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The Impact of Growth-Promoting Streptomyces Isolated from Rhizosphere and Bulk Soil on Oilseed Rape (*Brassica napus* L.) Growth Parameters

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Abstract: Inoculation of *Streptomyces* to improve oilseed rape (*Brassica napus* L.) yields and minimise the use of chemical fertilisers is a promising sustainable strategy. In this study, we isolated 72 actinobacterial strains from rhizosphere of oilseed rape and maize and from bulk soil for screening and characterising their antimicrobial activity. Nine promising strains, identified as *Streptomyces* sp. by morphology, physiological characteristics, and 16S rRNA gene sequencing, were selected for their plant growth-promoting traits and in planta experiments. The actinobacterial strains were positive for IAA production, siderophore production, and HCN production. In planta experiments were conducted by soaking the oilseed rape seeds in the actinobacterial suspension, followed by plant growth under controlled conditions in a cultivate chamber (22–28 °C, 8 h dark/16 h light, constant humidity 80%). We recorded root and shoot length (cm) and seedling fresh weight (g). For most of the abovementioned parameters, a significant enhancement was observed with strain KmiRC20A118 treatment. The length of the root increased by 53.14%, the shoot length increased by 65.6%, and the weight of the fresh plant by 60% compared to the control. The integrated application of PGPS (*Plant Growth Promoting Streptomyces*) from the rhizosphere of oilseed rape is a promising strategy to improve the growth of oilseed rape.

Keywords: *Streptomyces*; PGPS; oilseed rape

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1. Introduction

Oilseed rape (*Brassica napus* L.) is one of the valuable oilseed crops. It has been attracting attention in recent years as an important oil-producing plant [1] and as an alternative renewable energy source [2]. Due to the changes in temperature, rainfall, and other climatic conditions, various plant diseases have occurred on rapeseed plants in recent years, reducing their fertility [3]. Among the most important diseases are white rot (*Sclerotinia sclerotiorum*), stem cancer (*Leptosphaeria maculans*), alternarium spot (*Alternaria brassicae*) and grey mould (*Botrytis cinerea*) [4]. Oilseed rape plants lack resistance to most soil-borne pathogens and are therefore reliant on microbial-based defence mechanisms for their survival [5]. Beneficial plant-associated microorganisms are a valuable resource for the development of biological control agents and plant growth-promoting rhizobacteria (PGPR) [6]. Actinobacteria are one of the five most dominant bacterial phyla in soils and an important part of the microbial communities in both the rhizospheric and endospheric compartments of plant roots [7]. Actinobacteria are associated with the biological control of insect pests and pathogens, stress tolerance and growth promotion in plants [8]. Despite their popularity as biocontrol agents and their effectiveness in plant root system colonisation, it is surprising that they have not been adequately studied as plant growth promoters [9]. *Streptomyces* strains have evolved numerous potential mechanisms to directly stimulate plant growth, but in planta validation of these mechanisms is largely

lacking, although necessary as confirmation of their direct involvement in plant growth promotion [10]. There are several studies that have suggested that the application of rhizospheric actinomycetes isolated from different crops will significantly affect the growth of oilseed rape [11,12]. Some authors have also claimed that the growth of oilseed rape is influenced by bacterial strains which are isolated from oilseed rape and subsequently applied to it [13]. The in vivo application of actinomycetes to oilseed rape is mainly being tested to reduce phytopathogenic fungi [14–16], and not many studies are available to support rapeseed growth.

In this context, we hypothesised that the strain of *Streptomyces* isolated from the rhizosphere of oilseed rape (*Brassica napus* L.) promote oilseed rape growth under controlled conditions significantly more than strains from the rhizosphere of maize and bulk soil.

2. Materials and Methods

2.1. Isolation and Identification of Actinobacterial Strains

We collected 15 samples of rhizosphere soil of maize (genotype KESSOS) and the same number of samples of rhizosphere soil of oilseed rape (genotype SIDNEY) and of bulk soil. Soil samples were collected from fields at the university farm of the Slovak University of Agriculture (SUA) in Kolíňany (48°21'33" N, 18°11'28" E). According to the FAO classification, the soil type was haplic luvisol with a low to medium humus content (1.91%), an extremely low total nitrogen content (0.19%), and a neutral pH/H₂O (7.32) soil reaction. Soil samples were collected randomly from different locations of a field plot and conveyed to the laboratory in coolers. Rhizospheric actinobacteria were isolated as previously described [17]. Isolation and culturing of actinomycetes were performed by the serial dilution method (10^{-2} – 10^{-4}) and the spread plate technique on starch-casein medium (starch 10 g/L, casein 1 g/L, K₂HPO₄ 0.5 g/L, agar 13 g/L) [18] supplemented with cycloheximide (1 mg/mL). The plates were incubated at 30 °C for 7 days. Powdered colonies were picked up, transferred to ISP2 medium [19], and purified several times. To select *Streptomyces*-like bacteria, microscopic and macroscopic characteristics of the obtained colonies were assessed, resulting in a total of 72 isolates which were further purified and sub-cultured. We isolated 21 strains from the rhizosphere of oilseed rape, 17 strains from the rhizosphere of maize, and 34 strains from the bulk soil. Single colonies were characterised by their macroscopic morphology (colours of aerial and substrate mycelium, soluble pigments and melanin) on International *Streptomyces* Project media (ISP2–ISP7). Physiological characteristics included the use of different carbon sources (glucose, arabinose, inositol, cellulose, mannose, fructose, galactose, rhamnose, sucrose, xylose) on ISP9 medium with final concentration of carbon sources adjusted to 1%. Nutrient medium without the addition of any carbon source was used as a negative control, and glucose-supplemented medium was used as a positive control. The assay was performed in 12-well microplates. Results of these assays were used in poly-phasic identification of strains.

2.2. Antimicrobial Activity Assay

The 72 streptomycetes strains were tested against 7 fungal pathogens and 4 bacterial plant pathogens that may be potentially pathogenic to oilseed rape. We tested *Fusarium poae* (KmiZM16H007), *Penicillium expansum* (KmiZB20H050), *Alternaria tenuissima* (KmiZM16H036), *Aspergillus niger* (KmiLR18H120), *Sclerotinia sclerotiorum* (KmiJM18H119), *Rhizoctonia solani* (KmiJM18H121) and *Leptosphaeria maculans* (KmiRC20H107), obtained from the Collection of Microorganisms of the Department of Microbiology, FBF, SUA, Nitra. For this assay, fungal strains were grown on Potato Dextrose Agar (PDA) plates for 3 days at 25 °C, and two 6-mm disks of mycelium from each phytopathogenic fungus were then placed on opposite edges of a new PDA plate. Following fungal inoculation, two 6-mm wells were opened on opposite sides of the PDA plate, containing 50 µL of a 5-day culture for each actinobacteria strain grown on ISP-2 medium. The plates were incubated at 28 °C for 5 days, and antagonistic activity was estimated by measuring the growth inhibition

zone. Antibacterial activity was tested against four bacterial plant pathogens, namely, *Xanthomonas campestris* (CCM 22), *Pseudomonas syringae* (CCM 2868), *Erwinia amylovora* (CCM 1114), and *Clavibacter michiganensis* subsp. *sepedonicus* (CCM 7014), obtained from the Czech Collection of Microorganisms.

Six-hour-old cultures of the tested bacteria in Mueller–Hinton (MH) broth were used for antimicrobial activity assessment. Bacterial cultures were diluted to 0.05 McFarland standard turbidity using MH and then were spread onto the surfaces on TSA (Tryptone Soya Agar) plates using sterile bacterial cell spreaders. Six millimetre disks of mycelium from each actinobacterial strain were placed in the centre of each plate [20]. Plates were incubated at 30 °C, and the inhibition zones were determined after 24 h. Based on antimicrobial activity, we selected the nine most active strains for further testing. Three strains were selected from the rhizosphere of maize, three from the rhizosphere of oilseed rape, and three from the bulk soil (Table 1).

2.3. Molecular Phylogeny

Strains were inoculated on ISP-2 agar and incubated at 28 ± 2 °C. The DNA was extracted from 24-h-old actinobacterial culture. Approximately 50 mg of actinobacterial culture was placed in 200 µL of PrepMan solution (Life Technologies, CA, USA) and homogenised with glass beads in a BeadBug homogeniser (Benchmark scientific, NJ, USA). Subsequently, 1 µL of the supernatant was used in the PCR (Polymerase Chain Reaction) reaction. The 16S rRNA gene of the DNA was amplified by using the forward primer 27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and the reverse primer 1492R (5'-TACGGTTACCTTGTACGACTT-3') [21]. Thirty microliters of the PCR mixture contained 3 µL of 10x Dream Taq DNA buffer, 3 µL of 2 mM dNTP mix, 1 µL of MgCl₂, 1.2 µL each of the opposing amplification primers (10 mM), 0.1 µL Dream Taq DNA polymerase, and 1 µL of genomic DNA. The PCR amplification was performed in a thermal-cycler MJ Mini (Biorad, CA, USA) programmed for an initial cycle at 95 °C for 3 min, followed by 40 cycles at 95 °C for 30 s, 56 °C for 30 s, 72 °C for 90 s, followed by a final extension at 72 °C for 10 min. The PCR-amplified 16S rDNA was checked in agarose gel. Products were purified by enzymatic treatment using the ExoI/FastAP mixture (ThermoFisher, MA USA) and sequenced by the MacroGen Company, South Korea. The similarity and homology of the 16S rRNA partial gene sequence were compared with available sequences by the BLAST search against the GenBank (www.genbank.com, accessed 15 April 2021) 16S rRNA gene database, limited to type strains [22]. The DNA sequences were aligned, and the phylogenetic tree was constructed using the maximum likelihood algorithm. A bootstrap analysis of 1000 replicates was carried out using Molecular Evolutionary Genetics Analysis (MEGA) 6.06 [23].

2.4. In-Vitro Screening of Actinobacteria Strains for Their Plant Growth-Promoting Traits

2.4.1. Production of Indole-3-Acetic Acid (IAA)

The production of indole-3-acetic acid was quantified using a method described previously [24]. All actinobacteria were grown in ISP-2 liquid medium with tryptophan (200 µg.mL⁻¹) for 10 days at 28 °C at 120 rpm. After incubation, 5 mL of the culture medium was centrifuged at 10,000 rpm for 10 min. The supernatant (4 mL) was mixed with 8 mL Salkowski reagent (35% HClO₄, FeCl₃) and incubated at 25 °C for 30 min in the dark. Development of a pink colour indicated IAA production, and the absorbance of the solutions was read at 530 nm. The concentration of indole-3-acetic acid in culture was determined using a calibration curve of pure IAA as a standard, following the linear regression analysis.

Table 1. Antimicrobial activity of isolated actinomycetes.

Isolation Source	Actinobacterial Strains	Antimicrobial Activity (mm)					Antifungal Activity (mm)				
		<i>Xantomonas campestris</i>	<i>Pseudomonas syringae</i>	<i>Erwinia amylovora</i>	<i>Clavibacter michiganensis</i>	<i>Fusarium poae</i>	<i>Penicillium expansum</i>	<i>Alternaria tenuissima</i>	<i>Aspergillus niger</i>	<i>Sslerotinia sclerotiorum</i>	<i>Leptosphaeria maculans</i>
Rhizosphere of maize	Kmi SK16A002	16	13	0	48	21	30	44	25	40	14
	Kmi SK16A004	24	13	16	44	0	11	36	0	0	28
	Kmi SK16A006	20	0	17	50	0	22	28	15	45	0
Bulk soil	Kmi ICH17A011	0	0	0	26	18	29	0	26	17	31
	Kmi ICH17A097	15	14	15	31	17	23	0	18	0	19
	Kmi ICH17A098	16	14	14	28	32	24	0	0	15	21
Rhizosphere of oilseed rape	Kmi RC20A118	22	0	0	42	16	14	12	10	0	41
	Kmi RC20A119	0	12	9	35	0	0	25	0	35	29
	Kmi RC20A120	24	0	10	39	15	8	45	0	28	37

2.4.2. Siderophore Production

The production of siderophores was qualified by Universal Chrome Azurol S (CAS) [25]. The actinobacterial strains were spot-inoculated on CAS agar and incubated for 7 days at 28 °C. Orange or yellow halo zones around the colony indicated siderophore production.

2.4.3. Phosphate Solubilisation

Actinobacteria strains were spot-inoculated on Pikovskaya agar [26] under aseptic conditions and incubated for 7 days at 28 °C. The clear zones around the colony were considered phosphate solubilisers. The phosphate solubilisation index (PSI) was calculated according to the following formula [27]:

$$\text{PSI} = \frac{\text{Colony diameter} + \text{Diameter of halo zone}}{\text{Colony diameter}}$$

2.4.4. Production of Hydrogen Cyanide (HCN)

Actinobacteria were spot-inoculated on ISP-2 medium and filter paper-dipped in the 2% sodium carbonate and in the 0.5% picric acid for 1 min; the filter paper was then placed on the media surface. Plates were incubated for 7 days at 28 °C. On orange to red colour of the filter indicated HCN production [28,29].

2.5. In-Situ Screening of Actinobacterial Strains for Their Plant Growth-Promoting Traits

2.5.1. Germination Test

The germination assay was conducted according to the protocols described for the standard germination test (Association of Official Seed Analysis, 1993). Groups of 100 rape-seed (*Brassica napus* L.) seeds were placed in a Petri dish with filter paper (Whatman No. 4) and moistened with sterile distilled water. Testing lasted for 7 days at 25 °C. Germination rate achieved 99%.

2.5.2. Preparation of Actinobacterial Inocula

To prepare the actinobacterial cultures, each bacterial strain was grown in a 250-mL flask containing 100 mL ISP-2 medium and incubated for 48 h at 28 °C. After incubation, actinomycetes were subsequently inoculated on the ISP-2 plates and cultivated at 28 °C for 5 days. The sporulated actinomycetes were scraped, poured into sterile distilled water, and filtered. The concentrations were standardised to 5×10^8 CFU.mL⁻¹ [30]. Oilseed rape seeds were sterilised as described elsewhere [31]. After sterilisation, seeds were soaked on spore suspension for 45 min with constant shaking at 120 rpm. For the control, the seeds were dipped in sterile distilled water.

2.5.3. In-Situ Experiment

This experiment was performed in sterile soil (haplic luvisol, humus content 1.91%, nitrogen content 0.19%, pH/H₂O 7.32). Actinobacterial-treated oilseed rape (genotype SYNDEY) seeds were placed in plastic pots with 200 g sterile soil to a depth of 1 cm. The soil was sterilised in an autoclave under the following conditions: pressure 0.1 MPa and temperature 121 °C for 20 min. The seeds dipped in sterile water were used as negative control. Each treatment included two biological replicates (two independent colonies for each bacterial strain), and six seeds were inoculated with each inoculum. Oilseed rape was grown under controlled conditions in a cultivation chamber (22–28 °C, 8 h dark/16 h light, constant humidity 80%). After 30 days, plants were removed from the soil, and the roots were rinsed with water. The root and shoot length and the weight of the fresh plant were measured and statistically evaluated.

2.5.4. Statistical Analysis

Statistical analysis was conducted by using analysis of variance, applying the statistical program Statgraphics XV. The differences among variances were compared using the Tukey HSD ($p \leq 0.05$).

3. Results and Discussion

A total of 72 actinomycete strains were isolated and subjected to primary screening (antimicrobial activity). Of these, we selected the nine most active ones (three from the maize rhizosphere, three from oilseed rape rhizosphere, and three from bulk soil) based on antimicrobial activity. The tested bacteria and fungi were among the important plant pathogens of wheat, potatoes, corn, beans, oilseed rape, and many others [32,33]. For example, strain KmiSK16A006 showed significant antimicrobial activity against the oilseed rape pathogen *Xanthomonas campestris* (20 mm), the causal agent of black rot disease [34] and *Sclerotinia sclerotiorum* (45 mm), the agent of white stem rot [35]. Also, strains KmiICH17A011, KmiRC20A118, KmiRC20A119, and KmiRC20A120 showed significant antifungal activity against the important oilseed rape pathogen *Leptosphaeria maculans*, the causal agent of phoma stem canker of oilseed rape (*Brassica napus* L.) [36]. According to a previous study, it is important to examine actinomycetes with antibacterial and antifungal effects as producers of new bioactive products in agriculture or for direct use as biocontrol in the soil environment [37]. According to the results in Table 1, the strains of actinomycetes selected here can be potential producers of bioactive substances important for the protection of oilseed rape from microbial pathogens [9,38,39].

Diameter of the inhibition zone using the agar plug method excluding the diameter of *Streptomyces* agar block (6 mm).

3.1. Characterisation of Actinomycete strains

Based on the morphological, physiological, and genotypic features, we characterised and identified all nine strains of actinomycetes (Tables 2 and 3). We confirmed that these are species of the genus *Streptomyces*, the most common genus in the soil. All strains were characterised based on 16S rRNA gene sequences and cultural taxonomy procedure. The dendrogram of the nine strains indicates species relatedness. The phylogenetic comparison of sequences with the 16S rRNA NCBI database limited to type species using the BLASTn is shown in Figure 1. For the correct identification of strains, we also compared all strains according to the available data presented in the Compendium of Actinobacteria [40]. Several type species (5–10) with highest sequence homology to particular strain were selected and their morphological and physiological data were accessed in compendium. These data were compared to strain properties and results to most likely identification.

Table 2. Morphological characteristics of actinobacterial strains.

Actinobacterial Strains	Colour of Aerial Mycelium	Colour of Substrate Mycelium	Soluble Pigment	Melanoid Pigment
KmiSK16A002	White	Yellow	—	+
KmiSK16A004	Green	Rusty red	Beige yellow	—
KmiSK16A006	Beige	Orange	—	+
KmiICH17A011	White	Bottle green	—	+
KmiICH17A097	White	Red	Olive green	+
KmiICH17A098	White	Ivory	Brown	—
KmiRC20A118	Ivory	Yellow	—	—
KmiRC20A119	Beige	Brown	—	+
KmiRC20A120	Ivory	Yellow	—	+

+ presence of pigment, — absence of pigment.

Table 3. Growth of actinobacterial strains in presence of various carbon sources.

Actinobacterial Strains	Glucose	Xylose	Sucrose	Mannose	Arabinose	Raffinose	Inositol	Fructose	Rhamnose	Cellulose
KmiSK16A002	+	—	—	+	+	—	—	+	—	—
KmiSK16A004	+	—	—	+	—	—	—	—	—	—
KmiSK16A006	+	—	—	+	+	—	—	+	+	+
KmiICH17A011	+	—	+	+	+	+	+	+	+	—
KmiICH17A097	+	+	+	+	+	+	+	+	—	—
KmiICH17A098	+	—	—	—	—	—	—	—	—	—
KmiRC20A118	+	—	+	+	+	+	—	+	—	+
KmiRC20A119	+	+	+	+	+	—	+	+	+	—
KmiRC20A120	+	+	+	+	—	+	+	—	—	—

+ growth, — absence of growth.

Figure 1. Phylogenetic tree based on the comparison of the 16S rDNA sequences of *Streptomyces* sp. strains and some of their closest phylogenetic relatives. The tree was created by ML algorithm with neighbour-joining starting tree. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications.

Using polyphasic identification systems actinobacterial strains were identified as *Streptomyces sampsonii* (KmiSK16A002), *Streptomyces puniceus* (KmiSK16A004), *Streptomyces olivochromogenes* (KmiSK16A006), *Streptomyces flavovariabilis* (KmiSK16A011), *Streptomyces spectabilis* (KmiICH17A097), *Streptomyces albidoflavus* (KmiICH17A098), *Streptomyces misionensis* (KmiRC20A118), *Streptomyces rishiriensis* (KmiRC20A119) and *Streptomyces peucetius* (KmiRC20A120). Accession numbers are shown in Table 4. According to data in the literature, all characterised species have already been confirmed as plant growth-promoting streptomycetes (PGPS). In the case of *Streptomyces albidoflavus* [41–43], *Streptomyces sampsonii* [44,45], *Streptomyces puniceus* [46], *Streptomyces cirratus* [47], *Streptomyces flavovariabilis* [48] and *Streptomyces rishiriensis* [49], there was a strong antagonistic activity against several phytopathogenic fungi such as *Aspergillus niger*, *Microsporium gypseum*, *Trichopyton* sp., *Fusarium* sp. and *Alternaria solani*. Strains identified as *Streptomyces olivochromogenes* [50], *Streptomyces spectabilis* [51], and *Streptomyces misionensis* [52] have already been confirmed as PGPS, which, in addition to antifungal activity, have also shown the ability to produce indolyl-3-acetic acid, solubilise phosphates, and produce siderophores. *Streptomyces peucetius* is one of the major producers of the new type of siderophores [53].

Table 4. GenBank accession numbers for tested *Streptomyces* strains, BLASTn best hit, and predicted taxonomic affiliation according polyphasic identification system.

Strain	GenBank Accessions No.	BLASTn Best Hit	Polyphasic Identification
KmiSK16A002	MZ172903	<i>S. sampsonii</i> KP096298 (772/773) ^a + 1 gap	<i>S. sampsonii</i>
KmiSK16A004	MZ172904	<i>S. cavourensis</i> NR_112345.1 (845/821) + 3 gaps	<i>S. puniceus</i>
KmiSK16A006	MZ172902	<i>S. olivochromogenes</i> KF317983 (646/651) + 2 gaps	<i>S. olivochromogenes</i>
KmiICH17A011	MZ172905	<i>S. pseudovenezuelae</i> NR_114832.1 (808/821)	<i>S. flavovariabilis</i>
KmiICH17A097	MZ172906	<i>S. spectabilis</i> NR_112398.1 (1341/1343) + 1 gap	<i>S. spectabilis</i>
KmiICH17A098	MZ172907	<i>S. albidoflavus</i> NR_041095.1 (1054/1057) + 3 gaps	<i>S. albidoflavus</i>
KmiRC20A118	MZ182311	<i>S. misionensis</i> NR_044138.1 (798/800)	<i>S. misionensis</i>
KmiRC20A119	MZ182310	<i>S. rishiriensis</i> NR_112392.1 (802/802)	<i>S. rishiriensis</i>
KmiRC20A120	MZ182312	<i>S. peucetius</i> NR_112574.1 (800/801) + 1 gap	<i>S. peucetius</i>

^a homology in base pairs.

3.2. Plant Growth Promotion Determination (IAA, Siderophore and HCN Production, Phosphate Solubilisation)

The streptomycetes from bulk soil and rhizosphere soil were determined for their plant growth-promoting activity, phosphate solubilisation, and the production of IAA, siderophores, and HCN. These capabilities of streptomycetes are particularly important for their use as biofertilisers and biostimulants, as oilseed rape requires high amounts of biogenic elements in the soil [12]. The ability of strains to produce IAA [54] results in improved plant growth via facilitating nutrient uptake, water absorption, germination, and root elongation [55]. All tested streptomycetes strains produced IAA within a range of 8–40 $\mu\text{g}\cdot\text{mL}^{-1}$ (Table 5). The most active strain was KmiRC20A118, isolated from the rhizosphere of oilseed rape. Other studies have also confirmed that *Streptomyces* sp. can produce large amounts of IAA [56], and 11.2% of rhizosphere streptomycetes produce IAA in the range of 11–144 $\mu\text{g}\cdot\text{mL}^{-1}$ [57]. The amount of IAA produced depends mainly on the presence of tryptophan [58]. In the strain *Streptomyces fradiae* NKZ-259, the production of IAA after adding various concentrations of L-tryptophan to the culture medium was significantly affected. After the addition of 2 g L⁻¹ tryptophan to the medium, which is the optimal amount, IAA production was 56.28 $\mu\text{g}\cdot\text{mL}^{-1}$, whereas when five times more tryptophan was added (10 g L⁻¹), the production decreased to 40.22 $\mu\text{g}\cdot\text{mL}^{-1}$ IAA, indicating that higher concentrations of tryptophan in the medium can also have an adverse effect on IAA production [59].

Table 5. Plant growth-promoting traits of Actinobacteria strains.

Strains	IAA Production ($\mu\text{g}\cdot\text{mL}^{-1}$)	Siderophore Production ^a	HCN Production ^b	Phosphate Solubilisation Index (mm)
KmiSK16A002	25.02 $\pm$ 0.002	—	+	0
KmiSK16A004	30.52 $\pm$ 0.01	+	+	0
KmiSK16A006	24.09 $\pm$ 0.004	+	+	0
KmiICH17A011	19.32 $\pm$ 0.002	+	+	0
KmiICH17A097	26.47 $\pm$ 0.005	+	+	11.9 $\pm$ 0.4
KmiICH17A098	8.12 $\pm$ 0.01	+	+	0
KmiRC20A118	40.34 $\pm$ 0.004	+	+	13.1 $\pm$ 0.04
KmiRC20A119	25.71 $\pm$ 0.003	+	+	0
KmiRC20A120	24.23 $\pm$ 0.005	+	+	12.7 $\pm$ 0.05

Values represent mean $\pm$ SE; ^a presence of orange zone: + present; — absent; ^b presence of orange-brown colour: + present; — absent.

The IAA production by streptomycetes can vary among different species and strains and is influenced by culture condition, growth stage, and substrate availability [60]. Moreover, strains from the rhizosphere are more efficient auxin producers than those from bulk soil [61]. According to our results, strains from rhizosphere soil showed high IAA production. These results are comparable with the results of a previous study [62]; the authors

tested 12 strains of actinomycetes from the rhizosphere soils of three crop plants (wheat, maize, and faba bean), using the same methodology, and found IAA production rates between 3.54 and 22.56 $\mu\text{g mL}^{-1}$. This is similar to the strains from bulk soil, and several studies have shown that *Streptomyces* sp. from various crop soils have this ability [55,57,63].

Siderophore production stimulates plant growth by forming complexes with iron (Fe^{3+}) in the rhizosphere, making iron unavailable to the phytopathogens [29]. In our study, we detected 88% of strains to be positive for siderophore production on CAS agar (Table 5). Similarly, in a previous study [64], 87.5% of strains from the rhizosphere soils of various crops (rice, chickpea, sorghum) and vermicompost were positive for siderophore production on CAS agar. In another study [65], *Streptomyces thermolilacinus*, *Streptomyces rochei*, and *Streptomyces carpinensis* showed the ability to produce siderophores on CAS agar medium. Actinomycetes, especially species of the genus *Streptomyces* isolated from various habitats, are the major producers of siderophores [66]; about 44% of soil actinobacteria provide positive results on chrome azurol sulphate (CAS) assay plates [67]. These findings confirm that strains from bulk soil can also produce siderophores, which is important for their potential use in agriculture.

Certain rhizosphere *Streptomyces* species have been reported to produce HCN [68,69]. All strains from the rhizosphere tested in this study were positive for HCN production. Strains from bulk soil also showed this ability, suggesting their potential use to support plant growth (Table 5). One study reported the production of HCN by rhizosphere *Streptomyces* [70]. Because of its toxicity to plant pathogens, HCN is recognised as a biocontrol agent [71], which is confirmed here because our strains showed excellent antimicrobial activity.

Also, actinobacteria can promote the growth of host plants by solubilising inorganic phosphate and enhancing the uptake of phosphorus by the plant when its bioavailability is low [72]. After 7 days of incubation on Pikovskaya agar, only three of the tested strains were able to solubilise phosphates based on the halo zones formed. The strain KmiICH17A097 (11.9 mm) from bulk soil, strain *Streptomyces misionensis* KmiRC20A118 (13.1 mm) and strain KmiRC20A120 (12.7 mm) from rhizosphere soil of oilseed rape showed low phosphate solubilisation index values, and we did not observe this ability in strains from the maize rhizosphere (Table 5). Similar results have been reported in studies where bacterial strains tested on Pikovskaya agar showed a low solubility index [73–75]. The ability of the same bacteria to dissolve phosphates on solid agar and liquid medium differs; some studies have found that the bacteria show a considerably higher phosphate production in liquid medium [76,77]. The low phosphate solubility index may be related to several factors, such as organic acid production or nutrient composition [76,78], but according to the available source, it is not related to origin of the strains.

3.3. Evaluation of *Streptomyces* Strains for Their PGP Potential under Controlled Conditions

Various *Streptomyces* strains have been described as efficient PGPS that can stimulate plant growth. Previously, researchers [79–82] have confirmed the promising effects of different PGPS species on various crops in laboratory and field experiments under varying ecological conditions. For instance, inoculation of the model plant *Arabidopsis thaliana* with several *Streptomyces* strains resulted in an increase in plant biomass [83,84]. For crops such as rice, wheat, sorghum, and tomato, an increase in biomass has been reported with several *Streptomyces* inoculants from rhizosphere soil [84–86]. In this study, all nine strains significantly stimulated oilseed rape growth ($p < 0.05$) in terms of root and shoot length as well as seedling weight with respect to the control (Table 6). Three strains (KmiRC20A118, KmiRC20A119, KmiRC20A120) from the rhizosphere of oilseed rape were more active compared to the control and other strains. The maximum value recorded for root length was 25.4 cm, which was observed for KmiRC20A118, followed by 23.45 cm for KmiRC20A120 and 21.15 cm for KmiRC20A119; the minimum value was 13.5 cm for the control. The increase in shoot length observed for KmiRC20A118, KmiRC20A119, and KmiRC20A120 was 65.6, 38.8, and 48%, respectively; for the remaining cultivars, shoot length ranged between 13 and 40%. Similar results have been reported elsewhere [12], with

a 35–40% increase in the length of oilseed rape shoots after 3 weeks of growth in a culture chamber. Seedling weight ($\text{g} \cdot \text{plant}^{-1}$) was also statistically higher for KmiRC20A118, KmiRC20A119, and KmiRC20A120 compared to the control and all other strains. The increase in seedling weight was 60% for KmiRC20A118 and KmiRC20A119 in regard to the control. Such enhanced growth can be attributed to the ability of PGPS to produce plant growth regulators such as IAA [87,88], which play a vital role in cell elongation, cell division, and root elongation, ultimately improving plant growth [89,90]. For some PGPR, such as *Pseudomonas fluorescens* and *Azospirillum brasilense*, analyses of both bacteria and *Arabidopsis thaliana* have revealed that bacterial auxin production and subsequent auxin signalling in the plant play a major role in growth promotion [91,92]. We can confirm this correlation, as the strain KmiRC20A118 produced a high amount of IAA ($40.34 \mu\text{g} \cdot \text{mL}^{-1}$). The response of oilseed rape to different strains was variable, which may be attributed to their individual traits and rhizospheric competencies. The significant increase in growth caused by strains from the rhizosphere of oilseed rape clearly indicates that these bacterial strains can colonise the plant host from which they had been isolated, resulting in an increase in plant biomass. The effect of strains from maize rhizosphere and bulk soil compared to the control was significant and suggests their potential in supporting plant growth. Thus, it is important to explore and identify region-specific PGPS strains as they are most suitable for enhancing crop growth and yield in that particular region [93]. Although, to date, data on the application of actinomycetes to oilseed rape are scarce, a significant improvement in rapeseed growth parameters after inoculation with actinomycetes in the culture chamber may be evidence for the designation of actinomycete strains as PGPS strains [12].

Table 6. Effect of *Streptomyces* strains to promote the growth of rapeseed (*Brassica napus* L.).

Treatment	Root Length (cm)	Shoot Length (cm)	Seedling Weight (g)
CONTROL	13.5 ± 1.84^a	16.20 ± 0.19^a	7.86 ± 0.92^a
KmiSK16A002	20.25 ± 3.18^{bc}	23.33 ± 0.62^c	10.22 ± 0.80^{bc}
KmiSK16A004	19.85 ± 3.89^{abc}	22.75 ± 0.56^c	10.14 ± 0.45^{bc}
KmiSK16A006	19.78 ± 3.78^{abc}	19.20 ± 1.25^b	11.08 ± 0.32^{cd}
KmiCH17A011	19.76 ± 1.77^{abc}	19.47 ± 0.56^b	10.58 ± 0.34^{bcd}
KmiCH17A097	20.18 ± 1.27^{bc}	19.20 ± 0.88^b	9.13 ± 0.34^{ab}
KmiCH17A098	14.75 ± 2.26^{ab}	18.38 ± 0.13^{ab}	10.20 ± 0.72^{bc}
KmiRC20A118	25.40 ± 1.82^c	26.83 ± 1.52^c	13.24 ± 0.99^e
KmiRC20A119	21.15 ± 2.35^{bc}	22.55 ± 0.47^c	13.27 ± 0.26^e
KmiRC20A120	23.45 ± 2.26^c	24.10 ± 0.13^c	12.00 ± 0.34^{de}

Mean values with the different letters (a–e) in superscript are significantly different according to Tukey's HSD test ($p < 0.05$).

4. Conclusions

This study confirmed our hypothesis that streptomyces strains from the rhizosphere of oilseed rape showed PGPS traits and significantly promoted oilseed rape growth under controlled conditions. The most effective strain was KmiRC20A118, in which we recorded the highest production of IAA ($40 \mu\text{g} \cdot \text{mL}^{-1}$); it produced siderophores and HCN and had a low phosphate solubility index, significantly affecting the total weight of fresh oilseed rape plants, with an increase of up to 68.45% compared to the control. We propose these strains for further testing in field experiments as a potential fertiliser and biostimulant for oilseed rape.

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