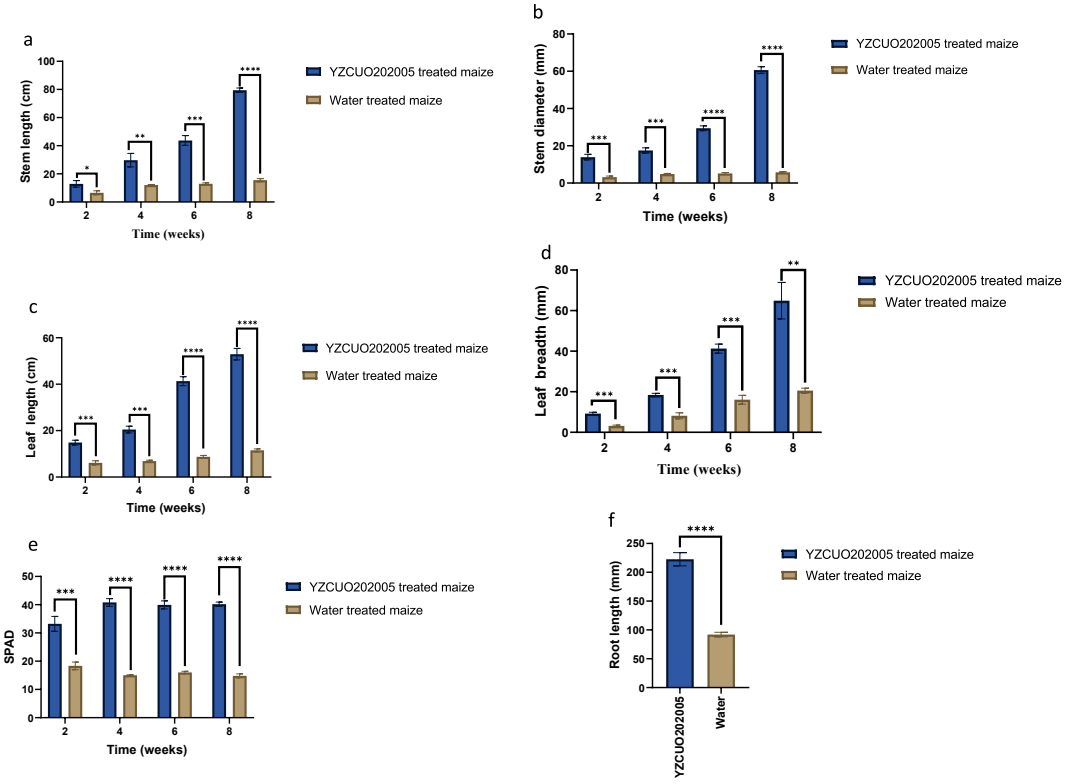

Fig. 5. The influence of the *B. licheniformis* YZCUO202005 strain on maize (a) stem length; (b) stem diameter (c) leaf length (d) lead breadth (e) chlorophyll level (f) root length. (10 maize plant samples from each treatment were used to measure stem length and stem diameter. 10 maize plant samples, 3 leaves per sample were used to measure leaf length, leaf breadth and chlorophyll levels. The variation was recorded as mean ± SE (****: $P < 0.0001$, ***: $P < 0.001$, **$P < 0.01$, *:$P < 0.05$).).

3.8. Antagonistic activity against pathogenic fungi and bacteria

The isolated YZCUO202005 strain inhibited the mycelia growth of pathogenic fungi when in a dual culture method is used, where a pathogen hyphal plug was placed in the center of each plate, and bacterial culture was injected into two of the three symmetrical holes. The pathogenic fungi include *F. oxysporum f.sp. vasinfectum, F. graminearum, N. sphaerica, R. solani,* and *S. rolfsii* (Fig. 6a–e). The average inhibition percentage of the YZCUO202005 strain against the pathogenic fungi ranges from 58.7% to 71.7% (Fig. 7). Furthermore, YZCUO202005 strain could inhibit the growth of *A. tumefaciens* and *R. solanacearum* bacterial pathogens (Fig. 6f and g).

4. Discussion

Auxins such as indole-3-acetic acid (IAA) regulate plant growth in several ways. IAA is soluble in aqueous solutions and, when protonated, diffuses passively across cell membranes without the need of a specific transporter [18,36]. The property of synthesizing IAA is considered an effective tool for screening beneficial microorganisms. Production of plant phytohormones is also one way in which plants use for their immediate growth as well as mitigation of biotic and abiotic stresses [19,37,38]. Furthermore, bacteria take advantage of their ability to produce IAA as a mechanism to interact with plants. IAA produced by bacteria is of paramount importance for root development in plants which in turn improves water and mineral uptake from the soil [39,40]. Based on previous studies on significance of IAA in enhancing plant and microbial interactions and plant growth, our study evaluated lichen associated non-photoautotrophic bacteria strain with abilities to produce indole acetic acid and was identified as *Bacillus licheniformis* YZCUO202005. In addition, beneficial microorganisms stimulate further plant growth by converting tryptophan to the phytohormone IAA [36]. In this study, when L-tryptophan was added into the culture media, more IAA was produced as compared to the tubes where no L -Tryptophan was added. These results are similar to what some researchers have reported before on the influence of L -Tryptophan in IAA biosynthesis and production [41]. Other studies reported that several Gram-positive bacteria including members of the *Bacilli* group produce a substantial amount of IAA [38], and our results are in agreement with such reports because lichen-associated *B. licheniformis* YZCUO202005 strain could produce up to 450 mg/L on the 9th day of incubation. Our results demonstrate that *B. licheniformis* YZCUO202005 strain from lichen provides IAA which would act as a growth precursor and defense mechanism in plants upon inoculation.

Plant growth-promoting bacteria are crucial in agriculture as they are determinants of soil quality and nutrients available to plants. In this study, *B. licheniformis* YZCUO202005 exhibit some of the plant growth-promoting attributes such as phosphate, potash, siderophores, and biofilms. Phosphorous and potassium form two of the three major plant nutrients. However, they both exist in forms that are unavailable to plants. As a result, many growers use synthetic fertilizers that are not environmentally friendly [42,43]. Previous studies have reported that phosphate solubilizing bacteria improves the nutrient availability in the soil, sparking plant growth and production of major crops [44]. Potassium solubilizing bacteria help to enrich the rhizosphere with potash that is needed to overcome abiotic stress and perform the synthesis of enzymes and proteins [45,46]. On the other hand, siderophores and biofilms produced by beneficial bacteria induce plant growth and help in controlling plant pathogens [47,48]. Our results suggest that the *B. licheniformis* YZCUO202005 would enhance the maize seed and plants' access to these nutrition elements for various metabolic processes, growth, and overcoming environmental stresses. It has been noted that the YZCUO202005 strain did not have *nif* genes responsible for nitrogen fixation though it shown growth on the minimal nitrogen media. However, previous studies reported that some lichens, particularly those without any contact with nitrogen-fixing bacteria, are usually supplied with nitrogenous compounds such as amino acids by lichen-associated bacteria [10]. Moreover, *Bacillus* spp. bacteria are commonly known to produce hydrolytic enzymes such as protease, amylase and cellulase that control how organisms function and facilitate nutrient availability respectively [49,50]. Proteases are

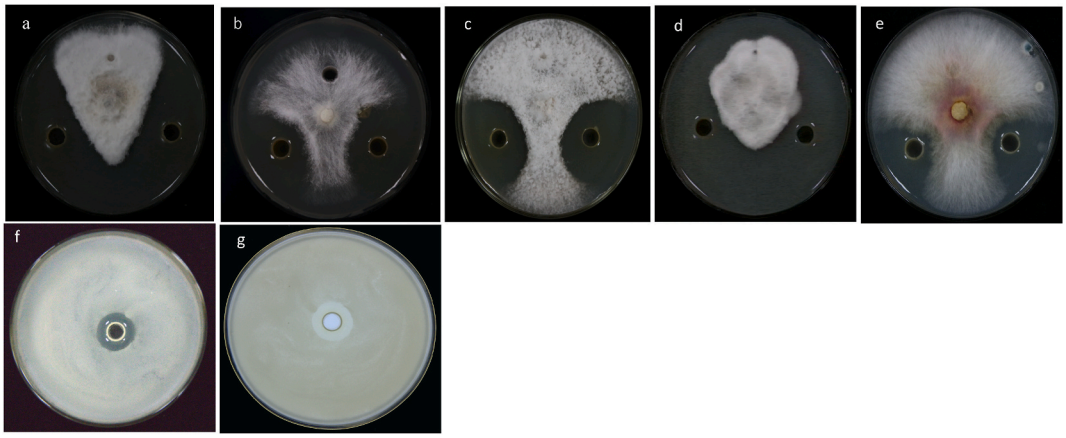

Fig. 6. The inhibition effect of the YZCUO202005 strain on 5 pathogenic fungi (A–E), two pathogenic bacteria (F and G) and inhibition percentage against fungal pathogens (A). *Fusarium oxysporum f.sp. vasinfectum,* (B). *Sclerotium rolfsii,* (C) *Nigrospora sphaerica,* (D) *Rhizoctonia solani,* and (E) *Fusarium graminearum*; (F) *Agrobacterium tumefaciens* (G) *Ralstonia solanacearum*).

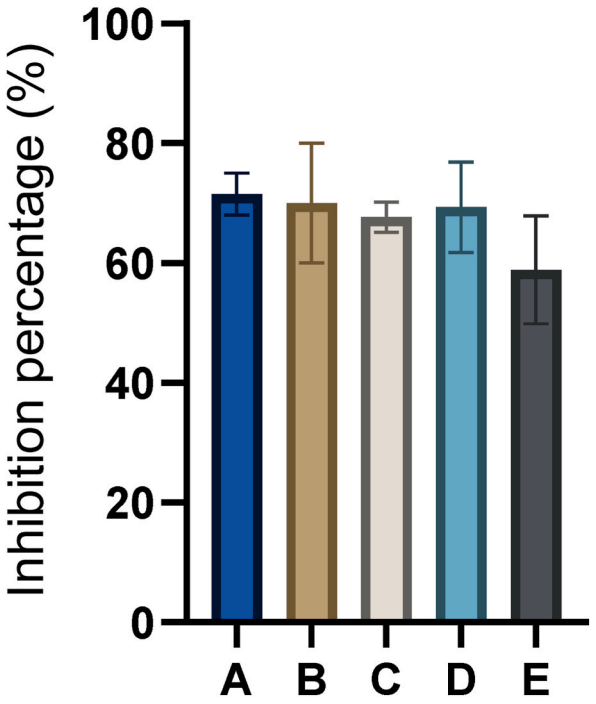

Fig. 7. The inhibition percentage against fungal pathogens (The variation was recorded as mean ± SE of triplicates.

involved in the breakdown of hydrolytic bonds in proteins and release either peptides or amino acids [51]. Our results found that the lichen-associated YZCUO202005 bacteria strain could produce protease enzymes and there is the possibility that this bacterial strain provides some nitrogen in form of nitrogenous compounds that enabled the bacteria to grow in the low nitrogen medium.

There were notable differences between YZCUO202005 strain-treated seeds and water-treated seeds. Major differences were noted on the root length and shoot length. Based on the previous research reports, beneficial bacteria initiate changes in the physiochemical forms of seeds, shoots, and roots, and improve seedling nutrient uptake which helps to overcome environmental stresses affecting seed germination and seedling growth [42,52]. Biofilms are extracellular matrices that plays a role in the root tip colonization by the beneficial bacteria such as *Bacillus*, *Pseudomonas*, *Salmonella* and *Staphylococcus* [53]. Biofilm formation allows PGPR to maintain high cellular levels attached to the roots of plants, from where they can exert their beneficial interactions in the rhizosphere [54]. In addition, as discussed earlier, IAA produced by bacteria helps improves water and mineral uptake from the soil by the plants [39,40]. These previous studies on biofilms and IAA are in agreement with our current study where YZCUO202005 bacteria strain promoted the growth of maize plants. Throughout maize vegetative stage, there were significant differences in overall growth performance between the YZCUO202005 strain-treated maize plants and water-treated maize plants. This might be contributed by the biofilms produced by *B. licheniformis* YZCUO202005 strains which allows adherence of the bacterial strain to the surface of plant roots [53] and ensure availability of other growth enhancers such as IAA, phosphates, potash, iron, and other plant growth-promoting traits that have not been demonstrated in this study such as 1-aminocyclopropane-1carboxylic acid (ACC) [55,56]. Further observation was that more adventurous roots were formed in the YZCUO202005 strain-treated maize plants as compared to the water-treated maize plants. Our results agree with the previous studies that found the correlation between inoculating IAA-producing bacteria with enhanced root growth and development. Other studies also recommended the bacteria strains of *Bacillus* genus to induce plant growth when inoculated in plants [57,58]. However, we recommend that further study be conducted to assess the YZCUO202005 bacterial contribution to other growth and yield performance parameters such as fresh and dry weight. Overall, the production of IAA and other growth-promoting attributes by the YZCUO202005 strain demonstrated that lichen-associated bacteria have the potential to promote plant growth even in poor nutritional environments.

In this study, the lichen-associated YZCUO202005 bacteria strain inhibits the growth of pathogenic fungal hyphae and pathogenic bacteria. Previous studies reported that *B. licheniformis* produce bacteriocins and other proteins which act against pathogens, particularly fungi and bacteria [49]. *B. licheniformis* lipopeptides, cell lytic enzymes, and siderophores aid in inhibiting the growth of pathogenic fungi [59]. Another study indicated that *B. licheniformis* bacteria strains have molecules known as quorum sensing molecules that regulate the exhibition of bio-control traits against fungi [60,61]. Moreover, previous studies have revealed that the stable dense biofilm matrix layer around the roots of plants not only facilitate plant growth, but also protect plants against pathogens and limit diffusion of biologically active compounds secreted by the beneficial bacteria [62]. For example, *Paenibacillus polymyxa* is generally considered as a great biofilm forming biocontrol agent which owning to its unique antibiotic spectra is even able to form single species root biofilms under natural conditions thereby providing protection against plant pathogens [63,64]. Furthermore *B. subtilis* produces biofilms in response to the antimicrobials produced by other microorganisms, thus constituting a defense

mechanism [65]. The extracellular hydrolytic enzymes excreted by the beneficial bacterial strains degrade cell wall components of plant pathogenic microbes by breaking down glycosidic linkages present in the polysaccharide of the cell walls of these pathogens, thus, providing a biological control mechanisms to the plants [66,67]. As discussed earlier, *B. licheniformis* YZCUO202005 strain was able to produce biofilms and four hydrolytic enzymes which include proteases, amylase, cellulose, and β −1,3-Glucanase. Therefore, it is suggested that the bacterial isolate use these traits and other mechanisms that have not been studied in our current study to inhibit the growth of pathogenic fungi and bacteria [68]. Our results suggest that the *B. licheniformis* YZCUO202005 strain is a potential biocontrol agent against plant pathogenic fungi and bacteria and can therefore be formulated into commercial bio-control products replacing synthetic chemical pesticides.

Based on previous studies, *Bacillus licheniformis* strains have been applied in health for infection treatments [69], in agriculture for livestock and plant health [70,71], and in food biotechnology to make various products [72,73]. To our knowledge, *Bacillus licheniformis* YZCUO202005 bacterial strain discussed in this study is very useful in dealing with environmental protection and improving sustainable agriculture. However, further studies are needed to evaluate its biosafety as a necessary process for ensuring the balanced use of this strain [74]. Moreover, not all the plant growth promotion and biocontrol traits that this strain can produce have been tested. Therefore, we are recommending that further study needs to be conducted to test other traits and the ability of this bacterial strain in plant growth promotion and biocontrol under field conditions. All in all, this study has demonstrated that lichens are naturally source of plant growth promotion and biocontrol agents which would alternate the use of synthetic chemical fertilizers and pesticides in agriculture.

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Author contribution statement

Rudoviko Galileya Medison; Jianwei Jiang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Milca Banda Medison; Litao Tan; Chicco D.M. Kayange: Analyzed and interpreted the data; Wrote the paper.
Yi Zhou; Zhengxiang Sun: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data associated with this study has been deposited at National Center for Biotechnology Information; The accession number of the 16S rRNA gene sequence in the GenBank is MW672343.1.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] S.D. Leavitt, E. Kraichak, J. Vondrak, M.P. Nelsen, M. Sohrabi, S. Perez-Ortega, et al., Cryptic diversity and symbiont interactions in rock-posy lichens, Mol Phylogenet Evol [Internet] 99 (2016) 261–274, https://doi.org/10.1016/j.ympev.2016.03.030.
[2] M. Ren, Q. Yang, X. Wei, Species diversity of cetrarioid and hypogymnioid lichens (parmeliaceae, ascomycota) from the greater and lesser khinggan mountains in nort-heast China, Biodivers. Sci. 27 (8) (2019) 833–841.
[3] B.P. Hodkinson, N.R. Gottel, C.W. Schadt, F. Lutzoni, Photoautotrophic symbiont and geography are major factors affecting highly structured and diverse bacterial communities in the lichen microbiome, Environ. Microbiol. 14 (1) (2012) 147–161.
[4] D. Armaleo, O. Müller, F. Lutzoni, Ó.S. Andrésson, G. Blanc, H.B. Bode, et al., The lichen symbiosis re-viewed through the genomes of Cladonia grayi and its algal partner Asterochloris glomerata, BMC Genom. 20 (1) (2019) 1–33.
[5] L. Selbmann, L. Zucconi, S. Ruisi, M. Grube, M. Cardinale, S. Onofri, Culturable bacteria associated with Antarctic lichens: affiliation and psychrotolerance, Polar Biol. 33 (1) (2010) 71–83.
[6] M.T. Suzuki, D. Parrot, G. Berg, M. Grube, S. Tomasi, Lichens as natural sources of biotechnologically relevant bacteria, Appl. Microbiol. Biotechnol. 100 (2) (2016) 583–595.
[7] A.H. Aguda, V. Lavallee, P. Cheng, T.M. Bott, L.G. Meimetis, S. Law, et al., Affinity crystallography: a new approach to extracting high-affinity enzyme inhibitors from natural extracts, J. Nat. Prod. 79 (8) (2016) 1962–1970.
[8] K. Motohashi, M. Takagi, H. Yamamura, M. Hayakawa, K. Shin-Ya, A new angucycline and a new butenolide isolated from lichen-derived Streptomyces spp, J. Antibiot. (Tokyo) 63 (9) (2010) 545–548.
[9] M. Grube, G. Berg, Microbial consortia of bacteria and fungi with focus on the lichen symbiosis, Fungal Biol Rev [Internet] 23 (3) (2009) 72–85, https://doi.org/10.1016/j.fbr.2009.10.001.
[10] M. Grube, M. Cardinale, J.V. De Castro, H. Müller, G. Berg, Species-specific structural and functional diversity of bacterial communities in lichen symbioses, ISME J. 3 (9) (2009) 1105–1115.
[11] Z. Zakeri, S. Junne, F. Jäger, M. Dostert, V. Otte, P. Neubauer, Lichen cell factories: methods for the isolation of photobiont and mycobiont partners for defined pure and co-cultivation, Microb Cell Fact [Internet] 21 (1) (2022) 1–11, https://doi.org/10.1186/s12934-022-01804-6.
[12] S.T. Bates, G.W.G. Cropsey, J.G. Caporaso, R. Knight, N. Fierer, Bacterial communities associated with the lichen symbiosis, Appl. Environ. Microbiol. 77 (4) (2011) 1309–1314.
[13] S. Hu, M. Wang, X. Zhang, W. Chen, X. Song, X. Fu, et al., Genetic basis of kernel starch content decoded in a maize multi-parent population, Plant Biotechnol. J. 19 (11) (2021) 2192–2205.

[14] H.A. Hussain, S. Men, S. Hussain, Q. Zhang, U. Ashraf, S.A. Anjum, et al., Maize tolerance against drought and chilling stresses varied with root morphology and antioxidative defense system, Plants 9 (6) (2020) 1–19.
[15] S.I. Sohn, S. Pandian, T.S. Kumar, Y.A.B. Zoclanclounon, P. Muthuramalingam, J. Shilpha, et al., Seed dormancy and pre-harvest sprouting in rice—an updated overview, Int. J. Mol. Sci. 22 (21) (2021).
[16] A. Tang, A.O. Haruna, N.M.A. Majid, M.B. Jalloh, Effects of selected functional bacteria on maize growth and nutrient use efficiency, Microorganisms 8 (6) (2020) 1–25.
[17] R.J. Ndeddy Aka, O.O. Babalola, Effect of bacterial inoculation of strains of pseudomonas aeruginosa, alcaligenes feacalis and bacillus subtilis on germination, growth and heavy metal (cd, cr, and ni) uptake of brassica juncea, Int. J. Phytoremediation 18 (2) (2016) 200–209.
[18] S. Spaepen, J. Vanderleyden, R. Remans, Indole-3-acetic acid in microbial and microorganism-plant signaling, FEMS Microbiol. Rev. 31 (4) (2007) 425–448.
[19] A.Y. Kim, R. Shahzad, S.M. Kang, C.W. Seo, Y.G. Park, H.J. Park, et al., IAA-producing Klebsiella variicola AY13 reprograms soybean growth during flooding stress, J Crop Sci Biotechnol 20 (4) (2017) 235–242.
[20] H. Etesami, H.A. Alikhani, H.M. Hosseini, Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents, MethodsX 2 (2015) 72–78, https://doi.org/10.1016/j.mex.2015.02.008.
[21] E. Glickmann, Y. Dessaux, A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria, Appl. Environ. Microbiol. 61 (2) (1995) 793–796.
[22] G.K. Narde, A. Kapley, H.J. Purohit, Isolation and characterization of Citrobacter strain HPC255 for broad-range substrate specificity for chlorophenols, Curr. Microbiol. 48 (6) (2004) 419–423.
[23] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, X. Mega, Molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (6) (2018) 1547–1549.
[24] J.I. Baldani, V.M. Reis, S.S. Videira, L.H. Boddey, V.L.D. Baldani, The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists, Plant Soil 384 (1–2) (2014) 413–431.
[25] S. Mehta, C.S. Nautiyal, An efficient method for qualitative screening of phosphate-solubilizing bacteria, Curr. Microbiol. 43 (1) (2001) 51–56.
[26] M.V.S. Rajawat, S. Singh, S.P. Tyagi, A.K. Saxena, A modified plate assay for rapid screening of potassium-solubilizing bacteria, Pedosphere [Internet] 26 (5) (2016) 768–773, https://doi.org/10.1016/S1002-0160(15)60080-7.
[27] P.R. Patel, S.S. Shaikh, R.Z. Sayyed, Modified chrome azurol S method for detection and estimation of siderophores having affinity for metal ions other than iron, Environ Sustain [Internet 1 (1) (2018) 81–87, https://doi.org/10.1007/s42398-018-0005-3.
[28] S. Saha, R.N. Roy, S.K. Sen, A.K. Ray, Characterization of cellulase-producing bacteria from the digestive tract of tilapia, Oreochromis mossambica (Peters) and grass carp, Ctenopharyngodon idella (Valenciennes), Aquac Res 37 (4) (2006) 380–388.
[29] Y.Y. Yu, C.H. Jiang, C. Wang, L.J. Chen, H.Y. Li, Q. Xu, et al., An improved strategy for stable biocontrol agents selecting to control rice sheath blight caused by Rhizoctonia solani, Microbiol. Res. 203 (July 2016) (2017) 1–9, https://doi.org/10.1016/j.micres.2017.05.006.
[30] S.A. Ahmed, F.A. Mostafa, W.A. Helmy, M.A. Abdel-Naby, Improvement of bacterial α-amylase production and application using two steps statistical factorial design, Biocatal. Agric. Biotechnol. 10 (November 2016) (2017), https://doi.org/10.1016/j.bcab.2017.03.004, 224–33.
[31] Q. Wu, X. Dou, Q. Wang, Z. Guan, Y. Cai, X. Liao, Isolation of β-1,3-glucanase-producing microorganisms from poria cocos cultivation soil via molecular biology, Molecules 23 (7) (2018).
[32] D. Sun, T. Zhuo, X. Hu, X. Fan, H. Zou, Identification of a Pseudomonas putida as biocontrol agent for tomato bacterial wilt disease, Biol Control [Internet 114 (2017) 45–50, https://doi.org/10.1016/j.biocontrol.2017.07.015.
[33] T. Zhuo, W. Rou, X. Song, J. Guo, X. Fan, G.G. Kamau, et al., Molecular study on the carAB operon reveals that carB gene is required for swimming and biofilm formation in Xanthomonas citri subsp. citri, BMC Microbiol [Internet] 15 (1) (2015) 1–8, https://doi.org/10.1186/s12866-015-0555-9.
[34] M. Zhang, J. Li, A. Shen, S. Tan, Z. Yan, Y. Yu, et al., Isolation and identification of Bacillus amyloliquefaciens IBFCBF-1 with potential for biological control of phytophthora blight and growth promotion of pepper, J. Phytopathol. 164 (11–12) (2016) 1012–1021.
[35] L. Chen, H. Shi, J. Heng, D. Wang, K. Bian, Antimicrobial, plant growth-promoting and genomic properties of the peanut endophyte Bacillus velezensis LDO2, Microbiol. Res. 218 (August 2018) (2019), https://doi.org/10.1016/j.micres.2018.10.002, 41–8.
[36] T. Lu, M. Ke, M. Lavoie, Y. Jin, X. Fan, Z. Zhang, et al., Rhizosphere microorganisms can influence the timing of plant flowering, Microbiome 6 (1) (2018) 1–12.
[37] S. Shilev, I. Babrikova, T. Babrikov, Consortium of plant growth-promoting bacteria improves spinach (Spinacea oleracea L.) growth under heavy metal stress conditions, J. Chem. Technol. Biotechnol. 95 (4) (2020) 932–939.
[38] D. Duca, J. Lorv, C.L. Patten, D. Rose, B.R. Glick, Indole-3-acetic acid in plant-microbe interactions. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 106 (1) (2014) 85–125.
[39] S.M. Kang, R. Shahzad, M.A. Khan, Z. Hasnain, K.E. Lee, H.S. Park, et al., Ameliorative effect of indole-3-acetic acid- and siderophore-producing Leclercia adecarboxylata MO1 on cucumber plants under zinc stress, J. Plant Interact. 16 (1) (2021) 30–41.
[40] N. Khan, S. Ali, M.A. Shahid, A. Mustafa, R.Z. Sayyed, J.A. Curá, Insights into the interactions among roots, rhizosphere, and rhizobacteria for improving plant growth and tolerance to abiotic stresses: a review, Cells 10 (6) (2021).
[41] V. Gravel, H. Antoun, R.J. Tweddell, Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: possible role of indole acetic acid (IAA), Soil Biol. Biochem. 39 (2007) 1968–1977.
[42] P. Panda, A. Choudhury, S. Chakraborty, D.P. Ray, S. Deb, P.S. Patra, et al., Phosphorus solubilizing bacteria from tea soils and their phosphate solubilizing abilities, Int J Bioresour Sci 4 (2) (2017) 113.
[43] M. Saha, B.R. Maurya, V.S. Meena, I. Bahadur, A. Kumar, Identification and characterization of potassium solubilizing bacteria (KSB) from Indo-Gangetic Plains of India, Biocatal. Agric. Biotechnol. 7 (2016), https://doi.org/10.1016/j.bcab.2016.06.007, 202–9.
[44] Y. Yan, W. Xu, Y. Hu, R. Tian, Z. Wang, Bacillus velezensis yyc promotes tomato growth through hormone and mapk signaling pathways, SSRN Electron. J. 172 (April) (2022), 104977, https://doi.org/10.1016/j.biocontrol.2022.104977.
[45] D. Kour, K.L. Rana, T. Kaur, N. Yadav, S.K. Halder, A.N. Yadav, et al., Potassium Solubilizing and Mobilizing Microbes: Biodiversity, Mechanisms of Solubilization, and Biotechnological Implication for Alleviations of Abiotic Stress [Internet]. New and Future Developments in Microbial Biotechnology and Bioengineering, Elsevier Inc., 2020, pp. 177–202, https://doi.org/10.1016/B978-0-12-820526-6.00012-9.
[46] P. Teotia, V. Kumar, M. Kumar, N. Shrivastava, A. Varma, Rhizosphere microbes: potassium solubilization and crop productivity – present and future aspects, in: V.S. Meena, B.R. Maurya, J.P. Verma, R.S. Meena (Eds.), Potassium Solubilizing Microorganisms for Sustainable Agriculture [Internet], Springer India, New Delhi, 2016, pp. 315–325, https://doi.org/10.1007/978-81-322-2776-2_22.
[47] A. Kumar, S. Singh, A.K. Gaurav, S. Srivastava, J.P. Verma, Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants, Front. Microbiol. 11 (July) (2020) 1–15.
[48] F.A. Ansari, I. Ahmad, J. Pichtel, Growth stimulation and alleviation of salinity stress to wheat by the biofilm forming Bacillus pumilus strain FAB10, Appl. Soil Ecol. 143 (May) (2019) 45–54, https://doi.org/10.1016/j.apsoil.2019.05.023.
[49] D. Fira, I. Dimkić, T. Berić, J. Lozo, S. Stanković, Biological control of plant pathogens by Bacillus species, J Biotechnol [Internet 285 (2018) 44–55, https://doi.org/10.1016/j.jbiotec.2018.07.044.
[50] M. Manasa, P. Ravinder, S. Gopalakrishnan, V. Srinivas, R.Z. Sayyed, H.A. El Enshasy, et al., Co-inoculation of bacillus spp. for growth promotion and iron fortification in sorghum, Sustain. Times 13 (21) (2021) 1–14.
[51] L.W. Theron, B. Divol, Microbial aspartic proteases: current and potential applications in industry, Appl. Microbiol. Biotechnol. 98 (21) (2014) 8853–8868.
[52] A.M. Eid, S.S. Salim, S.E.D. Hassan, M.A. Ismail, A. Fouda, Role of endophytes in plant health and abiotic stress management, Microbiome Plant Heal Dis (2019) 119–144.
[53] G. Santoyo, C.A. Urtis-Flores, P.D. Loeza-Lara, M.D.C. Orozco-Mosqueda, B.R. Glick, Rhizosphere colonization determinants by plant growth-promoting rhizobacteria (Pgpr), Biology 10 (6) (2021).

[54] N.S. Paulucci, L.A. Gallarato, Y.B. Reguera, J.C. Vicario, A.B. Cesari, M.B. García de Lema, et al., Arachis hypogaea PGPR isolated from Argentine soil modifies its lipids components in response to temperature and salinity, Microbiol Res [Internet (2015) (2015) 173, https://doi.org/10.1016/j.micres.2014.12.012, 1–9.
[55] D.P. Singh, H.B. Singh, R. Prabha, Microbial inoculants in sustainable agricultural productivity: vol. 1: research perspectives, Microb Inoculants Sustain Agric Product Vol 1 Res Perspect. 1 (2016) 1–343.
[56] R.P. Singh, P.N. Jha, A halotolerant bacterium Bacillus licheniformis HSW-16 augments induced systemic tolerance to salt stress in wheat plant (Triticum aestivum), Front. Plant Sci. 7 (DECEMBER2016) (2016) 1–18.
[57] M.A. El-Bendary, H.A. Hamed, M.E. Moharam, Potential of Bacillus isolates as bio-control agents against some fungal phytopathogens, Biocatal. Agric. Biotechnol. 5 (2016) 173–178, https://doi.org/10.1016/j.bcab.2016.02.001.
[58] S.J. Won, J.H. Kwon, D.H. Kim, Y.S. Ahn, The effect of bacillus licheniformis MH48 on control of foliar fungal diseases and growth promotion of Camellia oleifera seedlings in the coastal reclaimed land of Korea, Pathogens 8 (1) (2019).
[59] S. Nigris, E. Baldan, A. Tondello, F. Zanella, N. Vitulo, G. Favaro, et al., Biocontrol traits of Bacillus licheniformis GL174, a culturable endophyte of Vitis vinifera cv. Glera 06 biological sciences 0604 genetics 06 biological sciences 0607 plant biology 06 biological sciences 0605 microbiology, BMC Microbiol. 18 (1) (2018) 1–16.
[60] E. Esmaeilishirazifard, A. Dariush, S.A. Moschos, T. Keshavarz, A novel antifungal property for the Bacillus licheniformis ComX pheromone and its possible role in inter-kingdom cross-talk, Appl. Microbiol. Biotechnol. 102 (12) (2018) 5197–5208.
[61] E. Esmaeilishirazifard, D. De Vizio, S.A. Moschos, T. Keshavarz, Genomic and molecular characterization of a novel quorum sensing molecule in Bacillus licheniformis, Amb. Express 7 (1) (2017).
[62] S. Timmusk, S Bin Kim, E. Nevo, I.A. El Daim, B. Ek, J. Bergquist, et al., Sfp-type PPTase inactivation promotes bacterial biofilm formation and ability to enhance wheat drought tolerance, Front. Microbiol. 6 (MAY) (2015) 1–13.
[63] S. Timmusk, N. Grantcharova, E.G.H. Wagner, Paenibacillus polymyxa invades plant roots and forms biofilms, Appl. Environ. Microbiol. 71 (11) (2005) 7292–7300.
[64] W.M. Haggag, S. Timmusk, Colonization of peanut roots by biofilm-forming Paenibacillus polymyxa initiates biocontrol against crown rot disease, J. Appl. Microbiol. 104 (4) (2008) 961–969.
[65] D. López, M.A. Fischbach, F. Chu, R. Losick, R. Kolter, Structurally diverse natural products that cause potassium leakage trigger multicellularity in Bacillus subtilis, Proc Natl Acad Sci U S A 106 (1) (2009) 280–285.
[66] S. Rz, Hydrolytic enzymes of rhizospheric microbes in crop protection, MOJ Cell Sci Rep 3 (5) (2016) 4–6.
[67] L. Liu, R. Galileya, Zheng T. wen, Meng X. jia, Sun Z. xiang, Y. Zhou, Biocontrol potential of Bacillus amyloliquefaciens YZU-SG146 from Fraxinus hupehensis against Verticillium wilt of cotton, Biol. Control 183 (January) (2023), 105246, https://doi.org/10.1016/j.biocontrol.2023.105246.
[68] M.H. Jeong, Y.S. Lee, J.Y. Cho, Y.S. Ahn, J.H. Moon, H.N. Hyun, et al., Isolation and characterization of metabolites from Bacillus licheniformis MH48 with antifungal activity against plant pathogens, Microb. Pathog. 110 (2017) 645–653.
[69] C.M. Romo-Barrera, L.E. Castrillón-Rivera, A. Palma-Ramos, J.I. Castañeda-Sánchez, J. Luna-Herrera, Bacillus licheniformis and bacillus subtilis, probiotics that induce the formation of macrophage extracellular traps, Microorganisms 9 (10) (2021).
[70] Y. Zhao, D. Zeng, H. Wang, X. Qing, N. Sun, J. Xin, et al., Dietary probiotic Bacillus licheniformis H2 enhanced growth performance, morphology of small intestine and liver, and antioxidant capacity of broiler chickens against Clostridium perfringens–induced subclinical necrotic enteritis, Probiotics Antimicrob Proteins 12 (3) (2020) 883–895.
[71] M. Jamil, S. Zeb, M. Anees, A. Roohi, I. Ahmed, S. ur Rehman, et al., Role of Bacillus licheniformis in phytoremediation of nickel contaminated soil cultivated with rice, Int. J. Phytoremediation 16 (6) (2014) 554–571.
[72] A. Muras, M. Romero, C. Mayer, A. Otero, Biotechnological applications of Bacillus licheniformis, Crit. Rev. Biotechnol. 41 (4) (2021) 609–627, https://doi.org/10.1080/07388551.2021.1873239.
[73] V. Nithya, M. Prakash, P.M. Halami, Utilization of industrial waste for the production of bio-preservative from Bacillus licheniformis Me1 and its application in milk and milk-based food products, Probiotics Antimicrob Proteins 10 (2) (2018) 228–235.
[74] J.I. Vílchez, A. Navas, J. González-López, S.C. Arcos, M. Manzanera, Biosafety test for plant growth-promoting bacteria: proposed environmental and human safety index (EHSI) protocol, Front. Microbiol. 6 (JAN) (2016).