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Evaluation of *Mentha viridis* and *Opuntia ficus-indica* juices as plant-based media for beneficial rhizosphere microbes

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Abstract

Synthetic culture media are often costly due to their chemical constituents, prompting interest in developing affordable plant-based alternatives. This study evaluated the potential of *Mentha viridis* (*M. viridis*) and *Opuntia ficus-indica* (*O. ficus-indica*) juices as culture media for supporting microbial growth, due to their high content of amino acids, vitamins, and minerals. A total of seven bacterial and eight actinomycete isolates were obtained from the rhizosphere of *M. viridis*, while ten bacterial isolates were recovered from *O. ficus-indica*. Screening for plant growth-promoting traits revealed that isolate B5 (*Bacillus safensis* FO-36b) produced the highest levels of indole acetic acid (10.3 mg/mL) and gibberellic acid (28.25 mg/mL), and together with actinomycete isolate A3 (*Streptomyces afghaniensis* NR-114833.1), showed the strongest phosphate solubilization capacity (877.07 and 758.43 µg/mL, respectively). Additionally, 60% of isolates grown on *Mentha*-based medium and 40% on *Opuntia*-based medium produced high levels of ammonia. These findings highlight the potential of plant-based media as cost-effective platforms for isolating and screening beneficial microbes with biofertilizer properties.

Keywords: Plant-based culture media; *Mentha viridis*; *Opuntia ficus-indica*; Plant growth promoting microbes (PGPM); Indole acetic acid (IAA); Phosphate solubilization; Biofertilizer potential

1. Introduction

Culture media are essential for cultivating and maintaining microorganisms under laboratory conditions, but commercially available synthetic media such as Nutrient Agar, Cetrimide Agar, and MacConkey Agar are often costly. To reduce expenses, alternative media using locally available plant materials have been explored. Uthayasooriyan *et al*. [1] suggested cereals and legumes as nutrient sources for microbial culture, while fruits and vegetables have also been used successfully due to their rich content of amino acids, vitamins, and minerals [2]. For example, [3] demonstrated that rhizospheric microorganisms grew effectively on agar plates prepared with crude juice of *Mesembryanthemum crystallinum*. Plant-based extracts such as *M. viridis* and *O.ficus-indica* are promising alternatives. Peppermint leaves contain carbohydrates, proteins, and essential minerals that support microbial growth [4], while prickly pear cladodes are rich in sugars, amino acids, vitamins, and minerals, making them suitable for microbial cultivation [5, 6].

Plant-based media offer a useful platform for isolating plant growth-promoting rhizobacteria (PGPR). These beneficial microbes contribute to plant development through multiple mechanisms, including phosphate solubilization, phytohormone production, and the induction of systemic resistance [7, 8]. Among PGPR, *Bacillus safensis* has been recognized for its salt tolerance, phytohormone production, and root colonization ability [9-11]. Likewise, actinomycetes

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such as *Streptomyces afghaniensis* are known for producing phytohormones, antibiotics, and biocontrol metabolites [12-14]. This study, therefore, evaluates *M. viridis* and *O. ficus-indica* juices as cost-effective media for the isolation and characterization of PGPR with potential applications in sustainable agriculture.

2. Materials and methods

2.1. Samples collection

Samples of spearmint (*Mentha viridis* L.) and prickly pear (*O. ficus-indica* (L.) Mill.) were collected from the Agricultural Research Center Farm, Sirs El-Layan, El-Menoufia Governorate, Egypt. The vegetative parts were stored in sterile plastic bags at 4 °C until use.

2.2. Preparation of plant-based culture media

After 30 days of germination, vegetative parts (leaves and stems) of spearmint (*M. viridis*) and mature stem pads of prickly pear (*O. ficus-indica*) were harvested, thoroughly washed, sliced, and blended with equal volumes of distilled water (w/v) for 5 min using a laboratory blender. The resulting homogenate was filtered through cheesecloth to obtain plant juice, representing approximately 73-82% of the plant fresh weight. The pH values of the extracted juices ranged from 5.8-6.5 for spearmint and 3.6-5.2 for prickly pear.

Juices were diluted with distilled water (v/v) at ratios of 1:10, 1:20, 1:40, 1:80, and 1:100, then solidified with 2% (w/v) agar. The pH was adjusted to 7.0, and the media were sterilized by autoclaving at 121 °C and 1.5 atm for 20 min. The chemical composition of the plant juices was previously reported by [15, 16].

2.3. Soil sampling and preparation

Rhizosphere soil samples associated with *M. viridis* and *O. ficus-indica* plants were collected from the previously mentioned farm site. The samples were gently ground, passed through a 2 mm sieve, and air-dried for subsequent physical and chemical analyses (Table 1).

2.4. Isolation of rhizospheres microorganisms

Roots of spearmint (*M. viridis*) and prickly pear (*O. ficus-indica*) plants with adhering soil were collected. One gram of rhizosphere soil was suspended in 10 mL of sterile distilled water and vortexed (150 rpm, 10 min). Serial dilutions (10^{-1}-10^{-6}) were prepared, and 0.1 mL aliquots from each dilution were spread in triplicate onto spearmint- and prickly pear–based agar media using sterile L-shaped glass rods. Plates were maintained at 30 °C for an incubation period of 24-48h. Distinct bacterial and actinomycete colonies were repeatedly streaked onto fresh plant-based agar plates for purification [17], transferred to agar slants, incubated at 37 °C, and preserved at 4 °C as stock cultures for further studies.

2.5. Characterization of isolated rhizobacteria

2.5.1. Determination of phytohormones

- Auxin (Indole-3-Acetic Acid, IAA)

Bacterial isolates were cultured in 100 mL plant-based broth supplemented with 500 µg/mL tryptophan and incubated in 250 mL flasks on a rotary shaker at 150 rpm and 36 ± 2 °C for 72h. Actinomycetes isolates were cultivated under the same conditions without tryptophan supplementation, using 5 mm disks from 5-day-old cultures and incubating at 30 °C for 7 days. Culture broths were centrifuged (3,000-4,000 rpm, 15 min), and the supernatants were used for analysis.

IAA production was quantified by mixing 1 mL of supernatant with 2 mL of Salkowski reagent, incubating for 30 min in the dark, and measuring absorbance at 530 nm. Uninoculated medium served as a control. Concentrations were calculated against a standard IAA curve [18- 20].

- Gibberellic acid (GA)

Supernatants obtained as above (without tryptophan) were adjusted to pH 8.6 with 1% NaOH and extracted three times with equal volumes of ethyl acetate. The combined fraction was evaporated, and the aqueous phase was acidified to pH 2.8 with 1% HCl before re-extraction with ethyl acetate. The final fraction, containing GA, was used for quantification.

GA content was determined by mixing 1 mL of the ethyl acetate extract with 1 mL of HCl, 1 mL of Folin-Denis reagent, and 3 mL of distilled water. After heating in a boiling water bath (5 min) and cooling, absorbance was read at 750 nm and compared with a standard GA curve [21, 22].

2.6. Estimation of metabolites

2.6.1. Total carbohydrates

Soluble and insoluble sugars in culture supernatants of bacterial and actinomycete isolates were quantified as glucose using the phenol-sulfuric acid method [23].

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Table 1. Physical and chemical analysis of rhizosphere soil.

Parameters			
pH*	7.90	**ESP***	1.47
Sp*	32.5	**Organic matter (%)**	2.42
EC* ds/m	0.62	**$CaCO_3$ (%)**	2.10
Soluble Cations (meq/L)		**Available nutrients ($mg.kg^{-1}$)**	
Ca^{++}	3.02	**N**	18.90
Mg^{++}	1.02	**P**	8.23
Na^{+}	2.65	**K**	229.1
K^{+}	0.06	**Particles size distribution (%)**	
Soluble anions (meq/L)		**Sand**	28.4
Cl^{-}	3.37	**Silt**	30.9
HCO_3^{-}	1.86	**Clay**	40.7
SO_4^{2-}	1.52	**Textural class**	Clay
SAR*	1.86		

* pH= in suspension (1:2.5); SP= Saturation Percentage; EC= Electrical Conductivity; SAR= Sodium Absorption Ratio; ESP=Exchange Sodium Percentage.

One milliliter of the sample was mixed with 1 mL of 5% phenol and 5 mL of concentrated H_2SO_4. After cooling (25-30 °C, 20 min), absorbance was measured at 490 nm against a glucose standard curve.

2.6.2. Phosphate solubilization

The solubilization of inorganic phosphate was determined following Mehta and Nautiyal [24]. Isolates were inoculated into 50 mL of National Botanical Research Institute's phosphate growth medium (NBRIP) broth in 150 mL flasks and incubated (bacteria: 36 ± 2 °C, 3 days; actinomycetes: 30 °C, 7 days). Cultures were centrifuged (10,000 rpm, 10 min), and the supernatant was mixed with Barton's reagent [solution A: Ammonium molybdate (25 gm) was dissolved in 400 ml distilled water. Solution B: Ammonium metavanadate (1.25 gm) in 300 boiled distilled water and cooled, then 250 ml conc. HNO_3 was added. Solutions A and B were mixed and the volume was made up to 1000ml with distilled water. Absorbance was recorded at 420 nm, and soluble phosphate was estimated against KH_2PO_4 standards [25].

2.6.3. Ammonia production

Ammonia production was tested in peptone water [26]. Isolates were incubated (bacteria: 36 ± 2 °C, 3 days; actinomycetes: 30 °C, 7 days), followed by the addition of 1 mL Nessler's reagent (is prepared by mixing 2 g potassium iodide in 5 ml water. To this solution, 3 g of mercury (II) iodide is added, and the resulting solution is made to 20 ml. Finally, 40 g potassium hydroxide (30 %) is added to provide the alkaline base). Ammonia production was indicated by the development of yellow to brown coloration [27].

2.7. Identification of selected isolates

The most efficient bacterial (B5) and actinomycete (A3) isolates showing strong PGP activities were selected for identification. Preliminary characterization included Gram staining for the bacterial isolate [28] and morphological observation of the actinomycete by the coverslip culture method [29].

2.7.1. Molecular identification and phylogenetic analysis

Genomic DNA was extracted from pure cultures using a commercial kit (Zymo Research, USA) following the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using universal primers pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and pH (5′-AAGGAGGTGATCCAGCCGCA-3′) [30]. Amplification conditions were initial denaturation at 94 °C for 6 min, 35 cycles of 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. PCR products were separated by electrophoresis on 1% agarose gels.

Sequences were compared to those in the National Center for Biotechnology Information (NCBI) GenBank database using Basic Local Alignment Search Tool for Nucleotide sequences (BLASTN). Multiple sequence alignment was performed with Clustal X **[31]**. A distance-matrix method (with distance

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options according to Jukes-Cantor) **[32]** was employed, using clustering obtained with the neighbor-joining method **[33]**, and phylogenetic trees were constructed by the neighbor-joining method with bootstrap analysis (1,000 replicates) using MEGA v3.1.

2.7.2. Growth curve determination

The growth curve of the bacterial isolate B5 was assessed in Mentha-based broth by inoculating cultures and incubating at 30 °C for 1-6 days. Growth was monitored spectrophotometrically at 600 nm [34]. For actinomycete isolate A3, growth was measured according to Kumar and Kannabiran [35]. Cultures were incubated in Mentha-based broth at 30 °C for 1-10 days, and biomass was determined by filtering culture broth through pre-weighed dry filter papers, drying at 55 °C overnight, and recording the constant dry weight (mg/mL).

2.7.3. Antagonistic activity assay

The antagonistic potential of isolates B5 and A3 was evaluated against *Fusarium solani* and *Rhizoctonia solani* using a modified dual culture method on potato dextrose agar (PDA) [36, 37]. Fungal plugs (0.5 cm) from actively growing colonies were placed at the center of PDA plates. B5 was streaked around the fungal plug, while A3 was streaked centrally and confronted with fungal plugs at 90°. Plates were incubated at 28 °C for 4-7 days. Antagonistic activity was evaluated by measuring inhibition zones (mm) as the distance between fungal mycelial growth and the bacterial or actinomycete colony. Measurements were conducted in triplicate, and mean values were recorded.

3. Results and discussion

3.1. Isolation and growth of rhizospheric microorganisms

A total of ten bacterial isolates were recovered from the rhizosphere of prickly pear (*O. ficus-indica*) using different dilutions of *Opuntia*-based culture media, in accordance with Youssef *et al*. [15], who achieved the isolation of rhizobacteria from *O. ficus-indica* roots using diluted juices (1:20, v/v). In addition, seven bacterial and eight actinomycete isolates were sourced from the rhizosphere of spearmint (*M. viridis*), incubated on agar media prepared from diluted spearmint juices. The most efficient bacterial growth was observed at higher juice dilutions (up to 1:80, v/v), likely due to reduced osmotic stress and minimized inhibitory effects from antimicrobial compounds [38].

3.2. Influence of plant-derived nutrients and compounds

Both plant juices provided nutrient-rich environments favorable for microbial growth. *O. ficus-indica* contains vitamins, enzymes, sugars, lignin, saponins, salicylic acid, amino acids, and essential minerals (Ca, Cr, Cu, Se, Mg, Mn, K), all of which support microbial proliferation [5]. Similarly, *M. viridis* juices are abundant in carbohydrates, proteins, vitamin C, and minerals (K, Na, Ca, Mg, P), enhancing rhizobacterial growth [16]. However, actinomycetes exhibited better growth on spearmint-based media compared to prickly pear-based media. This difference may be attributed to the antimicrobial phytochemicals in *O. ficus-indica* (phenols, tannins, flavonoids, steroids, triterpenoids, alkaloids, saponins, salicylic acid), which suppress microbial development. Comparable effects were documented by McCutcheon *et al*. [39], who confirmed that certain plant extracts, such as *Aloe vera*, effectively inhibit Gram-positive bacteria due to the absence of an outer membrane barrier. These findings confirm that plant-based culture media not only support the growth of diverse rhizosphere microorganisms but can also act as selective substrates influenced by the nutritional and antimicrobial properties of the host plant.

3.3. Characterization of isolated rhizobacteria

3.3.1. Indole acetic acid (IAA) production

All bacterial and actinomycete isolates produced IAA in plant-based broth media, with concentrations ranging from 0.18 to 10.3 µg/mL (Table 2). The highest level was recorded for isolate B5 in *Mentha*-based broth (10.3 µg/mL), followed by isolate A3 (5.11 µg/mL). The lowest IAA production was observed in isolate O10 (0.18 µg/mL) grown on *Opuntia*-based broth. These results agree with Mirza *et al*. [40], who noted that IAA biosynthesis by microorganisms varies with species, strain, culture medium, and growth conditions. The relatively high IAA production in plant-based broths may be linked to the presence of vitamins, salts, carbon, nitrogen, and particularly tryptophan, an amino acid with an indole group that acts as the physiological precursor for IAA biosynthesis [41].

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3.3.2. Gibberellin production

Data presented in Table 2 show that isolate A5 from the *Mentha viridis* rhizosphere exhibited the highest gibberellin production (28.25 µg/mL), while the least value was detected in isolate O7 from *Opuntia ficus-indica* rhizosphere (0.37 µg/mL). The enhanced gibberellin production in plant-based media may be explained by their nutrient composition, which provides essential growth factors that stimulate phytohormone biosynthesis. Previous studies have shown that gibberellin production by rhizobacteria is influenced by strain type and culture conditions [42]. Environmental parameters such as pH, temperature, incubation time, aeration, and light availability can also affect both the quantity and type of gibberellins produced. For instance, Piccoli and Bottini [43] and Piccoli *et al*. [44] reported that nitrogen supply, oxygen availability, and osmotic potential significantly influence gibberellin biosynthesis in *Azospirillum* cultures.

3.4. Production of total carbohydrates, phosphate solubilization, and ammonia by microbial isolates

3.4.1. Carbohydrate production

Data presented in Table 3 indicate that all bacterial and actinomycete isolates produced moderate to low amounts of carbohydrates on plant-based culture media. Notably, isolates B5 and A3 from the *Mentha* rhizosphere exhibited the highest carbohydrate levels on *Mentha*-based medium (48 and 50 µg/mL, respectively). The relatively low yields across isolates may be explained by the nitrogen content of the media, which is known to suppress carbohydrate accumulation. These findings are consistent with [45], who reported that bacterial exopolysaccharide production is favored under high carbon and low nitrogen conditions.

3.4.2. Phosphate solubilization

All isolates demonstrated phosphate-solubilizing activity, with concentrations ranging from 90.41 to 877.07 µg/mL (Table 3). The highest solubilization was recorded for isolate B5, followed by A3. These results agree with [46], who estimated that 20-40% of cultivable soil bacteria possess phosphate-solubilizing capacity. The enhanced solubilization observed in isolates grown on plant-based media is likely due to the acidic nature of *Mentha* (pH 5.8-6.5) and *O. ficus-indica* (pH 4.5-6.5) juices. Such acidic conditions lower the medium pH, promoting the release of organic acids that enhance phosphorus solubilization [47]. Additionally, as noted by Pandey and Maheshwari [48], bacteria employ multiple mechanisms, including acid production, chelation, and siderophore secretion, to mobilize phosphorus.

3.4.3. Ammonia production

Table 3 also shows that approximately 60% of *Mentha* rhizospheric isolates produced high amounts of ammonia in peptone water following growth on *Mentha*-based medium, while about 40% of *Opuntia ficus-indica* rhizospheric isolates produced moderate amounts under similar conditions.

The efficiency of ammonia production may again be linked to the pH of the plant juices, with *Mentha* (pH 5.8-6.5) and *O. ficus-indica* (pH 4.5-6.5) providing favorable ranges for microbial activity.

These observations are in line with [49], who demonstrated that nitrate production is strongly correlated with pH.

Ammonia production represents an important PGPR trait, as it contributes to soil nitrogen enrichment. However, excessive accumulation may alter soil pH, creating alkaline conditions that disturb microbial community balance and inhibit fungal spore germination [50].

3.5. Molecular identification and phylogenetic analysis

Molecular characterization using 16S rRNA gene sequencing enabled accurate identification of the bacterial and actinomycete isolates. Sequence analysis confirmed their association with diverse genera frequently recognized as PGPR, such as *Bacillus*, *Pseudomonas*, *Streptomyces*, and related taxa. The reliability of 16S rRNA gene sequencing for microbial identification is well documented, as this conserved region allows resolution at the genus and, in many cases, species level [51, 52].

Phylogenetic trees were constructed using neighbor-joining methods to evaluate the evolutionary relationships among the isolates and their closest relatives retrieved from GenBank. The clustering patterns revealed that several isolates grouped tightly with reference strains of well-known PGPR, indicating their close genetic relatedness. Similar phylogenetic clustering has been reported in earlier studies of rhizospheric bacteria associated with medicinal and crop plants [53, 54].

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Table 2. Indole acetic acid (IAA) and gibberellic acid (GA) production by bacterial and actinomycete isolates on plant-based media.

Isolates on Mentha-based medium			Isolates on *O. ficus-indica* -based media		
	IAA (µg/ mL)	GA (µg/mL)		IAA (µg/mL)	GA (µg/mL)
B1	3.72	5.36	O1	1.32	1.53
B2	4.10	9.52	O2	1.02	3.87
B3	5.09	6.06	O3	3.00	0.66
B4	3.51	7.57	O4	2.78	0.90
B5	10.3	28.25	O5	0.99	2.98
B6	4.32	7.81	O6	1.56	7.54
B7	2.51	11.33	O7	2.33	0.37
A1	2.22	0.51	O8	1.76	11.98
A2	1.47	12.42	O9	0.53	8.43
A3	5.11	23.41	O10	0.18	0.67
A4	2.67	11.26	-	-	-
A5	1.23	1.40	-	-	-
A6	2.82	9.31	-	-	-
A7	1.54	0.72	-	-	-
A8	4.13	7.92	-	-	-

Table 3. Carbohydrate production, phosphorus solubilization, and ammonia production by bacterial and actinomycete isolates grown on plant-based media.

Isolates on Mentha-based medium				Isolates on *Opuntia ficus-indica* based media			
	Carbohydrate concentration (µg/ mL)	P. (µg/ mL)	Ammonia production		Carbohydrate concentration (µg/ mL)	P. (µg/ mL)	Ammonia production
B1	5	385.57	++	O1	13	198.7	+
B2	15	184.85	+++	O2	10	554.5	++
B3	20	519.63	+++	O3	7	209.0	+++
B4	8	428.91	+	O4	9	331.7	+
B5	48	877.07	+++	O5	23	730.1	+++
B6	30	415.52	+++	O6	19	472.8	+
B7	6	350.24	++	O7	44	390.2	++
A1	14	562.88	++	O8	23	397.4	+
A2	12	288.46	+++	O9	17	555.2	-ve
A3	50	758.43	++	O10	34	178.9	+
A4	10	90.41	+++	-	-	-	-
A5	31	690. 43	++	-	-	-	-
A6	20	321.85	+++	-	-	-	-
A7	6	260.63	+++	-	-	-	-
A8	26	534.11	-ve	-	-	-	-

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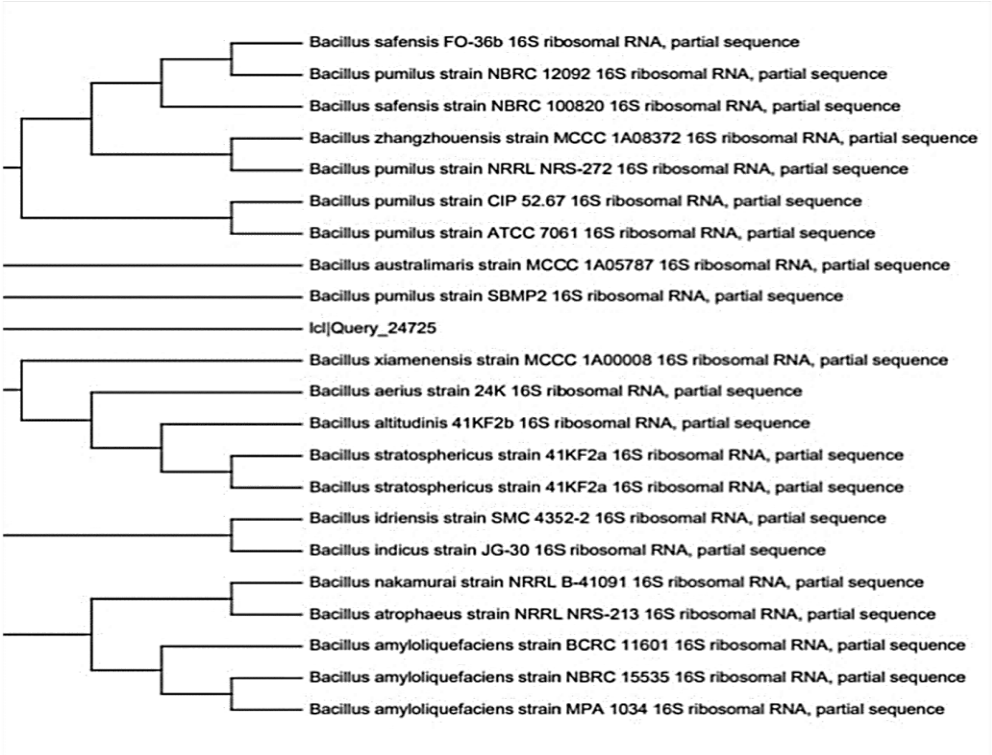

Figure 1. 16S rRNA-based phylogeny of *Bacillus safensis* isolate with related taxa.

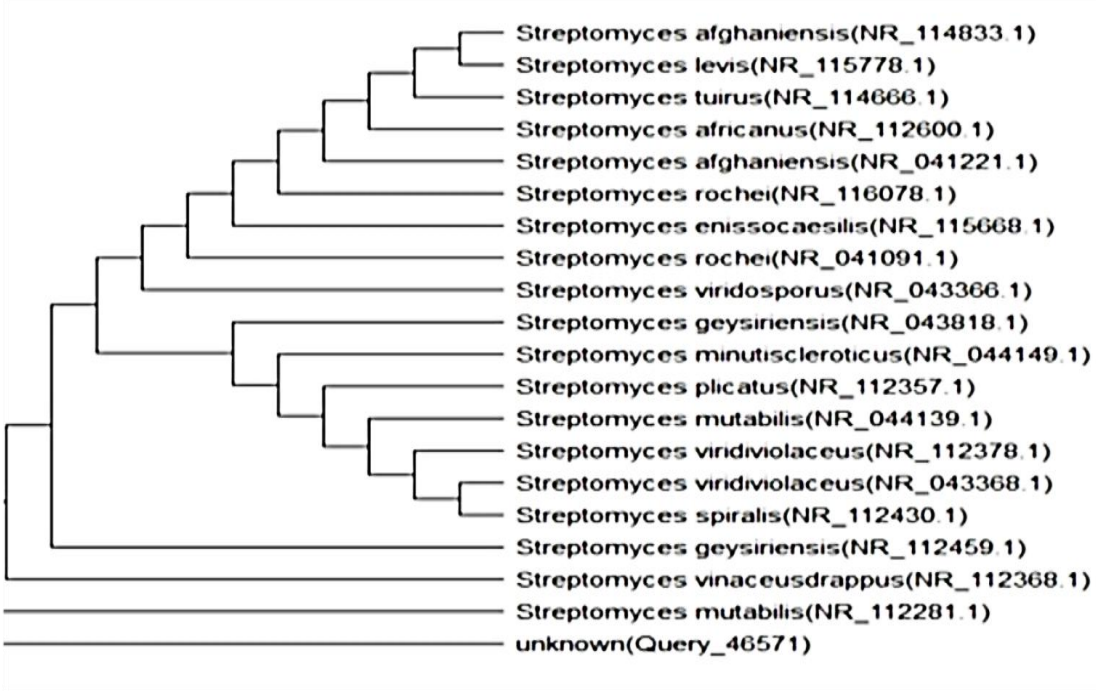

Figure 2. 16S rRNA-based phylogeny of *Streptomyces rochei* isolate and related reference strains.

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The phylogenetic positioning of these isolates supports their functional role as PGPR, as species belonging to these genera are widely recognized for attributes such as IAA biosynthesis, phosphate solubilization, and biocontrol activity. Moreover, phylogenetic diversity among isolates suggests that different bacterial lineages contribute synergistically to plant growth promotion. This diversity is of ecological significance, as a heterogeneous microbial community can enhance resilience under variable soil and environmental conditions [55, 56].

Taken together, molecular identification and phylogenetic analysis confirmed the taxonomic placement of the isolates and highlighted their prospective functional roles in the rhizosphere. These findings highlight the importance of integrating molecular tools with biochemical and physiological assays to achieve a comprehensive understanding of PGPR diversity and function (Figures 1 and 2).

3.6. Growth curve of the most efficient isolates

The growth dynamics of *Bacillus safensis* and *Streptomyces rochei* are shown in Figure 3 A and B. *B. safensis* displayed exponential growth between the second and third day of incubation, reaching its maximum density on the third day. Thereafter, a slight decline in growth was observed on the fourth day, marking the transition to the stationary phase (Figure 3A). In contrast, *S. rochei* exhibited a slower but more prolonged exponential phase, with rapid growth occurring between the fourth and seventh days of incubation, followed by a slight decrease on the eighth day (Figure 3B). These growth patterns highlight the distinct physiological behaviors of the isolates, reflecting their inherent metabolic and ecological adaptations.

The transition from exponential to stationary phase is typically correlated with the onset of secondary metabolite production in microorganisms. For *Bacillus* species, the stationary phase typically triggers the synthesis of antimicrobial lipopeptides and other metabolites that contribute to biocontrol activity [57]. Similarly, *Streptomyces* spp. is well known for producing antibiotics and antifungal compounds predominantly during the late exponential and stationary phases, when nutrient limitation induces secondary metabolism [58]. Thus, the observed growth patterns of *B. safensis* and *S. rochei* may directly relate to their antagonistic potential, with peak antifungal activity coinciding with phases of active metabolite production.

3.7. Antagonistic activities against root rot fungi

Both isolates demonstrated antagonistic potential against soilborne pathogenic fungi. *B. safensis* exhibited strong inhibitory activity against *Fusarium oxysporum*, producing a mean inhibition zone of 17 ± 2 mm (Table 4). These results align with earlier findings by [59], who reported effective antifungal activity of *Bacillus* spp. against *Fusarium graminearum*, contributing to the protection of durum wheat.

Similarly, *S. rochei* was highly effective against *Rhizoctonia solani*, producing an inhibition zone of 16 ± 0.8 mm (Table 4). This observation agrees with previous reports demonstrating the antagonistic potential of *Streptomyces* spp., including *S. rochei*, against *R. solani* [61] and *F. oxysporum* [60]. The antifungal efficacy of both isolates suggests that their biocontrol activity is likely linked to the formation of secondary metabolites occurring in the stationary phase. Such traits reinforce their promise as sustainable alternatives to chemical fungicides for crop protection.

Table 4. Biocontrol activity of selected isolates against *Fusarium oxysporum* and *Rhizoctonia solani*.

Isolates	*Fusarium oxysporum* Clear zone (mm)	*Rhizoctonia solani* Clear zone (mm)
Bacillus safensis	17 ± 2	13 ± 1.6
Streptomyces rochei	12 ± 1.2	14 ± 0.8

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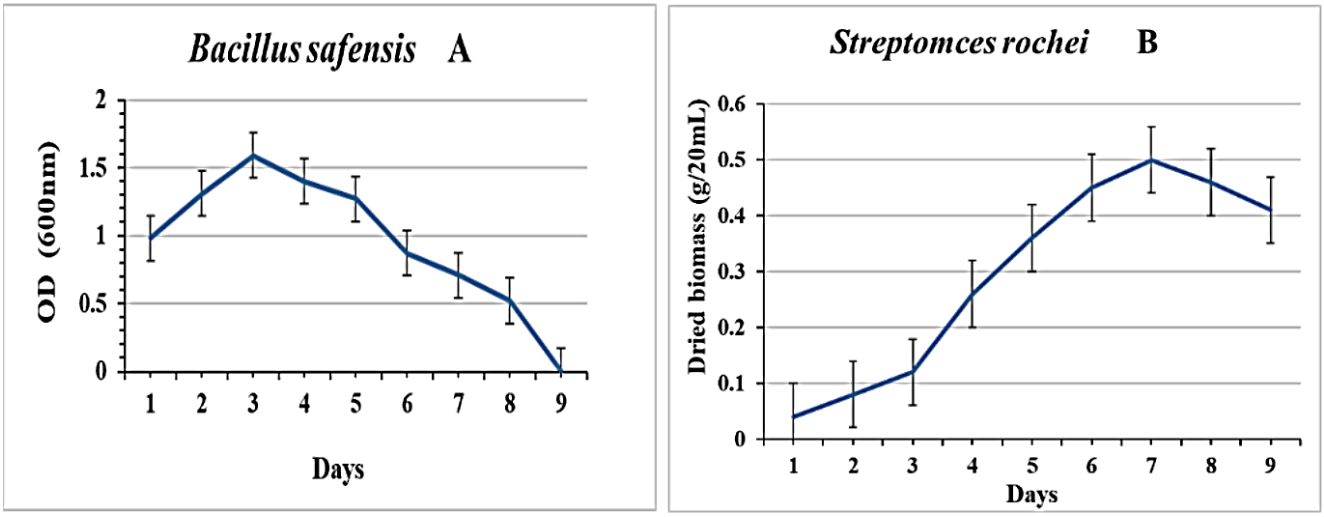

Figure 3. Growth patterns of the most efficient rhizospheric isolates, *Bacillus safensis* and *Streptomyces rochei*.

4. Conclusion

The results demonstrate that *Mentha viridis* and *Opuntia ficus-indica* juices can serve as effective, low-cost alternatives to synthetic culture media for supporting microbial growth. These plant-based media enabled the isolation of diverse bacteria and actinomycetes with strong plant growth-promoting traits, highlighting their promise for sustainable biofertilizer development and environmentally friendly microbial screening.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Data availability

Data will be available on request.

References

[1] Uthayasooriyan, M., Pathmanathan, S., Ravimannan, N., & Sathyaruban, S. (2016). Formulation of alternative culture media for bacterial and fungal growth. Der Pharmacia Lettre, 8(1), 431- 436. http://scholarsresearchlibrary.com

[2] Arulanantham, R., Pathmanathan, S., Ravimannan, N., & Niranjan, K. (2012). Alternative culture media for bacterial growth using different formulation of protein sources. Journal of Natural Product & Plant Resources, 2(6), 697-700. http://scholarsresearchlibrary.com/JNPPR-vol2-iss6/JNPPR-2012-2-6-697-700.pdf

[3] Nour, E., Hamza, M. A., Fayez, M., Monib, M., Ruppel, S., & Hegazi, N. (2012). The crude plant juices of desert plants as appropriate culture media for the cultivation of rhizospheric microorganisms. Journal of Advanced Research, 3(1), 35-43. https://doi.org/10.1016/j.jare.2011.03.002

[4] Mainasara, M. M., Abu Bakar, M. F., Waziri, A. H., & Musa, A. R. (2018). Comparison of phytochemical, proximate and mineral composition of fresh and dried peppermint (*Mentha piperita*) leaves. Journal of Science & Technology, 10(2), 85-91. https://doi.org/10.30880/jst.2018.10.02.014

[5] Upadhyay, R. K. (2018). Nutraceutical, therapeutic, and pharmaceutical potential of *Aloe vera*: A review. International Journal of Green Pharmacy, 12(1), S51-S70. https://doi.org/10.22377/ijgp.v12i01.1601

[6] Todhanakasem, T., Boonchuai, T., Ayutthaya, P. I. N., Suwapanich, R., Hararak, B., Wu, B., & Young, B. M. (2022). Development of bioactive *Opuntia ficus-indica* edible films containing probiotics as a coating for fresh-cut fruit. Polymers, 14(22), 5018. https://doi.org/10.3390/polym14225018

[7] Yadav, A. N., Verma, P., Singh, B., Chauhan, V.S., Suman, A. & Saxena, A. (2017). Plant growth promoting bacteria: Biodiversity and multifunctional attributes for

DOI: 10.62184/nhjbas.jnh10020253

sustainable agriculture. Advances in Biotechnology & Microbiology, 5(5), 555671. https://doi.org/10.19080/AIBM.2017.05.555671

[8] Benaissa, A. (2019). Plant growth-promoting rhizobacteria: A review. Algerian Journal of Environmental Science & Technology, 5(1), 873-880.

[9] Satomi, M., La Duc, M. T., & Venkateswaran, K. (2006). *Bacillus safensis* sp. nov., isolated from spacecraft and assembly-facility surfaces. International Journal of Systematic & Evolutionary Microbiology, 56(8),1735-1740. https://doi.org/10.1099/ijs.0.64189-0

[10] Chakraborty, U., Chakraborty, B. N., Dey1, P. L., & Chakraborty, A. P. (2018). *Bacillus safensis* from wheat rhizosphere promotes growth and ameliorates salinity stress in wheat. Indian Journal of Biotechnology, 17,466-479.

[11] Hasan, A., Tabassum, B., Hashim, M., & Khan, N. (2024). Role of plant growth promoting rhizobacteria (PGPR) as a plant growth enhancer for sustainable agriculture: A review. Bacteria, 3, 59-75. https://doi.org/10.3390/bacteria3020005

[12] Hussein, M. E. (1992). The effect of an Egyptian isolate of Streptomyces afghanensis on some plant viruses. Acta Virologica, 36(5), 479-482.

[13] Palaniyandi, S. A., Yang, S. H., Zhang, L., & Suh, J. W. (2013). Effects of actinobacteria on plant disease suppression and growth promotion. Applied Microbiology & Biotechnology, 97(22), 9621-9636. https://doi.org/10.1007/s00253-013-5206-1

[14] Nonthakaew, N., Panbangred, W., Songnuan, W., & Intra, B. (2022). Plant growth-promoting properties of *Streptomyces* spp. isolates and their impact on mung bean plantlets' rhizosphere microbiome. Frontiers in Microbiology, 13, 2022. https://doi.org/10.3389/fmicb.2022.967415

[15] Youssef, H. H., Hamza, M. A., Fayez, M., Mourad, E. F., Saleh, M. Y., Sarhan, M. S., Suker, R. M., Eltahlawy, A. A., Nemr, R. A., El-Tahan, M., Ruppel, S., & Hegazi, N. A. (2016). Plant-based culture media: Efficiently support culturing rhizobacteria and correctly mirror their in-situ diversity. Journal of Advanced Research, 7(2), 305-316. https://doi.org/10.1016/ j.jare.2015.07.005

[16] Ibrahim, S. E., Ahmed, E. A., Shehata, H., El-Shahat, R. M., & Salah El Din, R. A. (2016). Isolation of rhizobacteria and micro algae from saline soil and production of plant growth promoters. Global Advanced Research Journal of Microbiology, 5(3), 21-31.

[17] Reddy, N. G., Ramakrishna, D. P. N., & Raja Gopal, S. V. (2011). A morphological, physiological and biochemical studies of marine *Streptomyces rochei* (MTCC 10109) showing antagonistic activity against selective human pathogenic microorganisms. Asian Journal of Biological Sciences, 4(1), 1-14. https://doi.org/10.3923/ajbs.2011.1.14

[18] Bric, J. M., Bostock, R. M., & Silverstone, S. E. (1991). Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied & Environmental Microbiology, 57(2), 535-538. https://doi.org/10.1128/aem.57.2.535-538.1991

[19] Glickmann, E. & Dessaux, Y. (1995). A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Applied & Environmental Microbiology, 61(2),793-796. https://doi.org/ 10.1128/aem.61.2.793-796.1995

[20] Rafik, E., Rahal, E., & Ahmed, L. (2014). Isolation and screening of actinomycetes strains producing substances plant growth promoting. Indo-American Journal of Agricultural & Veterinary Sciences, 2(4), 1-10.

[21] Udagwa, K., & Kinoshita, S. (1961). A colorimetric determination of gibberellin A3. Part I. Journal of the Agricultural Chemical Society of Japan, 35(3), 219- 223. https://doi.org/10.1271/nogeikagaku1924.35.3_219

[22] Shindy, W. W., & Smith, O. F. (1975). Identification of plant hormones from cotton ovules. Plant Physiology, 55(3), 550-554. https://doi.org/10.1104/pp.55.3.550

[23] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3), 350-356. https://doi.org/10.1021/ac60111a017

[24] Mehta, S., & Nautiyal, C. S. (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. Current Microbiology, 43(1), 51-56. https://doi.org/10.1007/s002840010259

[25] Jackson, M. L. (1959). Soil chemical analysis. Prentice Hall of Englewood Cliffs. Zeitschrift für Pflanzenernährung, Düngung, Bodenkunde, 85(3), 251-252. https://doi.org/10.1002/jpln.19590850311

DOI: 10.62184/nhjbas.jnh10020253

[26] Dye, D. W. (1962). The inadequacy of the usual determinative tests for the identification of Xanthomonas spp. New Zealand Journal of Science, 5(4), 393-416.

[27] Cappuccino, J. C., & Sherman, N. (2005). Microbiology: A laboratory manual, 7th edition. San Francisco: Pearson/Benjamin Cummings.

[28] Vincent, J. M. (1970). A manual for the practical study of the root nodule bacteria. Blackwell Scientific Publishers, Oxford, 164.

[29] Kawato, M., & Shinobu, R. (1959). A simple technique for the microscopical observation. Memoirs of the Osaka University Liberal Arts & Education, 8, 114.

[30] Edwards, U., Rogall, T., Blöcker, H., Emde, M., & Böttger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Research, 17(19), 7843-7853. https://doi.org/10.1093/nar/17.19.7843

[31] Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22(22), 4673-4680. https://doi.org/10.1093/nar/22. 22.4673

[32] Jukes, T. H., & Cantor, C. R. (1969). Evolution of protein molecules. Mammalian protein metabolism, 21-123. https://doi.org/10.1016/b978-1-4832-3211-9.50009-7

[33] Saitou, N., & Nei, M. (1987). The Neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4, 406-425. DOI: https://doi.org/10.1093/oxfordjournals.molbev.a040454

[34] Koch, A. L. (1970). Turbidity measurements of bacterial cultures in some available commercial instruments. Analytical Biochemistry. 38(1), 252-259. https://doi.org/10.1016/0003-2697(70)90174-0

[35] Kumar, S., & Kannabiran, K. (2010). Antifungal activity of Streptomyces VITSVK5 spp. against drug resistant Aspergillus clinical isolates from pulmonary tuberculosis patients. Journal of Medical Mycology, 20(2), 101- 107. https://doi.org/ 10.1016/j.mycmed.2010.04.005

[36] Silo-Suh, L. A., Lethbridge, B. J., Raffet, S. J., He, H., Clardy, J., & Handelsman, J. (1994). Biological activities of two fungistatic antibiotics produced by Bacillus cereus UW85. Applied and Environmental Microbiology, 60(6), 2023-2030. https://doi.org/10.1128/aem.60.6.2023-2030.1994

[37] Madigan, M. T., Martinko, J. M., & Parker, J. (1997). Brock Biology of Microorganisms, 11th (ed). Pearson Prentice Hall: Upper Saddle River, NJ.

[38] Pellizzoni, M., Ruzickova, G., Kalhotka, L., & Lucini, L. (2012). Antimicrobial activity of different *Aloe barbadensis* Mill. and *Aloe arborescens* Mill. leaf fractions. Journal of Applied Medicinal Plant Research, 6(10), 1975-1981. https://doi.org/10.5897/JMPR011.1680

[39] McCutcheon, A. R., Ellis, S. M., Hancock, R. E. W., & Towers, G. H. N. (1992). Antibiotic screening of medicinal plants of the British Columbian native people. Journal of Ethnopharmacology, 37(3), 213-223. https://doi.org/10.1016/0378-8741(92)90036-Q

[40] Mirza, M. S., Ahmad, W., Latif, F., Haurat, J., Bally, R., Normand, P., & Malik, K. A. (2001). Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane in vitro. Plant & Soil, 237, 47-54. https://doi.org/10.1023/A:1013388619231

[41] Apine, O. A., & Jadhav, J. P. (2011). Optimization of medium for indole-3-acetic acid production using *Pantoea agglomerans* strain PVM. Journal of Applied Microbiology, 110(5), 1235-1244. https://doi.org/10.1111/j.1365-2672.2011.04976.x

[42] Karakoç, S., & Aksöz, N. (2004). Optimization of carbon-nitrogen ratio for production of gibberellic acid by *Pseudomonas* sp. Polish Journal of Microbiology, 53(2), 117-120.

[43] Piccoli, P., & Bottini, R. (1994). Effects of C/N relationships, N content, pH, and incubation time on growth and gibberellin production by *Azospirillum lipoferum*. Symbiosis, 17, 229-236.

[44] Piccoli, P., Masciarelli, O., & Bottini, R. (1999). Gibberellin production by *Azospirillum lipoferum* cultured in chemically-defined medium as affected by oxygen availability and water status. Symbiosis (Rehovot), 27, 135-146.

[45] Kimmel, S. A., & Robert, R. F. (1998). Development of a growth medium suitable for exopolysaccharide production by *Lactobacillus delbrueckii* ssp. bulgaricus RR. International Journal of Food Microbiology, 40(1-2), 87-92. https://doi.org/10.1016/S0168-1605(98)00023-3

DOI: 10.62184/nhjbas.jnh10020253

Research Article

[46] Chabot, R., Antoun, H., & Cescas, M. P. (2011). Stimulation de la croissance du maïs et de la laitue romaine par des microorganismes dissolvant le phosphore inorganique. Canadian Journal of Microbiology, 39(10), 941-947. https://doi.org/10. 1139/m93-142

[47] Khan, M. S., Zaidi, A., & Ahmad, E. (2014). Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms. Phosphate Solubilizing Microorganisms, 31–62. https://doi.org/10.1007/978-3-319-08216-5_2

[48] Pandey, P., & Maheshwari, D. K. (2007). Bioformulation of *Burkholderia* sp. MSSP with a multispecies consortium for growth promotion of *Cajanus cajan*. Canadian Journal of Microbiology, 53(2), 213-222. https://doi.org/10.1139/ w06-118

[49] Sahrawat, K. L. (1982). Nitrification in some tropical soils. Plant & Soil, 65, 281- 286. https://doi.org/10.1007/bf02374659

[50] Patel, T., & Saraf, M. (2017). Exploration of novel plant growth promoting bacteria *Stenotrophomonas maltophilia* MTP42 isolated from the rhizospheric soil of *Coleus forskohlii*. International Journal of Current Microbiology & Applied Sciences, 6 (11), 944-955. https://doi.org/10.20546/ijcmas. 2017.611.111

[51] Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173(2), 697-703. https://doi.org/10.1128/jb.173.2.697-703.1991

[52] Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. Journal of Clinical Microbiology, 45(9), 2761-2764. https://doi.org/10.1128/JCM. 01228-07

[53] Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology, 28(4), 1327-1350. https://doi.org/10.1007/s11274-011-0979-9

[54] Singh, R. P., & Jha, P. N. (2016). The multifarious PGPR *Serratia marcescens* CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (*Triticum aestivum* L.). PLoS ONE, 11(6), e0155026. https://doi.org/10.1371/journal.pone.0155026

[55] Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biology & Biochemistry, 42(5), 669-678. https://doi.org/10.1016/j.soilbio.2009.11.024

[56] Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Reviews, 37(5), 634-663. https://doi.org/10.1111/1574-6976.12028

[57] Ongena, M., & Jacques, P. (2008). *Bacillus lipopeptides*: versatile weapons for plant disease biocontrol. Trends in Microbiology, 16(3), 115-125. https://doi.org/10.1016/j.tim.2007.12.009

[58] van der Meij, A., Worsley, S. F., Hutchings, M. I., & van Wezel, G. P. (2017). Chemical ecology of antibiotic production by actinomycetes. FEMS Microbiology Reviews, 41(3), 392-416. https://doi.org/10.1093/femsre/fux005

[59] Zalila-Kolsi, I., Mahmoud, A. B., Ali, H., Sellami, S., Nasfi, Z., Tounsi, S., & Jamoussi, K. (2016). Antagonist effect of *Bacillus* spp. strains against *Fusarium graminearum* for the protection of Durum wheat (*Triticum turgidum* L. subsp. *durum*). Microbiological Research, 192, 148-158. https://doi.org/ 10.1016/j.micres.2016.06.012

[60] Zamoum, M., Yacine, G., Sabaou, N., Mathieu, F., & Zitouni, A. (2017). Development of formulations based on *Streptomyces rochei* strain PTL2 spores for biocontrol of *Rhizoctonia solani* damping-off of tomato seedlings. Biocontrol Science & Technology, 27(6), 1-16. https://doi.org/10.1080/09583157. 2017.1334257

[61] Kanini, G. S., Katsifas, E. A., Savvides, A. L., & Karagouni, A. D. (2013). *Streptomyces rochei* ACTA1551, an indigenous Greek isolate studied as a potential biocontrol agent against *Fusarium oxysporum* f. sp. *lycopersici*. BioMed Research International, 2013, 387230. https://doi.org/10.1155/2013/ 387230