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Research Article

Characterisation of the Endophytic and Rhizospheric *Bacillus licheniformis* Strains Isolated from Sweet Potato with Plant Growth-Promoting and Yield Enhancing Potential

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The primary aim of the present study was to identify bacterial isolates with yield-enhancing potential for application as biofertilisers in the cultivation of sweet potato. Therefore, endophytic and rhizospheric strains were isolated from sweet potato plantations in Hungary to identify bacterial strains with plant growth-promoting and antifungal potential. In total, seven *Bacillus licheniformis* strains were identified and subjected to detailed ecophysiological investigations. Experiments have been conducted to investigate the tolerance of selected strains to different limiting factors such as pH, temperature, and water activity, which affect survivability in various agricultural environments. The majority of tested *B. licheniformis* strains exhibited plant growth-promoting potential (e.g., production of indole-3-acetic acid up to 40.42 μg mL^{-1}, production of ammonia up to 0.87 mg mL^{-1}, phosphorus solubilising activity, siderophore production), with two strains (SZMC 27713 and SZMC 27715) demonstrating inhibitory activity (ranging between 7% and 38%) against plant pathogenic fungi prevalent in sweet potato cultivation. Furthermore, strain SZMC 27715 induced accelerated germination and a significantly higher germination rate in tomato seeds compared to the control. In a field study, it was observed that strain SZMC 27715 had a potent yield enhancing effect in sweet potato, where a significant yield per plant increase was observed in all treatments (1.13, 1.09 and 1.40 kg) compared to the control plants (0.92 kg). The highest yield per plant was observed when the cuttings were soaked combined with two additional foliar treatments. To the best of our knowledge, this is the first report of the successful utilisation of the *B. licheniformis* strain as a biofertiliser for yield enhancement in sweet potato cultivation. Based on our results, strain SZMC 27715 has potential for application as a biofertiliser in sweet potato cultivation either as a standalone option or in a microbial consortium.

1. Introduction

One of today's most urgent challenges pertains to the escalating chemical pollution of the environment, a concern exacerbated by the substantial contribution of agricultural practices through the extensive application of pesticides and fertilisers [1]. Consequently, there is a growing global imperative to prioritise ecological and alternative approaches as the foundation for establishing sustainable agriculture in the long term [2]. Plant growth-promoting bacteria (PGPB) present a promising alternative to chemical pesticides and fertilisers, given their effectiveness, environmental safety, and non-toxic nature [3]. PGPB are efficient stimulators of plant growth, nutrition, and production which makes them suitable for use as bioinoculants, replacing the aforementioned agrochemicals [4].

Typically residing in the rhizosphere, PGPB are soil bacteria that play a pivotal role in enhancing plant growth and development by secreting diverse regulatory molecules. PGPB encompass both epiphytes and endophytes, inhabiting plant surfaces and internal tissues, respectively [5]. Among these, plant growth-promoting endophytes (PGPEs), a subgroup of PGPB, are particularly effective in driving growth due to their internal presence, which facilitates efficient interaction with host plants and the delivery of beneficial effects. Notably, PGPEs encounter fewer fluctuations in the soil's biotic and abiotic conditions [6].

Endophytic bacteria do not cause any harm or disease while living inside the plants, and they can contribute to the growth and development of plants through various direct and indirect processes. Direct mechanisms include the production of various phytohormones (e.g. auxins, gibberellins), protection against abiotic stresses (e.g. salinity, drought), and the supply of various nutrients to plants (e.g., N_2 fixation, phosphorus solubilisation). Indirect processes include the suppression of various plant pathogens (e.g., production of antibiotics, volatile organic compounds), protection against pests and herbivores, or the induction of plant systemic resistance (ISR) [7]. Endophytic bacteria are also capable of specifically altering the composition of plants, an excellent example being the ability to increase the oil content of thyme (*Thymus vulgaris* L.) with the bioinoculation of the consortium of endophytic bacteria (*B. licheniformis* T11r and *B. velezensis* T13r strains) [8]. However, it is important to highlight that the success of inoculated endophytes in colonisation, growth, and the expression of their antifungal and plant stimulating activities depend on the host plant [9], and it may therefore be more beneficial to test an endophytic microorganism isolated from a particular host on the same species.

Prominent among reported PGPB genera are *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Agrobacterium*, *Rhizobia*, *Alcaligenes*, *Azotobacter*, *Mycobacterium*, *Flavobacterium*, *Cellulomonas*, and *Micrococcus* [10]. Of these, the *Bacillus* genus—a frequent endophyte—commands significant attention due to its rapid growth in different media, formation of highly tolerant endospores, and synthesis of a diverse range of bioactive metabolites [11]. *Bacillus*-based biocontrol agents are notably more potent than counterparts rooted in other PGPB organisms (e.g., *Pseudomonas*). This is largely attributed to their remarkable efficiency in metabolite production and spore formation, which enhances the stability of commercially formulated products [12]. Abundant in soil, *Bacillus* species generate metabolites with antimicrobial properties, enabling them to suppress or eliminate other microorganisms [13]. They can exert their antimicrobial activity through the production of lipopeptides, antibiotics, and various enzymes that can promote plant growth and inhibit plant pathogens, in addition, they can produce volatile compounds that can promote plant defence mechanisms [14].

Ipomoea batatas [L.] Lam., commonly known as sweet potato, holds global significance as a staple crop and a nutritional cornerstone in many regions. In Hungary, its cultivation and consumption have burgeoned over the last 8–10 years, making sweet potato readily available in major grocery stores. In addition to its good nutritional value, it also contains a number of compounds with beneficial physiological effects (e.g., β-carotene, polyphenols) [15]. Nonetheless, like most plants, sweet potato is susceptible to several field pathogens [16] and postharvest diseases [17] worldwide. Consequently, protection against these diseases is crucial both on the field and during the storage of the harvested tubers. In recent years, experiments have been reported in Hungary in sweet potato cultivation using different biocontrol organisms against the larvae of the white grub cockchafer (*Melolontha melolontha*) [18] and the southern European marshland pyralid (*Duponchelia fovealis*) [19]. Recently, our laboratory reported the successful augmentation of yields through a multicomponent microbiological soil inoculant for sweet potato cultivation [20].

Given these circumstances, the present study was specifically designed with a clear set of objectives aimed at harnessing the agricultural potential of *Bacillus licheniformis* for sweet potato cultivation in Hungary. The primary aim was to isolate *B. licheniformis* strains directly from sweet potato plantations, thereby ensuring the relevance and adaptability of these strains to local cultivation conditions. Subsequent characterisation of these strains focused on identifying and elucidating their plant growth-promoting traits and antifungal capabilities. This included assessing their ability to produce phytohormones, siderophores, and ammonia, alongside evaluating their antagonistic effects against common fungal pathogens of sweet potato, such as those from the *Aspergillus*, *Fusarium*, *Rhizoctonia*, and *Phomopsis* genera. The culmination of this research aimed to evaluate the practical application potential of these *B. licheniformis* isolates in the field, with the ultimate objective of establishing their efficacy as biofertilisers in enhancing sweet potato yields. Through this targeted approach, the study seeks to contribute to the broader goals of sustainable agriculture by providing an eco-friendly alternative to chemical fertilisers.

2. Materials and Methods

2.1. Isolation and Identification of Rhizospheric and Endophytic Bacteria. Samples were collected from sweet potato plantations in Southern Hungary prior to harvest. Common isolation techniques, such as dilution plate method and selective media, were applied. For the isolation of rhizospheric bacteria, soil collected from the rhizosphere of sweet potato (Bordány, coordinates: 46°19′N 19°55′E) was prepared by mixing 1 g of soil in 10 mL sterile 0.9% NaCl for 10 min, serially diluted (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}). For *Bacillus* isolation, each dilution step was incubated at 90°C for 15 min [21]. Heat treatment was followed by spreading 50 μL of each dilution step on yeast extract glucose (YEG) medium containing yeast extract, 5 g L^{-1}; glucose, 10 g L^{-1}; and agar, 20 g L^{-1} in distilled water. The YEG medium was supplemented with 0.01 g L^{-1} nystatin and carbendazim to suppress fungal growth enabling bacterial selection. The plates were incubated at 25°C for 2 days. Afterwards, single colonies were randomly picked and separated until homogeneous on YEG medium.

Table 1: Primers and temperature parameters used for polymerase chain reactions.

Primers (5′–3′)	PCR parameters	References
16S rRNA gene		
Eub 341-F	95°C, 2 min (1 cycle)	
CCTACGGGAGGCAGCAG	95°C, 30 s; 57°C, 45 s	[23]
Eub 1060-R	72°C, 1 min (32 cycles)	
CGACACGAGCTGACGACA	72°C, 7 min (1 cycle)	
DNA gyrase alpha subunit gene		
gyrA-F	95°C, 5 min (1 cycle)	
CAGTCAGGAAATGCGTACGTCCTT	94°C, 30 s; 50°C, 45 s	[22]
gyrA-R	94°C, 30 s (30 cycles)	
CAAGGTAATGCTCCAGGCATTGCT	72°C, 2 min (1 cycle)	

The DNA sequences were submitted to NCBI GenBank (accession numbers OP620082-88).

For isolation of endophytic bacteria, plant samples (tuber, stem, and leaf samples collected in Madaras, coordinates: 46°03′18″N 19°15′43″E) were washed thoroughly under tap water for 10 min and then left to dry on paper towels. The plant tissues were then cut into pieces of <1 cm which were soaked in 0.1% $HgCl_2$ solution for 30 s and then placed in 96% ethanol for further 30 s. The surface sterilisation steps were repeated three times. After the third ethanol wash, the samples were left to dry, and each piece was cut to expose its inner tissue and placed on YEG medium supplemented with 0.01 g L^{-1} nystatin and carbendazim. The plates were incubated at 25°C for 7–10 days and checked daily for the growth of bacterial colonies. Colonies were selected that were growing from the centre of the tissue placed on the culture medium and separated until homogeneous on YEG medium. The endophytic isolation was performed from three simultaneous samples.

Cell suspensions were prepared from bacterial colonies cultured overnight on solid YEG medium suspended in 50 μL double distilled water (ddH_2O) to serve as DNA templates for amplification of partial sequences of DNA gyrase alpha subunit (*gyrA*) [22] and the 16S rRNA (*Eub*) [23] genes. PCR reactions consisted of 2 μL 10x Taq Buffer with 20 mM $MgCl_2$ (Thermo Fisher Scientific, Waltham, MA, USA), 2 μL 2 mM dNTP Mix (Thermo Scientific), 0.1 μL 5 U μL^{-1} Dream Taq DNA Polymerase (Thermo Scientific), 4–4 μL 1 μM primers, 7 μL ddH_2O, and 1 μL DNA template. PCR reactions were performed using a VWR Doppio Thermal Cycler (VWR International, Darmstadt, Germany), the primer sequences and temperature profiles are given in Table 1. Amplicons were subjected to horizontal agarose gel electrophoresis using 1% agarose gel in 1x TAE buffer (50x TAE buffer was prepared by dissolving Tris base, 242 g; 0.5 M EDTA, 100 mL; and glacial acetic acid, 57.1 mL in 1 L distilled water) for 15 min at 90 V and visualised with ethidium bromide staining using UV-illumination. PCR products were further purified using a purification kit (E.Z.N.A. Cycle Pure Kit, Omega BIO-TEK, USA). Sequencing was performed by an external service (Eurofins Genomics Germany, Ebersberg, Germany). Raw sequences were read using FinchTV software and analysed in the database of the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov) in "standard nucleotide blast" mode, based on their identity values (>99%). The identification targeted isolates belonging to the *Bacillus* genus due to the aforementioned advantages of the genus' species in agricultural applications.

2.2. Temperature Dependence Test. To investigate temperature tolerance at 25 and 37°C, the strains were first incubated overnight at 25°C with shaking at 140 rpm in 2 mL of YEG liquid nutrient solution. Subsequently, they were inoculated at a density of 10^6 CFU mL^{-1} into 100 mL Erlenmeyer flasks containing 50 mL of YEG liquid medium. As a negative control, noninoculated liquid YEG medium was used. The growth performance of the strains was evaluated at 25 and 37°C, respectively, by subjecting them to shaking at 140 rpm for 24 hr using a MaxQTM 8000 shaker (Thermo Fisher Scientific, USA). To determine the optical density of samples, 200 μL of bacterial suspension was transferred in a 96-well polystyrene microtiter plate (Greiner Bio-One Cellstar® microplate, No.655185, Greiner Bio-One, Mosonmagyaróvár, Hungary) and measured using a SPECTROstar Nano microplate reader (BMG Labtech, Offenburg, Germany) at 620 nm (OD_{620}). Temperature tolerance of strains was estimated based on the cell density observed at 620 nm. The experiment was conducted in triplicate.

2.3. Water Activity Tolerance Test. The impact of water activity (a_w) [24] was evaluated using liquid YEG medium fortified with specific NaCl concentrations. Following the previously mentioned bacterial suspension preparation, the strains were inoculated at a concentration of 10^6 CFU mL^{-1} into 100 mL Erlenmeyer flasks containing 50 mL of liquid YEG medium supplemented with 1% ($a_w = 0.991$), 2% ($a_w = 0.980$), and 5% ($a_w = 0.968$) NaCl. Positive control samples consisted of inoculated liquid YEG medium devoid of NaCl, while negative control samples were non-inoculated liquid YEG medium containing corresponding NaCl concentrations. The cultures were incubated at 25°C, at 140 rpm for 1 day. Optical density determination was executed as outlined earlier. This trial was conducted in triplicate.

2.4. pH Dependence Test. To investigate the pH dependence of the bacterial strains, liquid YEG media with varying pH values (pH 4.0, pH 5.0, pH 6.0, pH 7.0, and pH 8.0) were prepared utilising McIlvaine buffer solutions. The pH values

were adjusted by mixing two stock solutions (solution A (0.2 M Na_2HPO_4): 25.59 g L^{-1} of $Na_2HPO_4 \times 2H_2O$ in distilled water and solution B (0.1 M $C_6H_8O_7$): 21.01 g L^{-1} of citric acid $\times H_2O$ in distilled water) in proportions: 7.71, 10.30, 12.63, 16.47, and 19.45 mL of solution A and 12.29, 9.70, 7.37, 3.53, and 0.55 mL of solution B, respectively [25]; pH Duotest® paper (Macherey–Nagel, Düren, Germany) was used to check pH values. Both buffer solutions and media were formulated at double concentrations and then blended in equal ratios. Following the aforementioned bacterial suspension preparation, the strains were inoculated at a concentration of 10^6 CFU mL^{-1} into 100 mL Erlenmeyer flasks containing 50 mL of YEG liquid nutrient solution with the specified pH values. Subsequent steps, including sample preparation and optical density measurement at 620 nm (OD_{620}) after 24 hr, followed the procedures outlined earlier. This investigation was conducted in triplicate.

2.5. Qualitative Determination of Indole-3-Acetic-Acid Production. The capacity of the *B. licheniformis* strains to produce indole-3-acetic-acid (IAA) was evaluated using a method based on Shrivastava and Kumar [26], with slight adjustments. Holes, 8 mm in diameter, were introduced into YEG media supplemented with 1 g L^{-1} L-tryptophan as the IAA precursor, using a sterile cork borer. Subsequently, 200 μL of bacterial cultures, grown overnight in 30 mL of liquid YEG, were pipetted into these holes. Each inoculation was replicated three times. Negative controls encompassed noninoculated sample holes and holes filled with sterile YEG media. Following this, the cultures were incubated for 2 days at 25°C. For analysis, colonies were gently fully removed from the culture medium's surface using unsterile paper towels. Then, 200 μL of Salkovski reagent (12 g L^{-1} $FeCl_3$ dissolved in 37% H_2SO_4) [27] were pipetted into the holes. Subsequently, the dishes were incubated in darkness for 20 min. A distinctive pink zone surrounding the colonies indicated IAA production. This test was executed in triplicate.

2.6. Quantitative Determination of the IAA Production. To achieve a more precise assessment of IAA production, we employed a spectrophotometric method [28] with slight adjustments to quantify the strains' IAA production in the presence and absence of L-tryptophan. Strains were initially inoculated on solid YEG medium. On the following day, colonies were collected from the medium surface using 5 mL of sterile 0.9% NaCl and subsequently transferred into sterile test tubes. The optical density of these suspensions was gauged at 620 nm (OD_{620}). Next, the strains were inoculated at a concentration of 10^5 CFU mL^{-1} in 30 mL of pure liquid YEG medium, both with and without L-tryptophan. For the induction of IAA production, 0.1 g/L^{-1} L-tryptophan was added to the liquid YEG medium. As controls, noninoculated liquid YEG medium, with and without L-tryptophan, was utilised. The cultures were then maintained at 25°C and 140 rpm. During the incubation period, bacterial growth was monitored at 620 nm (OD_{620}), while IAA production was assessed at 530 nm (OD_{530}). At specific time intervals (0 hr, 1, 2, and 3 days of incubation), 1 mL samples were collected in 1.5 mL Eppendorf tubes and subjected to centrifugation at 10,000 *g* for 10 min. To initiate the colorimetric reactions, 500 μL of the supernatant was transferred to new 1.5 mL Eppendorf tubes, with an additional 500 μL of Salkovski reagent. These samples were incubated in darkness for 20 min, and the optical density of the reactions was gauged at 530 nm (OD_{530}) using a SPECTROstar Nano microplate reader (BMG Labtech, Offenburg, Germany). IAA production manifested as a pink colouration in the samples. This test was conducted in triplicate. To quantify IAA concentration, we employed a standard curve based on the IAA standard (CAS-No.: 87-51-4, Merck, Darmstadt, Germany), encompassing a range from 0.006 to 0.4 mg mL^{-1}. The IAA standard was dissolved in high-performance liquid chromatography (HPLC) grade purity methanol (MeOH; Chem-Lab NV, B-8210 Zedelgem, Belgium).

2.7. Phosphorus Solubilisation Ability Test. The ability of the *B. licheniformis* strains to solubilise phosphorus (P) was tested on Pikovskaya medium (glucose, 10 g L^{-1}; $(NH_4)_2SO_4$, 0.5 g L^{-1}; NaCl, 0.2 g L^{-1}; KCl, 0.2 g L^{-1}; yeast extract, 0.5 g L^{-1}; $Ca_3(PO_4)_2$, 5 g L^{-1}; $MgSO_4 \times 7H_2O$, 0.1 g L^{-1}; $MnSO_4 \times H_2O$, 0.002 g L^{-1}; $FeSO_4 \times 7H_2O$, 0.002 g L^{-1}; and agar, 20 g L^{-1} in distilled water) [29], following the method outlined by Oves et al. [30]. Strains were pregrown overnight on solid YEG medium then were spot inoculated on Pikovskaya medium using a sterile toothpick. Plates were incubated at 25°C for 7 days after inoculation. P-solubilisation capacity was indicated by the appearance of a clear halo zone around the colonies. The solubilisation index (S.I.) was calculated by the following formula:

$$\text{S.I.} = \left(\frac{Cd + Zd}{Cd}\right), \tag{1}$$

where *Cd* indicates the colony diameter, and *Zd* represents diameter of the clear halo zone (solubilised zone). S.I. was calculated in Microsoft Excel® 2013. The test was carried out in triplicate.

2.8. Ammonia Production Test. The ammonia production capacity of the *B. licheniformis* strains was investigated in peptone water (meat peptone from beef, 10 g L^{-1} and NaCl, 5 g L^{-1}) based on the method of Goswami et al. [31], using Nessler's reagent. The Nessler reagent was prepared as follows: 50 g KI was dissolved in 50 mL distilled water, then a saturated solution of $HgCl_2$ (22 g in 350 mL distilled water) was added until no orange precipitate was formed. In the next step, 200 mL of 5 N NaOH was added to the mixture and made up to 1 L final volume by adding distilled water. A loopful of freshly grown culture was inoculated in 5 mL of peptone water and incubated for 7 days at 25°C. Noninoculated peptone water served as negative control. This test was conducted in triplicate. To quantify ammonia production, 2 mL of bacterial culture was taken and centrifuged at 10,000 *g* for 10 min. Then, 1 mL Nessler's reagent was added to 1 mL of cell-free supernatant and the volume of this mixture was made up to 10 mL using distilled water. The optical density of the reactions was measured at 530 nm (OD_{530}) [32]. The presence of ammonia was indicated by the solution's orange

colouration. The concentration of ammonia was estimated based on a standard curve of ammonium sulphate $(NH_4)_2SO_4$ (Molar Chemicals, Budapest, Hungary) ranging from 5 to 50 μM mL^{-1}. Both the test and calibration curve were conducted in triplicate.

2.9. Siderophore-Producing Ability Test. Our strains failed to produce siderophores on Chrome Azurol S (CAS) agar [33] (data not shown), therefore, siderophore production was assessed using a modified spectrophotometric method based on the reaction with CAS solution, as described by Arora and Verma [34] with adjustments. The CAS solution was prepared by mixing three solutions as follows: in the first step, 10 mL of $FeCl_3$ solution (1 mM $FeCl_3 \times 6H_2O$ in 10 mM HCl) was mixed with 50 mL of CAS solution (2 mM CAS in distilled water), and then 40 mL of HDTMA solution (5 mM hexadecyltrimethylammonium bromide in distilled water) was added to the mixture. All glassware was soaked overnight in 10% HNO_3 to remove iron residues [35]. Strains were grown on solid iron-free standard succinate medium (SSM; K_2HPO_4, 6 g $L^{-1;}$, KH_2PO_4, 3 g L^{-1}; $(NH_4)_2SO_4$, 1 g L^{-1}; $MgSO_4 \times 7H_2O$, 0.2 g L^{-1}; $C_4H_6O_4$, 4 g L^{-1}; and agarose, 20 g L^{-1} in distilled water) [36] for 2 days. The pH was adjusted to 7.0 by addition of 0.2 M NaOH, prior to sterilisation. After incubation, the colonies were collected from the surface of the medium with sterile 0.9% NaCl, then inoculated at 10^5 CFU mL^{-1} in 30 mL of liquid iron-free SSM medium and incubated at 140 rpm for 2 days at 25°C. Noninoculated SSM served as negative control and reference sample, while SSM supplemented with $FeCl_3 \times 6H_2O$ 0.029 g L^{-1} served as positive control. After the incubation period, 1 mL of bacterial cell suspensions were centrifuged at 10,000 *g* for 10 min, then 100 μL of cell-free supernatant and 100 μL of CAS formulation was mixed for each reaction on a 96-well microtiter plate. CAS reactions were incubated for 20 min in the darkness, then optical density was observed at 630 nm (OD_{630}) (SPECTROstar Nano, BMG Labtech, Offenburg, Germany). The CAS solution presents as a bright blue compound, which becomes colourless when the siderophores present in the culture supernatant remove Fe from the solution. Siderophore production was indicated by a change in the reactions from dark blue to yellow–orange colour. The percent siderophore unit (PSU) was calculated from the data observed at 630 nm (OD_{630}) using the following formula:

$$\text{PSU} = \left(Ar - \frac{As}{Ar}\right) \times 100, \quad (2)$$

where *Ar* represents the absorbance of the reference sample and *As* represents the absorbance of the inoculated sample [37]. This test was carried out in triplicate.

2.10. In Vitro Antagonism Tests on Solid Media. To evaluate the antagonistic potential of the *B. licheniformis* strains against various plant pathogenic fungi (including *Aspergillus niger* - SZMC 0050, *Fusarium oxysporum* - SZMC 27512, *Fusarium solani* - SZMC 11058F, *Fusarium solani* - SZMC 11059F, *Phomopsis sp.* - SZMC 27124, and *Rhizoctonia solani* -SZMC 6252J), *in vitro* confrontation assays were performed on potato dextrose agar (PDA) medium (VWR Chemicals, Leuven, Belgium) supplemented with 10 g L^{-1} agar following the method of Vörös et al. [38]. For the procedure, mycelial disks measuring 6 mm in diameter were cut under sterile conditions from fungal colonies aged 4–5days and grown on PDA medium. These disks were then placed in the centre of PDA plates, and 5 μL of 24-hr-old shaken (MaxQTM 8000, 8000-1CE, Thermo Fisher Scientific, USA) bacterial suspension was inoculated in spots 25 mm away from the fungal inoculation point. Plates containing only the fungus served as control samples. Plates were incubated at 25°C until the colony radius of the control fungal colony reached 25 mm. The incubation times varied based on the growth characteristics of the tested fungi. The diameter of the inhibition zone between the edge of the bacterial colony and the inhibited fungal colony was measured. The percentage (%) of inhibitory activity (I.A.) was calculated using the following formula:

$$\text{I.A.} = \left(\frac{Cc - Ci}{Cc}\right) \times 100, \quad (3)$$

where *Cc* represents the diameter of the control fungal colony and *Ci* represents the diameter of the inhibited fungal colony. This test was conducted in triplicate.

2.11. In Vitro Antagonism Tests on Sweet Potato Slices. The selected *B. licheniformis* strains (SZMC 27713 and SZMC 27715) were tested for their antagonistic effects against the same six plant pathogenic fungal strains used in previous *in vitro* antagonism assays (subsection 2.10). This assessment was performed on sterilised sweet potato slices as the growth medium. Plant pathogenic fungal strains were inoculated on PDA medium (VWR Chemicals, Leuven, Belgium) supplemented with 10 g L^{-1} agar and incubated for 5 days at 25°C. On the 6^{th} day, the conidia were washed with 10 mL of sterile 0.9% NaCl solution and pipetted into sterile Falcon tubes. The cell concentration of conidia suspensions was adjusted to 1×10^6 mL. *B. licheniformis* strains SZMC 27713 and SZMC 27715 were cultured overnight in 4 mL of liquid Mueller–Hinton medium (VWR Chemicals, Leuven, Belgium) and the optical density of the suspensions was determined at 620 nm (OD_{620}). The final cell concentration of the bacterial suspensions was adjusted to 1×10^6 mL^{-1}. Sweet potato tubers were first washed under running tap water and then subjected to surface sterilisation by washing in 10% NaOCl solution (2 min), 70% EtOH solution (2 min), and sterile distilled water (2 min). The sterilisation steps were repeated in two subsequent rounds. The sterile tubers were then cut into 1 cm thick slices under sterile conditions and the sweet potato slices were placed in glass Petri dishes containing sterile wet filter paper (Whatman filter paper no. 1 of area 70.541 cm^2). For the slices to be inoculated with fungus, 50 μL of conidia suspension was pipetted into the centre of the sterilised slices and then dried. To test the inhibitory effect of the bacterial

Table 2: Bacterial treatments applied in the field experiment.

Variant	Description
Treatment 1 (Tr1)	Soaking the lower part of the cuttings in the bacterial suspension for 15 min, then soaking the upper part of the cuttings in the bacterial suspension for another 15 min
Treatment 2 (Tr2)	Tr1+ additional inoculation of the leaves with the bacterial suspension at a dosage of 20 mL per plant on the 18th day after planting
Treatment 3 (Tr3)	Tr2+ secondary additional inoculation of the leaves with the bacterial suspension at a dosage of 20 mL per plant on the 39th day after planting.

strains against plant pathogens, 50 μL of bacterial suspension was first pipetted on the slices then dried and 50 μL of conidia suspension was pipetted at the same spot on the slice. Sterile sweet potato slices inoculated with 50 μL of sterile 0.9% NaCl solution served as negative control. The plates were checked daily, and photographic evaluation of the plates was performed on day 21. For the visual assessment of sweet potato slices, three levels of disease severity were distinguished: symptomless slice (–), slice with slight symptoms (+), and slice with severe symptoms (++). The test was performed in three replicates.

2.12. Germination Test. The germination test evaluated the effects of two promising bacterial strains (SZMC 27713 and SZMC 27715), which demonstrated favourable outcomes in previous assessments, on tomato (*Solanum lycopersicum* L.) seeds (cultivar "Zömök", Rédei Kertimag Zrt., Hungary). Overnight bacterial suspensions were prepared as previously described above, and shaken (MaxQTM 8000, 8000-1CE, Thermo Fisher Scientific, USA) overnight at 25°C at 140 rpm. The optical density of these bacterial suspensions was measured at 620 nm (OD_{620}). Tomato seeds were surface sterilised in 70% ethanol for 1 min then the seeds were soaked in sterile distilled water for 5 min. In the next step, the seeds were soaked for 10 min in 10% NaOCl then washed four times with sterile distilled water. Sterilised seeds (10 seeds in each of three plates) were placed in sterile glass Petri dishes containing three layers of sterile filter paper (Whatman filter paper no. 1 of area 70.541 cm^2) drenched with 5 mL of sterile distilled water. For the treatment, 50 μL of 10^5 CFU mL^{-1} suspension diluted with 0.9% NaCl was pipetted on each seed, while sterile distilled water was used instead of bacterial suspension for the control samples. The plates were then placed in the dark at 25°C and incubated for 6 days. All Petri dishes were sprayed daily with 2 mL of sterilised distilled water. The number of emerging seedlings was checked daily, and germination rates (GR) were calculated.

The GR (%) were calculated using the following formula described by Ranganathan and Thavaranjit [39]:

$$\mathrm{GR} = \left(\frac{\mathrm{NGS}}{\mathrm{TNS}}\right) \times 100, \tag{4}$$

where NGS represents the number of germinated seeds and TNS signifies the total number of seeds. This test was conducted in triplicate.

2.13. Preparation of Bacterial Inoculum for Field Experiment. For the field experiment, freshly grown colonies of strain SZMC 27715 were inoculated in 250 mL Erlenmeyer flasks containing 50 mL YEG medium and were shaken at 140 rpm overnight at 25°C. The grown cultures were used to inoculate 1 L of liquid YEG media and were shaken at 140 rpm for 3 days at 25°C. The bacterial suspension was diluted with sterile tap water to a final cell concentration of 10^6 cells/mL for soaking of sweet potato secondary cuttings and foliar treatment of plants. A 10 L bucket was used to soak the sweet potato cuttings and a manual pressure sprayer (Stihl® SG 11 Plus) was used to spray the leaves.

2.14. Field Experiment. To investigate the yield enhancing potential of the strain SZMC 27715 in sweet potato cultivation, a field experiment was designed in which the candidate strain was applied in various treatments (Table 2). The experiment was carried out in Zsombó (coordinates: 46°20′N 19°59′E), South Hungary, on alkaline (pH 8.1) sandy soil of poor humus (0.4%), very good AL-soluble P_2O_5 (258.7 mg kg^{-}1) and good AL-soluble K_2O (161.5 mg kg^{-1}) content. The preceding crop was pepper (*Capsicum annuum* var. *grossum*; cultivar "Bravia F1"). No soil disinfection was applied before the sweet potato planting. Irrigation was managed by a drip system. Sweet potato (cultivar "Ásotthalmi 12") secondary cuttings with an average length of 20–30 cm were planted in ridges with plastic mulch cover, with 1 m row spacing and 0.3 m plant-to-plant distance on 13 June 2023. Three treatments were used in the test (Table 2): Treatment 1 (Tr1): soaking the lower part of the cuttings in the bacterial suspension for 15 min, then soaking the upper part of the cuttings in the bacterial suspension for another 15 min. Treatment 2 (Tr2): the same as Tr1 with an additional inoculation of the leaves with the bacterial suspension at a dosage of 20 mL per plant on the 18th day after planting. Treatment 3 (Tr3): the same as Tr2 with a secondary additional inoculation of the leaves with the bacterial suspension at a dosage of 20 mL per plant on the 39th day after planting. Control (C): no bacterial inoculation applied. Sweet potato storage roots were harvested on 6 October 2023. Within each treatment, the storage root weight per plant was measured one by one with two decimal places using a digital scale (Gebo Tools, Cluj-Napoca, Romania), and the storage root number per plant was counted one by one during harvest. The control plot consisted of 50 plants, the Tr1 plot 102 plants, the Tr2 plot 57 plants, and the Tr3 plot 50 plants.

Table 3: Endophytic strains isolated from surface-sterilised sections of sweet potato.

	L1	L2	L3	S1	S2	S3	T1	T2	T3
1.	EL1/B1	EL2/B1	EL3/B1	ES1/B1	ES2/B1	—	ET1/B1	ET2/B1	ET3/B1
2.	EL1/B2	—	EL3/B2	—	—	—	ET1/B2	ET2/B2	ET3/B2
3.	EL1/B3	—	EL3/B3	—	—	—	—	—	ET3/B3
4.	—	—	EL3/B4	—	—	—	—	—	—
5.	—	—	EL3/B5	—	—	—	—	—	—
6.	—	—	EL3/B6	—	—	—	—	—	—
7.	—	—	EL3/B7	—	—	—	—	—	—
8.	—	—	EL3/B8	—	—	—	—	—	—
9.	—	—	EL3/B9	—	—	—	—	—	—

The table presents the laboratory codes for each isolate. Samples were collected in Madaras, Hungary. L1 = leaf sample 1; L2 = leaf sample 2; L3 = leaf sample 3; total: 13 isolates S1 = stem sample 1; S2 = stem sample 2; S3 = stem sample 3; total: 2 isolates T1 = tuber sample 1; T2 = tuber sample 2; T3 = tuber sample 3; total: 7 isolates.

Table 4: Rhizospheric and endophytic bacterial strains isolated and identified from sweet potato.

Bacterial species	Laboratory code	SZMC number	Isolation source	GenBank accession number
B. licheniformis	ET3/B2	SZMC 27712	Tuber	OP620082
B. licheniformis	ET2/B2	SZMC 27713	Tuber	OP620083
B. licheniformis	EL1/B1	SZMC 27714	Leaf	OP620084
B. licheniformis	ES1/B1	SZMC 27715	Stem	OP620085
B. licheniformis	EL3/B1	SZMC 27716	Leaf	OP620086
B. licheniformis	S1/B3	SZMC 27717	Rhizosphere	OP620087
B. licheniformis	EL2/B1	SZMC 27718	Leaf	OP620088

2.15. Statistical Analysis and Data Visualisation. Before applying statistical analysis, normal distribution of the datasets was checked. Statistical analysis and data visualisations were created using GraphPad Prism 10.0.1 (https://www.graphpad.com/) software. One-way Analysis of Variance (ANOVA) was used to analyse the results of the plant growth promotion tests (IAA production, phosphorus solubilising ability, ammonia production, siderophore production, and germination test) and the field test. Regarding the data proven to be significant by ANOVA, the averages of the factors were compared by Tukey test at the probability level of 95% ($p < 0.05$). Unless specified, values represent the means of three replicates with standard deviations (SD).

3. Results

3.1. Isolation and Identification of Rhizospheric and Endophytic Bacteria. Strains were isolated from two distinct sweet potato plantations located in southern Hungary. The endophytic strains were obtained from surface-sterilised sections of sweet potato leaves, stems, and tubers (Table 3).

Rhizospheric strain was isolated from the rhizosphere of sweet potato (Bordány, Hungary). In total, 44 strains were isolated from endophytic and rhizospheric sources in the collected sweet potato and soil samples. Among these, a subset of 14 strains underwent identification (details not presented here) involving sequence analysis of a fragment from both the 16S rRNA and DNA gyrase alpha subunit genes. Given our specific interest in the *Bacillus* genus, our subsequent investigations were focused on the *B. licheniformis* strains (Table 4), which were then subjected to a battery of diverse tests.

3.2. Effect of Temperature on Bacterial Growth. B. licheniformis strains' growth response to two temperatures, 25 and 37°C, showed variations. The observed optical density values suggest that the strains have different temperature tolerances at the two tested temperatures. In contrast, similar OD values were observed for strain SZMC 27715 at both tested temperatures. Among the strains, SZMC 27712, SZMC 27713, SZMC 27714, SZMC 27716, and SZMC 27718 preferred 25°C, while SZMC 27717 performed better at 37°C Figure 1. Overall, all strains were able to tolerate both tested temperatures.

3.3. Effect of Water Activity on Cell Density. In terms of water activity, all strains thrived in the presence of 1% ($a_w = 0.991$) or 2% ($a_w = 0.980$) NaCl. However, only SZMC 27715 tolerated 5% NaCl ($a_w = 0.968$). Notably, SZMC 27713 exhibited the highest growth in both 1% and 2% NaCl conditions. On the other hand, strains SZMC 27712, SZMC 27714, SZMC 27716, SZMC 27717, and SZMC 27718 displayed reduced cell densities with increasing NaCl concentration. Strain SZMC 27712 showed consistent cell density regardless of NaCl presence Figure 2.

3.4. pH Dependence. The *B. licheniformis* strains' growth was assessed at various pH levels: pH 4.0, pH 5.0, pH 6.0, pH 7.0, and pH 8.0. As depicted in Figure 3, none of the strains exhibited growth at acidic levels including pH 4.0 and pH 5.0. Specifically, only SZMC 27714 and SZMC 27715 strains displayed growth at pH 6.0. Conversely, all strains demonstrated growth,

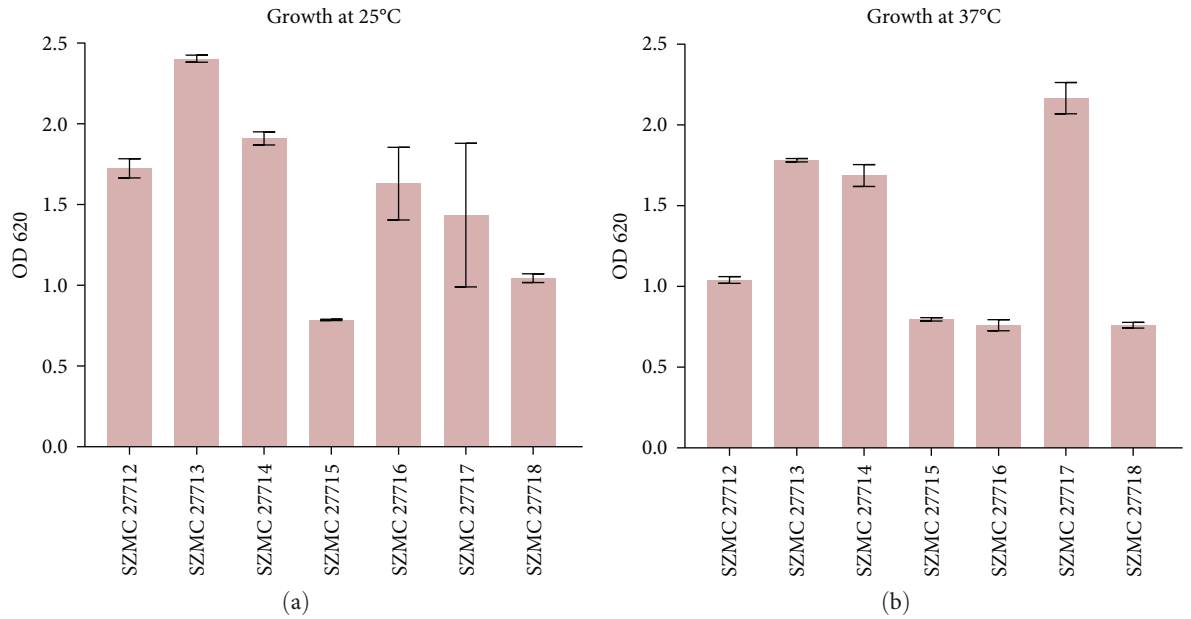

Figure 1: Effect of temperature on the cell density of the *B. licheniformis* strains at: (a) 25°C and (b) 37°C. The data represents the mean ± standard deviation of three replicates.

albeit varying, at pH 7.0 and pH 8.0. Notably, SZMC 27713 and SZMC 27715 strains displayed the highest cell density at pH 8.0. Evidently, most strains exhibited a preference for alkaline pH conditions.

3.5. Qualitative Determination of IAA Production. In the qualitative test on YEG media amended with L-tryptophan, a total of six strains exhibited IAA-synthesising ability manifested by the emergence of a pink halo zone around the colonies (Figure 4). Conversely, the SZMC 27713 isolate did not produce a halo zone, indicating a lack of IAA production.

3.6. Quantitative Determination of the IAA Production. The spectrophotometric method effectively facilitated the quantification of IAA concentration in the culture supernatants. Across both conditions of presence and absence of L-tryptophan, most of the examined strains exhibited the capability to produce IAA (Table 5). In alignment with the qualitative assay outcomes, six strains displayed IAA synthesis potential when supplemented with L-tryptophan, although substantial variations were apparent among strains (Figure 5). Among the tested isolates, strains SZMC 27714 and SZMC 27715 exhibited the highest IAA production (36.20 and 40.42 $\mu g/mL^{-1}$) in the presence of L-tryptophan. These two isolates exhibited significantly higher ($p < 0.05$) IAA production compared to the other strains. The highest IAA production was observed for strain SZMC 27715 in the presence of L-tryptophan after 3 days of incubation (40.42 $\mu g/mL^{-1}$), although, this isolate exhibited no IAA production in the absence of L-tryptophan. No significant difference ($p > 0.05$) in the IAA production between strain SZMC 27714 and SZMC 27715 was observed in the presence of L-tryptophan after 3 days of incubation. Isolates SZMC 27712, SZMC 27716, SZMC 27717, and SZMC 27718 demonstrated comparable IAA production (19.75, 18.05, 20.53, and 26.20 $\mu g/mL^{-1}$) after 3 days of incubation in the presence of L-tryptophan, no significant differences ($p > 0.05$) were observed among these strains. Strains SZMC 27712, SZMC 27714, SZMC 27716, SZMC 27717, and SZMC 27718 exhibited the capacity for IAA production under both conditions. In the absence of L-tryptophan, SZMC 27714 displayed the greatest IAA concentration (15.84 $\mu g\,mL^{-1}$) after 2 days of incubation. Strain SZMC 27713 demonstrated a lack of IAA production in both presence and absence of L-tryptophan. The highest IAA concentrations were generally attained after 2 or 3 days of incubation.

3.7. Phosphorus Solubilisation Activity. All tested strains exhibited the capability to solubilise tricalcium phosphate from the medium, although their phosphate solubilisation efficiencies varied, the solubilisation index (S.I.) ranged between 1.1 and 1.7 (Table 6, Figures 6 and 7). Strain SZMC 27715 showed a significantly higher ($p < 0.05$) P-solubilisation capacity (S.I.: 1.7) compared to all other tested isolates, which displayed the most substantial phosphate solubilising zone (5.5 mm). A remarkable S.I. (1.4.) was observed for strain SZMC 27717, which was significantly higher ($p < 0.05$) than for isolates SZMC 27712, SZMC 27713, SZMC 27714, SZMC 27716, and SZMC 27718. Isolates SZMC 27712, SZMC 27713, SZMC 27714, SZMC 27716, and SZMC 27718 exhibited comparable S.I. (1.2; 1.1; 1.2; 1.3, and 1.2), no significant differences ($p > 0.05$) were observed among these strains. The lowest S.I. (1.1) and the smallest zone (0.2 mm) were observed for SZMC 27713 strain.

3.8. Ammonia Production. Among the seven tested *B. licheniformis* strains, five strains (SZMC 27712, SZMC 27713, SZMC 27714, SZMC 27715, and SZMC 27716) exhibited a certain level of ammonia production, while two strains (SZMC 27717 and SZMC 27718) did not produce ammonia under the

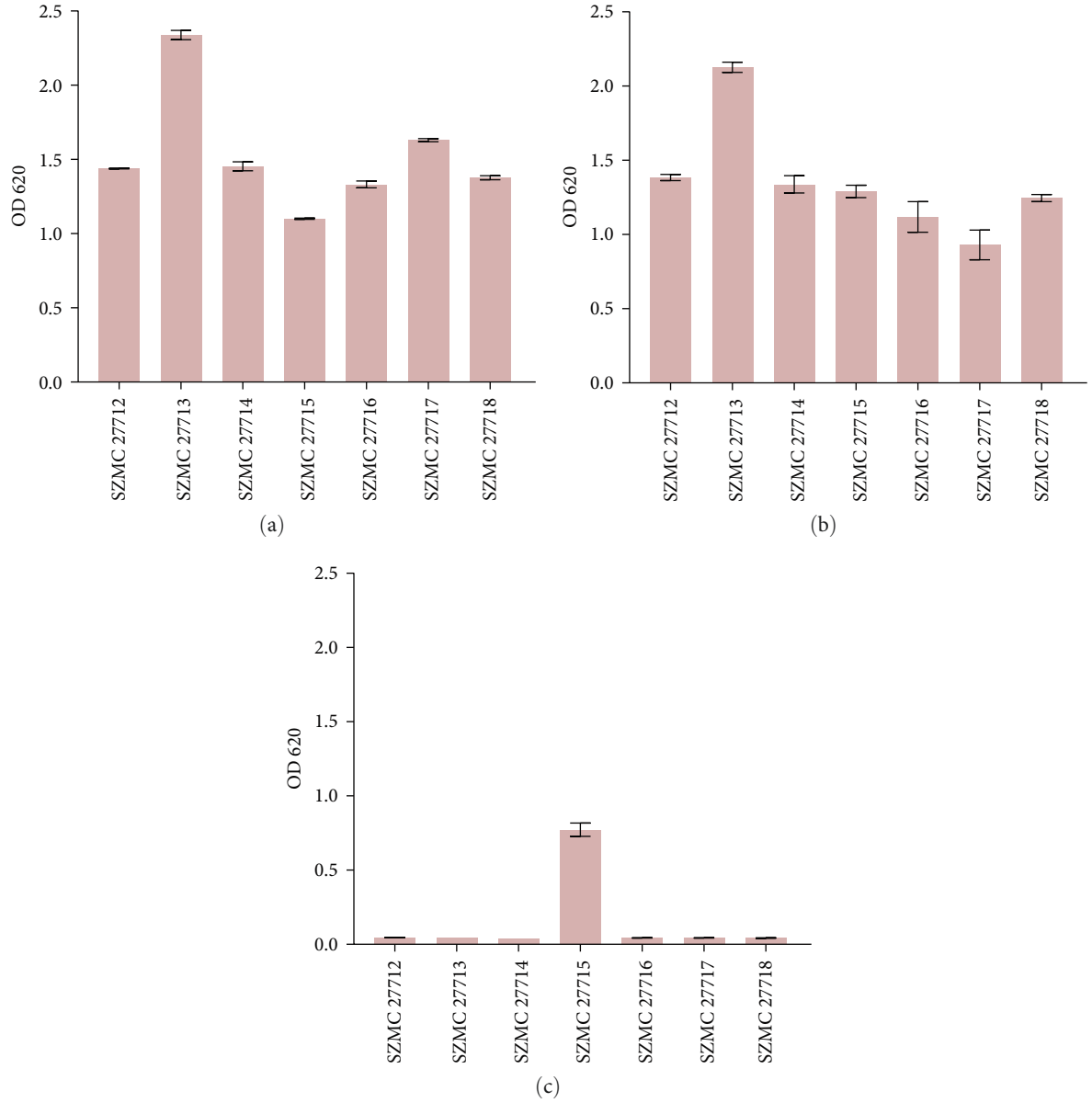

Figure 2: Effect of water activity on the cell density of *B. licheniformis* strains: (a) $a_w = 0.991$; (b) $a_w = 0.980$; and (c) $a_w = 0.968$. Values represent the mean ± S.D. of three replicates.

experimental conditions (Figure 8). Generally, the concentration of ammonia ranged from 55 to 870 $\mu g\,mL^{-1}$. Notably, strains SZMC 27713 and SZMC 27715 demonstrated significantly higher ($p < 0.05$) levels of ammonia production (870 and 839 $\mu g\,mL^{-1}$) in comparison to all other tested isolates, however, no significant differences ($p > 0.05$) were found between the two strains. Isolates SZMC 27712, SZMC 27714, and SZMC 27716 demonstrated comparable ammonia production (117; 121; and 55 $\mu g/mL^{-1}$), no significant differences ($p > 0.05$) were observed among these strains.

3.9. Siderophore Production. Initial tests using CAS agar plates did not reveal any siderophore production by the strains (data not shown). However, the spectrophotometric method confirmed the siderophore-producing ability of five *B. licheniformis* strains. This was evident from the decrease in OD_{630} values compared to the reference sample. Conversely, strains SZMC 27713 and SZMC 27715 exhibited no siderophore production (Figure 9). PSU values ranged from 0.90% (SZMC 27712) to 90% (SZMC 27718), though SZMC 27712's siderophore production was minimal and not considered for further analyses. The siderophore production of strains SZMC 27714, SZMC 27717, and SZMC 27718 was significantly higher ($p < 0.05$) than the other strains exhibiting siderophore production (SZMC 27712 and SZMC 27716), however, no significant differences ($p > 0.05$) were found among these strains. The highest PSU value (89.95%) was observed for strain SZMC 27718.

3.10. In Vitro Antagonism Tests. The inhibitory activity (I.A.) of the *B. licheniformis* strains was assessed against six different plant pathogenic fungi occurring in sweet potato

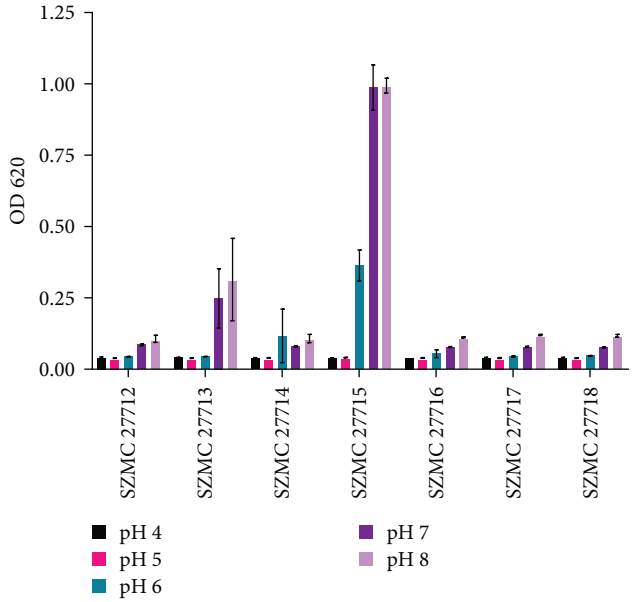

FIGURE 3: Effect of pH on the cell density of the *B. licheniformis* strains. Bars represent the mean ± standard deviation (S.D.) of three replicates.

cultivation, the observed I.A. was calculated using the formula described above and expressed as percentages (Table 7). Among the tested bacterial strains, SZMC 27715 displayed I. A. against *A. niger* SZMC 0050 (22%), *F. oxysporum* SZMC 27512 (35%), *F. solani* SZMC 11058F (18%), *F. solani* 11059F (25%), *Phomopsis* sp. SZMC 27124 (36%), and *R. solani* SZMC 6252J (35%). In the case of strain SZMC 27713, I.A. against five plant pathogenic fungi was observed: *A. niger* SZMC 0050 (7%), *F. oxysporum* SZMC 27512 (23%), *F. solani* SZMC 11058F (35%), *F. solani* 11059F (7%), and *Phomopsis* sp. SZMC 27124 (38%) (Table 7 and Figure 10). Notably, out of the seven tested *B. licheniformis* strains, five strains (SZMC 27712, SZMC 27714, SZMC 27716, SZMC 27717, and SZMC 27718) did not demonstrate any growth inhibition effect against the tested fungal plant pathogens.

These findings have led to the selection of isolates SZMC 27713 and SZMC 27715 as strains with promising biocontrol activity for further studies.

3.11. In Vitro Antagonism Tests on Sweet Potato Slices. Visual assessment of the slice test showed that inoculation of sweet potato slices with plant pathogenic fungal strains such as *A. niger* (SZMC 0050), *F. solani* (SZMC 11058F and SZMC 11059), and *R. solani* (SZMC 6252J) led to evident infection symptoms (++) on the slices by day 21 when compared to the symptomless control (−). Conversely, pre-inoculation of sweet potato slices with the candidate biocontrol strain SZMC 27715 resulted in limited symptoms (+) of fungal infection, yielding slices with visual similarity to the control. In contrast, the inoculation of slices with *F. oxysporum* (SZMC 27512) and *Phomopsis* sp. (SZMC 27124) on sweet potato slices did not exhibit distinct disease symptoms of fungal infections (+). Notably, the protective effect of the SZMC 27715 strain was not observed in these cases (+; Figure 11). No protective or inhibitory effects were observed for *B. licheniformis* strain SZMC 27713 in any of the cases (data not shown).

3.12. Accelerated Germination of Tomato Seeds by Strain SZMC 27715. The germination test examined the impact of the two potential biocontrol strains (SZMC 27713 and SZMC 27715) on sterilised tomato seeds. The two strains were selected on the promising results of the preliminary tests (IAA and ammonia production, P-solubilisation, antagonism tests). Germination rates (GR) were calculated for control and bacterial treated tomato seeds by counting germinating seeds daily. Although higher germination rates were observed on day 3 (86.7%) and day 4 (93.3%) when treated with strain SZMC 27715 compared to the control (66.7% and 80%), but these values were not significantly different from the control data ($p > 0.05$). On day 6, the germination-promoting effect of strain SZMC 27715 was significantly confirmed (96.7%) ($p < 0.05$) compared to the control seeds (80%), thereby revealing the positive effect of the strain on tomato seed germination. Conversely, strain SZMC 27713 did not exhibit any stimulating effect on germination; in such instances, germination rates were comparable to the control (Figure 12). Our results suggest that strain SZMC 27713 has no remarkable effect on tomato seed germination, whereas strain SZMC 27715 is presumably involved in tomato seed germination through certain mechanisms.

3.13. Yield Enhancing Effect of SZMC 27715 Isolate in Sweet Potato Cultivation In Vivo. In the field experiment, we assessed the yield enhancing performance of *B. licheniformis* strain SZMC 27715 in the cultivation of sweet potato, based on its promising plant growth-promoting traits and biocontrol potential. Therefore, three different treatments with the isolate SZMC 27715 were set up in the field experiment; in each treatment (Tr1, Tr2, and Tr3), the sweet potato secondary cuttings were soaked in the bacterial suspension before planting, while in the subsequent treatments (Tr2 and Tr3) the bioinoculant was applied as a foliar spray using a manual sprayer. As depicted in Figure 13, all treatments resulted in increased yield per plant compared to the control group (C), however, notable disparities in the effects of each treatment were observed. In comparison to the average yield per plant of untreated control plants (0.92 kg), Treatment 3 (Tr3), which involved soaking along with two foliar treatments, demonstrated significantly different ($p < 0.05$) yield increase, reaching 1.40 kg per plant, this resulted in 0.48 kg more harvested sweet potato tubers per plant. Furthermore, Tr3 treatment resulted significantly higher ($p < 0.05$) yield increase than Tr1 and Tr2 treatments. Treatment 1 (Tr1) and Treatment 2 (Tr2) exhibited comparable effects on yield enhancement (1.13 and 1.09 kg per plant, respectively) in comparison to the control (0.92 kg). Nevertheless, Tr1 appeared to be slightly more effective in boosting yield than Tr2, however, no significant difference ($p > 0.05$) was found between the two treatments. Based on our results, both Tr1 and Tr2 treatments resulted in a notable difference in yield increase compared to the control, but two additional treatments were

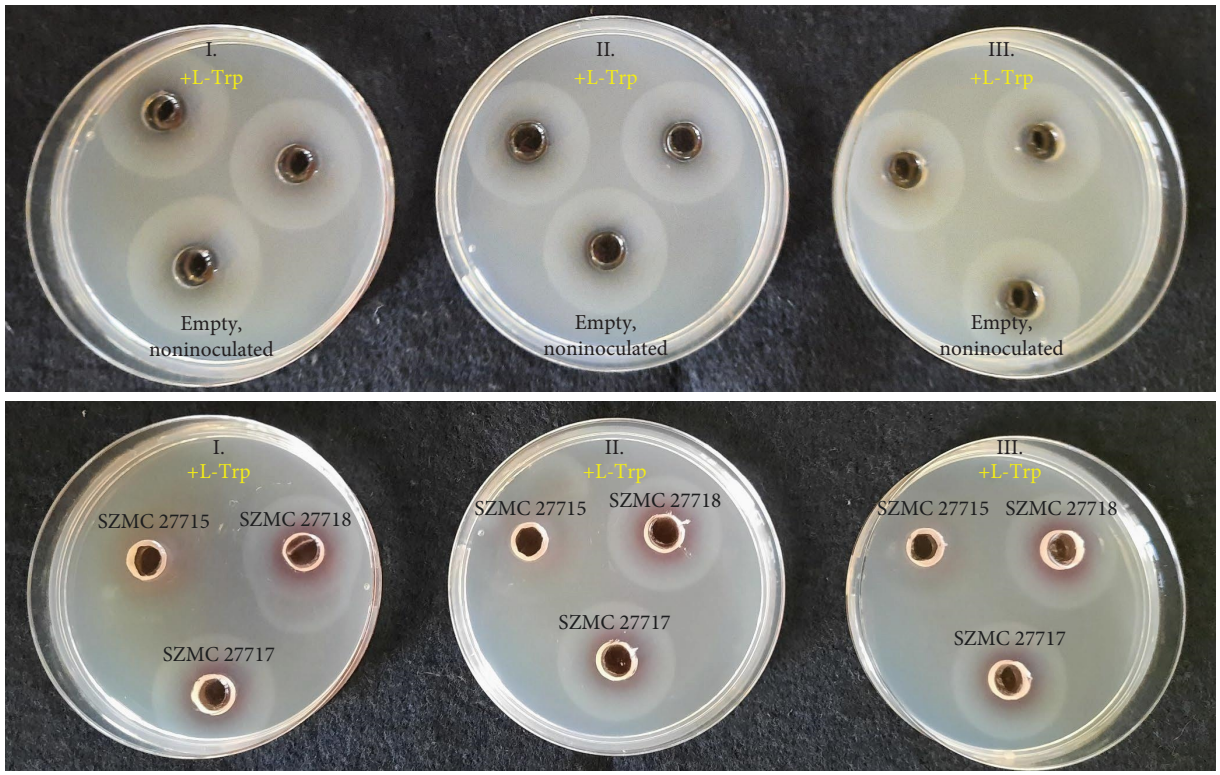

Figure 4: IAA production of the *B. licheniformis* strains on solid YEG media amended with L-tryptophan. The ability to produce IAA was confirmed by the pink halo zone around the colonies.

Table 5: IAA production by *B. licheniformis* strains in the presence and absence of L-tryptophan (Trp).

Isolate	IAA production ($\mu g\,mL^{-1}$)					
	Day 1		Day 2		Day 3	
	Trp+	Trp−	Trp+	Trp−	Trp+	Trp−
SZMC 27712	9.13 ± 3.565	5.68 ± 1.221	17.89 ± 0.727	2.27 ± 0.000	19.75 ± 0.897	—
SZMC 27713	—	—	—	—	—	—
SZMC 27714	9.71 ± 0.312	2.27 ± 1.259	35.41 ± 1.693	15.84 ± 1.448	36.20 ± 0.120	0.52 ± 0.599
SZMC 27715	1.23 ± 0.215	—	2.55 ± 1.033	—	40.42 ± 5.302	—
SZMC 27716	6.98 ± 0.683	1.00 ± 0.727	21.77 ± 5.538	14.33 ± 0.727	18.05 ± 7.378	2.43 ± 9.280
SZMC 27717	8.59 ± 0.379	—	21.49 ± 0.469	4.41 ± 1.797	20.53 ± 0.286	2.98 ± 1.561
SZMC 27718	2.14 ± 0.379	2.67 ± 1.924	31.98 ± 1.622	5.28 ± 6.476	26.20 ± 2.344	0.13 ± 7.223

In this table, values are mean ± S.D. of three replicates.

required to achieve a statistically significant difference. This means that a second foliar treatment on the 39th day after planting was essential to obtain a prominent yield enhancing effect.

Calculating with the experimental setting of 33.333 plants ha^{-1}, the difference between the control (30.7 tons ha^{-1}) and the treated (Tr1 : 37.7, Tr2 : 36.3, Tr3 : 46.7 tons ha−1) plots can be between 5.7 and 16.0 tons at the hectare level (Table 8). These findings highlight the substantial impact of *B. licheniformis* strain SZMC 27715 on sweet potato cultivation, particularly when incorporated into Tr3 (soaking of sweet potato cuttings along with two foliar treatments), which showed significant results in terms of yield increase in comparison to the untreated control plants (C).

As shown in Figure 14, there was no significant difference ($p > 0.05$) in the number of tubers per plant between the applied treatments (average range: 5.79–6.90). However, the Tr3 treatment resulted in the highest number of tubers per plant. Our results suggest that the average size of tubers increased with the frequency of treatments, which can be the reason for the increase in yield per plant for each treatment.

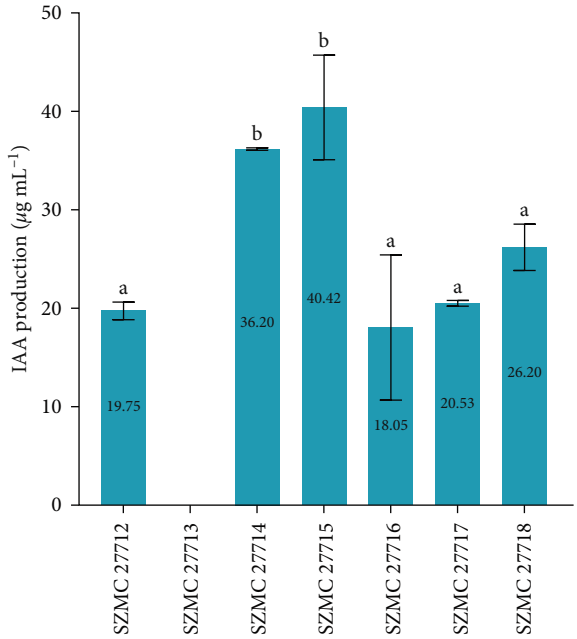

Figure 5: IAA production of the *B. licheniformis* strains after 3 days of incubation in the presence of L-tryptophan. The same letters above the data in the columns mean no significant difference ($p > 0.05$). Different letters indicate significant differences ($p < 0.05$). Values are presented as mean ± S.D. of three replicates.

Table 6: Phosphorus solubilisation activity of *B. licheniformis* strains.

Strain	Colony diameter (mm)	Zone diameter (mm)	Total diameter (mm)	Solubilisation index (S.I.)
SZMC 27712	9.7 ± 0.58	2.3 ± 0.58	12.0 ± 0.00	1.2 ± 0.08
SZMC 27713	7.3 ± 1.53	0.6 ± 0.4	7.9 ± 1.85	1.1 ± 0.04
SZMC 27714	10.0 ± 0.00	2.8 ± 0.29	12.8 ± 0.29	1.3 ± 0.03
SZMC 27715	7.2 ± 0.76	5.2 ± 0.29	12.3 ± 0.58	1.7 ± 0.11
SZMC 27716	9.8 ± 0.76	2.5 ± 0.50	12.3 ± 0.58	1.3 ± 0.07
SZMC 27717	9.3 ± 0.58	3.8 ± 0.29	13.2 ± 0.29	1.4 ± 0.05
SZMC 27718	13.3 ± 0.58	2.2 ± 1.04	15.5 ± 0.50	1.2 ± 0.08

Values in this table represent the mean ± standard deviation (S.D.) of three replicates.

Figure 6: Tri-calcium phosphate ($Ca_3(PO_4)_2$) solubilisation activity of *B. licheniformis* strains grown on Pikovskaya medium.

4. Discussion

4.1. Ecophysiological Investigations. A series of experiments were carried out to test the selected *B. licheniformis* strains for their tolerance to different limiting factors such as pH, temperature, and water activity (detailed in Subsection 3.2, 3.3, and 3.4) influencing their survivability in different agricultural environments. This examination is particularly important as global warming increasingly positions sweet potato as an alternative to traditional crops in continental climates. Soil salinisation has become a global problem in recent decades, affecting more than 400 million hectares worldwide in 2015 [40] including Europe. Therefore, in the PGPB screening process, it is essential to test the salinity tolerance of the isolates. In our study, all tested strains were able to tolerate the presence of 10 and 20 g/L^{-1} NaCl ($a_w = 0.991$ and 0.980), furthermore, our candidate strain

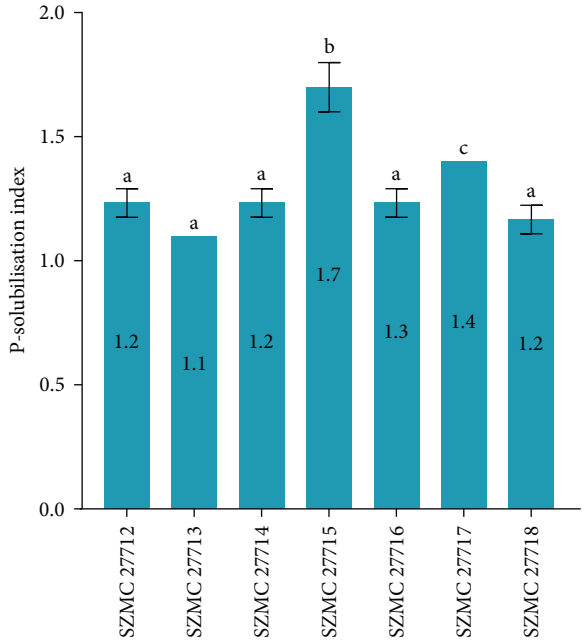

Figure 7: Phosphorus solubilisation activity of the *B. licheniformis* strains. The same letters above the data in the columns mean no significant difference ($p > 0.05$). Different letters indicate significant differences ($p < 0.05$). Values are presented as mean ± S.D. of three replicates.

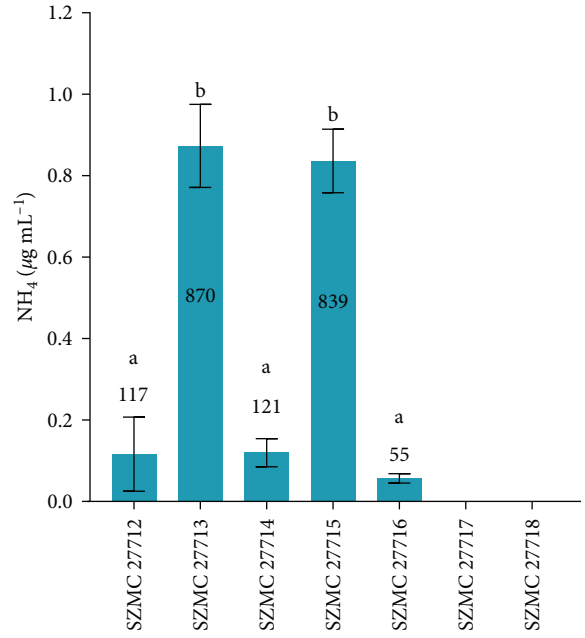

Figure 8: Ammonia production of *B. licheniformis* strains. The same letters above the data in the columns mean no significant difference ($p > 0.05$). Different letters indicate significant differences ($p < 0.05$). Values are presented as mean ± S.D. of three replicates.

(SZMC 27715) was able to survive in the presence of 50 g/L^{-1} NaCl ($a_w = 0.968$), which allows the application of the isolate on saline soils. Since the pH tolerance of certain microorganisms is very narrow, the pH of a given soil has a major influence on the survival of the PGPB [41]. In the pH

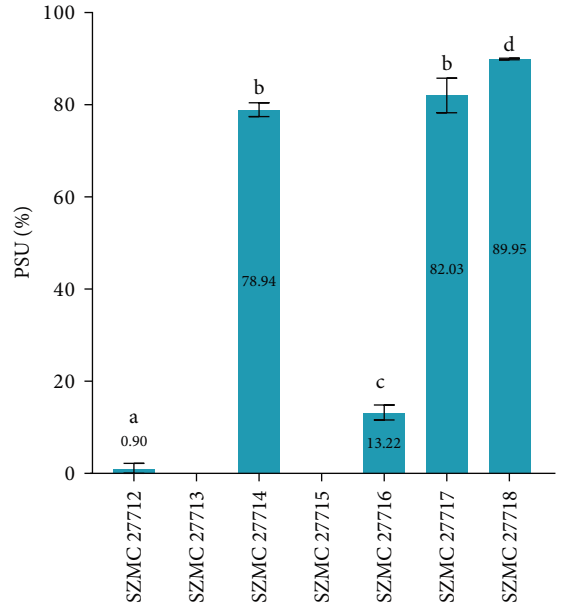

Figure 9: Siderophore production (PSU) of *B. licheniformis* strains after 48 hr. The same letters above the data in the columns mean no significant difference ($p > 0.05$). Different letters indicate significant differences ($p < 0.05$). In this figure, values represent the mean ± S.D. of three replicates.

tolerance test, we found that strain SZMC 27715 could grow intensively at pH 7 and 8, suggesting that neutral and slightly alkaline environments would be optimal for its growth. Global warming put additional abiotic stress factors challenging microorganisms to adapt to an increase in temperature. Furthermore, temperature has remarkable impact on beneficial plant-microbe interactions [42]. Testing our isolates revealed that all strains could grow at 25°C and also at a higher temperature (37°C) which enhances their application potential in different agricultural environments. This temperature range allows the application of the isolates in sweet potato production both at planting and throughout the growing season at tuber development.

4.2. IAA Production. Auxins (e.g., IAA) produced by bacteria can change the auxin pool of plants to optimal or supra-optimal levels, most typically inducing root growth, especially secondary roots, thus increasing the total root surface area and consequently resulting in more intensive growth and higher yields [43]. Bacteria can stimulate plant growth by producing auxins via both L-tryptophan-dependent and L-tryptophan-independent pathways [44]. Previous observations indicated that plants treated with *B. licheniformis* B12 exhibited enhanced vegetative growth due to elevated levels of auxin-like compounds (1.79 μg mL^{-1}) produced by the strain [45]. In our study, we successfully demonstrated IAA production by *B. licheniformis* strains, both in the presence and absence of L-tryptophan (Table 5), suggesting the possibility of L-tryptophan-independent IAA production in these strains. Not surprisingly, higher IAA levels were observed in the presence of L-tryptophan. In contrast, IAA production by strain SZMC 27713 could not be detected by either qualitative or

Table 7: Inhibitory activity (I.A.) of *B. licheniformis* strains against plant pathogenic fungi.

Strain	I.A. (%)					
	Aspergillus niger SZMC 0050	*Fusarium oxysporum* SZMC 27512	*Fusarium solani* SZMC 11058F	*Fusarium solani* SZMC 11059F	*Phomopsis sp.* SZMC 27124	*Rhizoctonia solani* SZMC 6252J
SZMC 27712	0	0	0	0	0	0
SZMC 27713	7 ± 0.03	23 ± 0.05	35 ± 0.03	7 ± 0.02	38 ± 0.03	0
SZMC 27714	0	0	0	0	0	0
SZMC 27715	22 ± 0.06	35 ± 0.05	18 ± 0.06	25 ± 0.13	36 ± 0.03	35 ± 0.02
SZMC 27716	0	0	0	0	0	0
SZMC 27717	0	0	0	0	0	0
SZMC 27718	0	0	0	0	0	0

Values in this table represent the mean ± S.D. of three replicates.

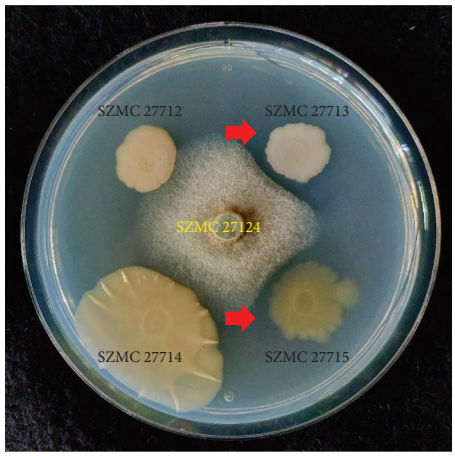

Figure 10: Inhibitory activity (I.A.) of strains SZMC 27713 and SZMC 27715 against *Phomopsis sp.*-SZMC 27124 on PDA.

quantitative methods. The highest auxin production was observed in strain SZMC 27715 ($40.42\,\mu g\,mL^{-1}$) after 3 days of incubation in the presence of L-tryptophan. Rawat et al. [46] reported that a *B. licheniformis* strain isolated from rice rhizosphere was found to produce $16.85\,\mu g/mL^{-1}$ under similar experimental conditions in the presence of L-tryptophan, compared to our results, isolate SZMC 27715 showed more than 2x fold greater IAA production. In another report [47], higher IAA production was reported compared to our results, in which *B. licheniformis* and *Bacillus* spp. strains isolated from potato rhizosphere were able to produce remarkably high levels (78 and $101\,\mu g\,mL^{-1}$) of IAA. Goswami et al. [48] reported a *B. licheniformis* A2 strain exhibiting IAA production above 5 $\mu g\,mL^{-1}$ in the presence of L-tryptophan, furthermore, significant increases in fresh biomass, total length and root length were observed in groundnut plants (*Arachis hypogea*), when plants were treated with the strain. IAA production, as one of the most important PGP traits, plays a crucial role in promoting plant growth and development. Therefore, IAA production of strain SZMC 27715 is expected to play a remarkable role in the sweet potato yield increase observed in the field test.

4.3. Phosphorus Solubilisation. Phosphorus ranks among the primary essential nutrients for plants, alongside nitrogen (N) and potassium (K), thus adequate phosphorus levels are vital for plant growth [49]. Phosphorus accounts for 0.2%–0.8% of the dry weight of plants [50] and is required for nucleic acids, phospholipids, and enzymes. However, the availability of soluble phosphorus to plants in soils is limited, as most of the P-fertilisers applied will rapidly convert to insoluble phosphates [51]. Hence, P solubilisation is a key aspect of the plant growth-promoting properties of PGPB. In our study, all tested strains exhibited the ability to solubilise tricalcium phosphate (the most common form of insoluble P present in soil) from the medium, yet significant differences ($p < 0.05$) were observed among the strains. Notably, strain SZMC 27715 displayed the highest S.I. value (1.7) (Figures 6, and 7, and Table 6). In the work of Mahdi et al. [52], the *B. licheniformis* strain QA1 demonstrated remarkable phosphate solubilising capability ($346\,mg\,L^{-1}$) through a quantitative plate assay, indicating potential for stimulating germination and promoting growth in *Chenopodium quinoa* Willd. seedlings. In the report of Rawat et al. [46], a *B. licheniformis* strain with P-solubilising ability was isolated from rice rhizosphere, which resulted in a significant increase in root length of rice plants compared to control plants. Furthermore, a *B. licheniformis* CKA1 strain isolated from the rhizosphere of apple with P-solubilising ability was reported and parameters (e.g. temperature, inoculum size) affecting P-solubilisation were investigated extensively [53]. In the study by Oves et al. [30] comparable S.I. values to our results were observed, where the phosphate solubilising potential of *Ensifer adhaerens* OS3 was investigated under heavy metal stress, yielding S.I. values ranging from 1.4 to 2.2. The results reported in the literature suggest that PGPB strains capable of P-solubilisation may be able to stimulate plant growth efficiently. Although the soil analysis in our study revealed a very good AL-soluble P_2O_5 ($258.7\,mg\,kg^{-}1$) content in the soil at the field trial location (Zsombó), strain SZMC 27715 could have an important role in providing additional phosphorus source to the sweet potato plants, as it could be incorporated into the soil during treatments, both at planting and during additional foliar treatments. As suggested above, P-solubilisation could have a potential role in the yield enhancing effect of strain SZMC 27715.

4.4. Ammonia Production. In addition to soluble phosphate, ammonia produced by PGPB can directly support plant growth [54], through the nitrogen fertilisation of plants.

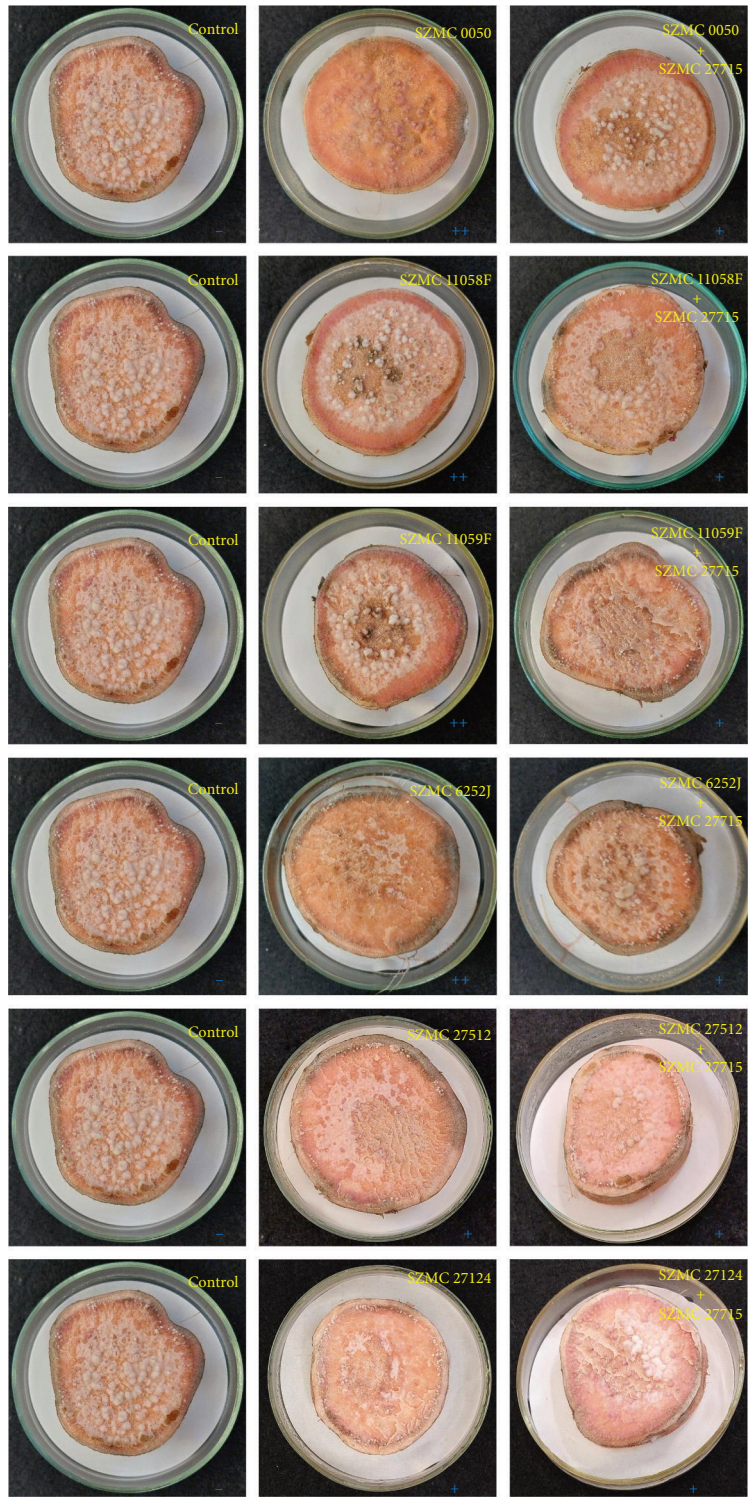

Figure 11: *In vitro* antagonism tests on sterilised sweet potato slices. Left column: noninoculated control slices. Middle column: slices inoculated with fungal pathogens. Right column: SZMC 27715 pre-treated slices plus fungal pathogens. The blue plus or minus symbols located in the bottom right corner of the images signify the severity of symptoms: "–" for symptomless, "+" for slight symptoms, and "++" for severe symptoms.

For an organism to be classified as PGPB, it must possess characteristics such as IAA production, P-solubilisation, and ammonia production [55, 56]. Therefore, in addition to IAA production and P-solubilisation, we also examined the ammonia production ability of the isolates to ascertain their PGPB potential. In our study, out of the seven tested *B. licheniformis* strains, five isolates exhibited varying levels of ammonia production, while two strains (SZMC 27717 and SZMC 27718) did not

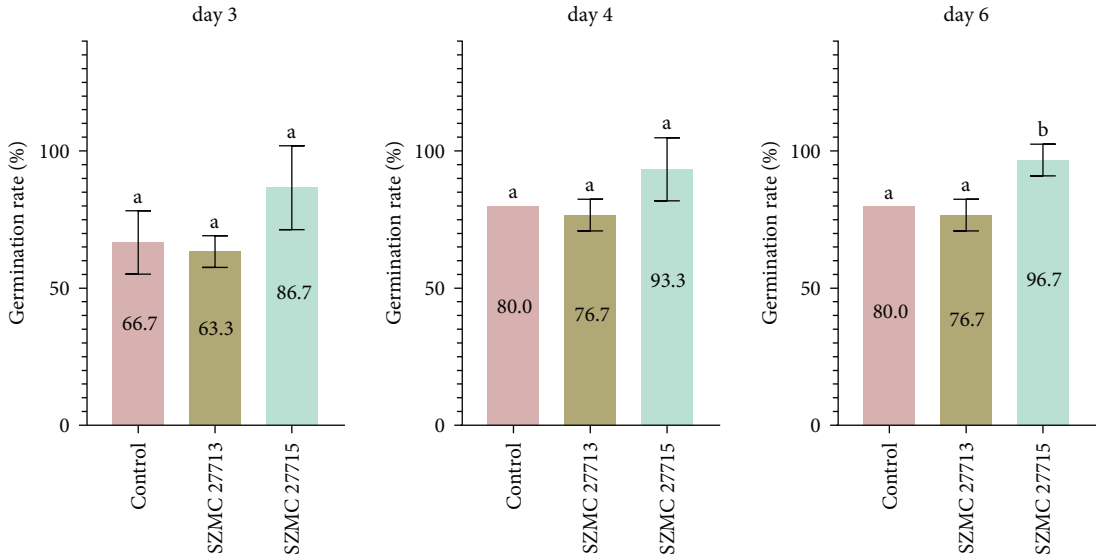

Figure 12: Effect of two candidate *B. licheniformis* strains on tomato seed germination on the 3rd, 4th, and 6th days. The same letters above the data in the columns mean no significant difference ($p > 0.05$; mean ± SD). Different letters indicate significant differences ($p < 0.05$).

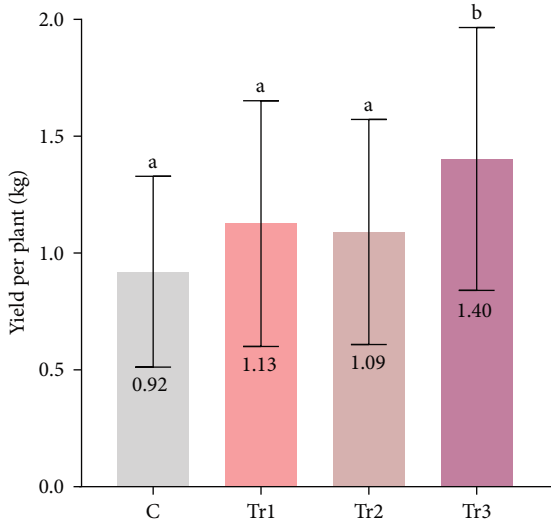

Figure 13: Effect of different SZMC 27715 treatments on the performance of sweet potato storage root yield per plant. The same letters above the data in the columns mean no significant difference ($p > 0.05$; mean ± SD). Different letters indicate significant differences ($p < 0.05$). C: control, no bacterial treatments applied; Tr1: treatment 1, soaking sweet potato secondary cuttings in bacterial suspension before planting; Tr2: treatment 2; same treatment as Tr1 with an additional treatment of leaves; Tr3: treatment 3, same treatment as Tr2 with an additional secondary treatment of leaves.

produce ammonia under the applied test conditions. The ammonia concentrations ranged from 55 to 870 $\mu g\,mL^{-1}$ (Figure 8). Strains SZMC 27713 and SZMC 27715 produced significantly higher ($p < 0.05$) levels of ammonia (870 and 839 $\mu g\,mL^{-1}$) than all remaining tested strains. In the research by Goswami et al. [48], a *B. licheniformis* strain A2 capable of producing ammonia (4 $\mu mol\,mL^{-1}$) displayed pronounced plant growth-promoting traits on *Arachis hypogaea* plants. Similarly, in another publication from the same research group [31],

Table 8: Estimated yields per plant and per hectare values for each treatment, calculated with the experimental setting of 33.333 plants ha^{-1}.

	Variant			
	C	Tr1	Tr2	Tr3
Yield per plant (kg)	0.92	1.13	1.09	1.4
Extra yield per plant (kg)	—	0.21	0.17	0.48
Yield per hectare (tons)	30.7	37.7	36.3	46.7
Extra yield per hectare (tons)	—	7	5.7	16

C: control, no bacterial treatments applied; Tr1: treatment 1, soaking sweet potato secondary cuttings in bacterial suspension before planting; Tr2: treatment 2; same treatment as Tr1 with an additional treatment of leaves; Tr3: treatment 3, same treatment as Tr2 with an additional secondary treatment of leaves.

the ammonia-producing *Pseudomonas aeruginosa* BG strain (27 $mg\,mL^{-1}$) was recognised for its excellent plant growth-promoting properties. Ammonia production by strains SZMC 27713 and SZMC 27715 has the potential to contribute significantly to plant growth and development. The production of ammonia by strain SZMC 27715 could contribute to an increase in vegetative mass of sweet potato plants, which may result in increased nutrient accumulation in sweet potato tubers through enhanced photosynthetic activity, thereby increasing the yield.

4.5. Siderophore Production. Siderophores represent organic, low molecular weight compounds with a strong affinity for chelating iron [57], and they play a pivotal role in facilitating iron uptake by plants. In our study, we successfully confirmed the siderophore-producing ability of five *B. licheniformis* strains using a modified spectrophotometric method [34]. Conversely, two strains (SZMC 27713 and SZMC 27715) did not demonstrate siderophore production (Figure 9). In line with the findings of previous studies of our laboratory [58, 59], no siderophore production was observed by the *Pseudomonas resinovorans* SZMC 25875

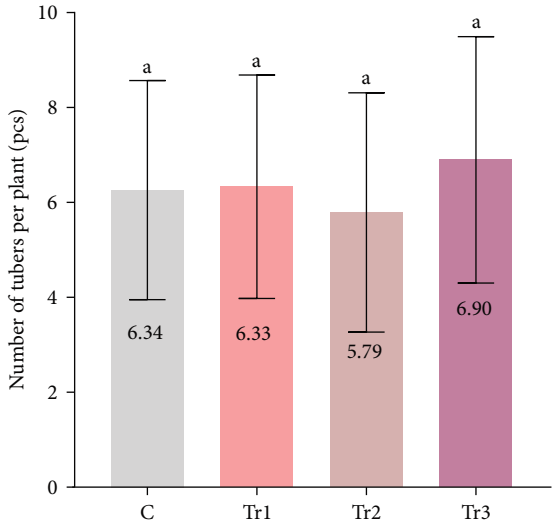

Figure 14: Effect of different SZMC 27715 treatments on the number of sweet potato storage roots per plant. The same letters above the data in the columns mean no significant difference ($p > 0.05$; mean ± SD). Different letters indicate significant differences ($p < 0.05$). C: control, no bacterial treatments applied; Tr1: treatment 1, soaking sweet potato secondary cuttings in bacterial suspension before planting; Tr2: treatment 2; same treatment as Tr1 with an additional treatment of leaves; Tr3: treatment 3, same treatment as Tr2 with an additional secondary treatment of leaves.

strain on CAS agar, though siderophore production became evident when the strain was cultured in liquid SSM. The composition of culture medium can have a significant effect on siderophore production. Utilisation of succinic acid as a carbon source instead of glucose can enhance siderophore production [37]. In the present study, siderophore production was particularly prominent in three strains (SZMC 27714 : 78.9%; SZMC 27717 : 82%; and SZMC 27718 : 90%). Previously, *B. licheniformis* strains capable of siderophore production have been reported [47, 60, 61], however, only the qualitative CAS agar method was applied to determine the siderophore production of the strains. Goswami et al. [48] used the spectrophotometric method to investigate siderophore production by a *B. licheniformis* A2 strain, where similar PSU values (above 70%) were observed. To the best of our knowledge, this is the first study to report the siderophore producing ability of endophytic *B. licheniformis* strains isolated from sweet potato using spectrophotometric method. Although the two candidate strains (SZMC 27713 and SZMC 27715) failed to produce siderophores under the applied test conditions, the potential for the future use of an outstanding siderophore-producing strain (e.g., SZMC 27718) in microbial consortium with strain SZMC 27715 is conceivable.

4.6. In Vitro Antagonism Tests. Among many other pathogens, species belonging to the *Aspergillus* [62], *Fusarium* [16], *Phomopsis* [63, 64], and *Rhizoctonia* [65] genera can cause several diseases during sweet potato production, both in cultivation and during storage of the tubers. Therefore, we tested the inhibitory activity (I.A.) of the *B. licheniformis* strains against some of the abovementioned plant pathogens available in our strain collection (Szeged Microbiology Collection—SZMC). Two *B. licheniformis* strains, namely SZMC 27713 and SZMC 27715, demonstrated remarkable I.A. against fungi associated with sweet potatoes. This inhibitory effect was successfully demonstrated through *in vitro* confrontation assays (Table 7 and Figure 10), furthermore, strain SZMC 27715 also exhibited inhibitory activity on sterilised sweet potato slices (Figure 11). Nigris et al. [66] reported on the inhibitory activity of an endophytic *B. licheniformis* GL174 strain isolated from grapevine against plant pathogenic fungi (*Phaeoacremonium aleophilum*, *Phaeomoniella* spp, *Botryosphaeria* spp, *Botrytis cinerea* and *Sclerotinia sclerotiorum*), although the inhibition indexes were generally higher compared to our present observations. Maheshwari et al. [67] reported a *B. licheniformis* strain isolated from soil exhibited outstanding inhibitory activity against *S. sclerotiorum* (76%) and *F. oxysporum* (77%), while the other tested *B. licheniformis* MTCC 57 type strain did not demonstrate any inhibitory activity against the tested fungal pathogens. *Bacillus* species can exert their antimicrobial activity through the production of lipopeptides (e.g., fengycin, iturin, surfactin), antibiotics and various enzymes that can inhibit fungal pathogens [14], however, these properties can vary significantly among isolates, which will strongly affect the inhibitory activity of a given *Bacillus* strain against pathogens. While the inhibitory activity against the tested plant pathogens observed in this study was modest, the application of SZMC 27713 and SZMC 27715 isolates could still contribute to helping sweet potato plants resist pathogen attacks and reduce the reliance on chemical pesticides in sweet potato cultivation.

4.7. Germination Test. In present study, the germination-promoting effect of strain SZMC 27715 was confirmed on tomato seeds on day 6, in which the germination rate (GR) of SZMC 27715 treated seeds was significantly higher ($p < 0.05$) (96.7%) compared to control seeds (80%). Conversely, strain SZMC 27713 did not exhibit any stimulating effect ($p > 0.05$) on tomato seed germination (Figure 12). Rawat et al. [46] reported a similar germination experiment on rice seeds, where seed bacterization with *B. licheniformis* resulted in a higher GR (92%) on day 7 compared to the control (80%). In a manner consistent with our findings, a prior study revealed that treatment with *B. licheniformis* led to accelerated germination of *Arabidopsis thaliana* seeds compared to the control, resulting in a significant increase in leaf development of seedlings [68]. In the study of Singh et al. [69], it was found that *Acacia senegal* seeds displayed improved germination upon inoculation with a *B. licheniformis* strain isolated from *A. senegal* root nodules. Another investigation reported that PGPB strains of *B. pumilus* and *B. licheniformis*, isolated from the rhizosphere of *Alnus glutinosa* [L.] Gaertn., synthesised substantial amounts of physiologically active gibberellins (GA) [70], a family of plant growth-promoting hormones also produced by PGPBs, which has a crucial role in breaking seed dormancy. However, auxins, serving as phytohormones, influence not only plant growth but also seed dormancy through abscisic acid (ABA) signalling pathways, thereby

impacting germination [71]. The production of IAA by the SZMC 27715 strain may account for the accelerated germination of tomato seeds, potentially through interactions with other phytohormones involved in germination, such as ABA and gibberellins. The variation in the germination-promoting ability between the SZMC 27713 and SZMC 27715 strains could be attributed to differences in their seed colonizing capabilities and the quantity of bacterial metabolites that penetrate the seed [72].

4.8. Field Study. Based on our previous tests (IAA production, P-solubilisation, ammonia production, *in vitro* antagonism tests, germination test), SZMC strain 27715 was selected for field testing. In the field experiment, it was found that strain SZMC 27715 had a potent yield enhancing effect in sweet potato, where a prominent yield per plant increase was observed in all treatments compared to the control plants. Significant ($p < 0.05$) yield increase was observed when used in combination with soaking of sweet potato cuttings and double foliar application (Tr3). Similar yield per plant increases were observed for the soaked cuttings only (Tr1) and for the soaked and once-treated plants (Tr2), with no significant ($p > 0.05$) difference between the two treatments (Figure 13). As no significant difference ($p > 0.05$) in the number of tubers per plant was obtained between the treatments, our results suggest that the average size of tubers increased with the frequency of treatments, which explains the increase in yield per plant for each treatment (Figure 14). Like the results presented in our previous research [20], soaking sweet potato cuttings in the bacterial suspension as well as post-treatment was essential for yield increase, although, two additional treatments were required to achieve a significant yield increase in the present study. While there is evidence of strains from *B. licheniformis* enhancing plant growth and yields in various crops such as grapevines [66], Indian mustard [67], oil tea [73], potatoes [74], tomatoes, and peppers [75], the application of this bacterium specifically as a bioinoculant for sweet potatoes has not yet been explored. This study represents the first to investigate its potential as a foliar biofertiliser aimed at increasing sweet potato yields. Our results highlight the viability of using this selected *B. licheniformis* strain as a foliar treatment in sweet potato cultivation, offering an attractive alternative to chemical fertilisers. Given the cost-effective and environmentally friendly nature of such biocontrol products [76] compared to increasingly expensive agrochemicals, their application could offer farmers a significant economic advantage.

5. Conclusions

In this comprehensive study, we have successfully isolated, identified, and characterised *Bacillus licheniformis* strains from sweet potato, revealing their significant plant growth-promoting (PGP) properties for the first time. Notably, strains SZMC 27713 and SZMC 27715 demonstrated remarkable abilities to produce key compounds such as IAA, ammonia, and P, alongside exhibiting inhibitory activity against common sweet potato pathogens like *Fusarium oxysporum*, *F. solani*, and *Rhizoctonia solani*. Strain SZMC 27715, in particular, stood out for its pronounced impact on accelerating germination rates in tomato seeds, achieving a notable 96.7% germination.

Our field studies further highlight the yield-enhancing potential of SZMC 27715 in sweet potato cultivation, achieving a significant increase in yield per plant (1.40 kg) under optimised treatment conditions, compared to control plants (0.92 kg). This marks a pioneering contribution to the agricultural use of *B. licheniformis*, particularly in sweet potato cultivation, a context previously unexplored in existing literature.

The promising results from SZMC 27715 suggest its potential as a biofertiliser that could be used either individually or as part of a microbial consortium, aimed at promoting plant growth and controlling phytopathogens under specific environmental conditions. Ongoing and future research efforts are directed towards understanding the antifungal compounds produced by these strains and extending their application to other crops and geographical areas. Furthermore, work is underway to develop a commercial product that leverages the benefits of SZMC 27715 for practical agricultural use.

Given the novelty and efficacy of SZMC 27715, further investigations are warranted to explore its long-term effects on soil health and its interactions within the soil microbial ecosystem. Understanding the molecular mechanisms behind its PGP and antifungal activities will be crucial for enhancing its application in agriculture. The integration of such beneficial strains into holistic crop management strategies represents a promising avenue for promoting sustainable agriculture, thereby contributing to global food security and environmental sustainability.

Data Availability

DNA sequences are available in the NCBI GenBank Nucleotide database (http://www.ncbi.nlm.nih.gov/genbank/) under accession numbers OP620082-88. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Vágvölgyi Csaba, Monostori Tamás, and Ramteke Pramod Wasudeo contributed in the conceptualisation. Bordé-Pavlicz Ádám, Allaga Henrietta, Zhumakayev Anuar Rysbekovich, and Vörös Mónika contributed in the methodology. Bordé-Pavlicz Ádám contributed in the software, formal analysis, visualisation, and writing–original draft preparation. Bordé-Pavlicz Ádám and Zhumakayev Anuar Rysbekovich contributed in the validation. Bordé-Pavlicz Ádám, Allaga Henrietta, Zhumakayev Anuar Rysbekovich, and Vörös Mónika contributed in the investigation. Vágvölgyi Csaba contributed in the resources, project administration, and funding acquisition. Bordé-Pavlicz Ádám and Zhumakayev Anuar Rysbekovich contributed in the data curation. Vágvölgyi Csaba and Zhumakayev Anuar Rysbekovich contributed in the writing–review and editing. Vágvölgyi Csaba and Monostori Tamás contributed in the

supervision. All authors have read and agreed to the published version of the manuscript.

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