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The effect of seed bacterization with *Bacillus paralicheniformis* 2R5 on bacterial and fungal communities in the canola rhizosphere

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ABSTRACT

Bacillus sp. is one of the best-studied plant growth-promoting rhizobacteria (PGPR). However, more detailed studies targeting its effect on the rhizosphere microbial community are required for improving management practices regarding its commercial application in the field. Our earlier study showed that PGPR *Bacillus paralicheniformis* 2R5 stimulated canola growth. Hence, this study aimed to assess the time-course impact of *B. paralicheniformis* 2R5 on bacterial and fungal community structure and diversity. The results showed that inoculation with *B. paralicheniformis* 2R5 initially significantly decreased the observed bacterial richness compared to the control, while after 44 days of treatment this alpha diversity metrics increased. A linear discriminant analysis effect size showed that *B. paralicheniformis* 2R5 altered the soil bacterial and fungal community structure by increasing the abundance of plants' beneficial microorganisms such as *Nitrospira*, *Ramlibacter*, *Sphingomonas*, *Massilia*, *Terrimonas* as well as *Solicoccozyma*, *Schizothecium*, *Cyphellophora*, *Fusicolla*, *Humicola*. *B. paralicheniformis* 2R5 seems to be a promising alternative to chemical pesticides and can be considered for practical application in the field. Its ability to alter the rhizosphere microbiome by increasing the diversity and composition of bacterial communities and increasing plants' beneficial groups of fungi, appears to be important in terms of improving canola development. However, further studies on these increased microbial taxa are necessary to confirm their function in promoting canola growth.

1. Introduction

Plant-growth-promoting rhizobacteria (PGPR) have been studied as biofertilizers to enhance crop productivity and induce resistance in plants. PGPRs have been considered important components for promoting sustainable agriculture by reducing the application of fertilizers and pesticides (Bhattacharyya et al., 2018; Patel et al., 2021). In association with the plant rhizosphere, these microbes improve plant growth by secreting phytohormones, solubilizing phosphates, fixing nitrogen, increasing nutrients uptake, and acting as biocontrol agents of pathogens (Ciftci et al., 2021).

The effectiveness of PGPR inoculation is associated with their ability to colonize and survive, as well as their interactions with indigenous bacterial and fungal communities in the rhizosphere (Kong and Liu, 2022). *Bacillus* species are well known to form endospores which favor their survival in soil. *Bacillus* spp. can be harnessed in agricultural technologies as a bioinoculant because its properties result in a long shelf-life of the product before application (Hashmi et al., 2019). However, more detailed studies about the *Bacillus* effect on the rhizosphere microbial community are required to improve management practices regarding its commercial application in the field.

The maintenance of a healthy rhizosphere microbiome is essential for crop development and soil fertility (Santos and Olivares, 2021). The impact of introduced PGPRs on the native bacterial and fungal populations is still underexplored because of the bacterial inoculants' complex roles in the rhizosphere. PGPR can alter the rhizosphere microbiome by eliminating root-dwelling pathogens or removing other non-harmful microorganisms (Bhattacharyya et al., 2018). On the other hand, some native microbes can negatively affect the function and survival of introduced PGPR (Pacheco da Silva et al., 2022).

Our earlier study showed that PGPR *Bacillus paralicheniformis* 2R5 stimulated canola (*Brassica napus* L. var. *napus)* growth under sterile and non-sterile conditions (Świątczak et al., unpublished). Hence, the objective of the present study was to investigate the impact of *B. paralicheniformis* 2R5 on the composition and diversity of bacterial and fungal communities in the canola rhizosphere using next generation

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sequencing (NGS) of the 16S rRNA gene and internal transcribed spacer (ITS2). Additionally, the time-course impact on native microorganisms in the rhizospheres of bacteria-treated and untreated canola plants on the first, twenty-second and forty-fourth day after bacterization was evaluated.

2. Materials and methods

2.1. Seed bacterization and sampling

Bacillus paralicheniformis 2R5 strain was isolated from the canola (*Brassica napus* L. var. *napus*) rhizosphere (Świątczak et al., unpublished). Canola seeds were sterilized with 1 % sodium hypochlorite (NaOCl) for 30 min and washed three times with sterile distilled water, according to the method described by Rudolph et al. (2015). Sterilized seeds were agitated in *B. paralicheniformis* 2R5 suspension (10^8 CFU/ml), and 10 ml LB broth supplemented with 0.5 % carboxymethyl cellulose (CMC) for 30 min, while sterilized seeds agitated with 10 ml LB broth and 0.5 % CMC were the control. The seeds were germinated in pots (four seeds per pot) containing soil taken from the arable soil in Górsk, Poland (53°01′46.1"N18°26′59.4"E). Treated with *B. paralicheniformis* 2R5 and untreated rhizospheres samples (four replicates for each time-point) were collected by scraping the soil from the roots after 0, 22, and 44 days of sowing (total of 24 samples).

2.2. Rhizosphere DNA isolation, PCR, and sequencing

DNA was extracted from 0.25 g of rhizosphere soil using the DNeasy Power Soil Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The purity of the isolated DNA was measured with a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

The microbial community was determined through the next-generation-sequencing of bacterial 16S hypervariable region (V3-V4) and fungal internal transcribed spacer (ITS2). Bacterial libraries were obtained with the use of the following primers: 5′ CCTACGGGNGGCWGCAG (forward) and 5′ GACTACHVGGGTATCTAATCC (reverse) (Klindworth et al., 2013), while fungal libraries were obtained using 5′ GCATCGATGAAGAACGCAGC (ITS3 forward, without overhangs) and 5′ TCCTCCGCTTATTGATATGC (ITS4 reverse, without overhangs) primers, according to White et al. (1990). Library preparation was followed by Illumina Support Center (ISC) protocol with a slight modification: 2×Phanta Max Master Mix (Vazyme Biotech, Nanjing City, China) was applied instead of Kapa Hifi Hot Start Ready mix. The PCR products were checked using electrophoretic separation. Libraries were normalized based on band luminescence intensity on a 1.5 % agarose gel, pooled, and sequenced on Illumina MiSeq (Illumina, San Diego, CA, USA) using MiSeq Reagent Kit v2 (500-cycles) in 2 × 250 bps paired-end format.

2.3. Bioinformatic analyses

Sequence sets were merged and mothur v1.44.3 (Schloss et al., 2009) was used for quality processing, taxonomic assignments and OTU picking (at 97 % similarity threshold) of 16S rRNA amplicons based on mothur's MiSeq SOP (Kozich et al., 2013, downloaded at 22th May 2021). Deltaq was adjusted to 10 in the 'make.contigs' command. Ambiguous base calls and reads shorter than 300 nt and longer than 500 nt were excluded from the analysis. Primers were removed from the end of the sequences using trim.seqs(pdiffs=2, checkorient=T). Denoising was carried out with the pre.cluster command. Chimeric reads were filtered out using the mothur implementation of VSEARCH. Singleton reads were discarded from the sequence set according to Kunin et al. (2010). Read alignment and taxonomic assignment were carried out using the ARB-SILVA SSU Ref NR 138 reference database (Quast et al., 2012) with a minimum bootstrap confidence score of 80. Reads assigned to non-primer-specific taxonomic groups ('Chloroplast', 'Mitochondria' and 'unknown') were subsequently removed from the dataset. A random subsampling was performed based on the sample having the lowest sequence number. Richness estimators and diversity indices were also calculated with mothur.

For fungal ITS analysis, initial merging and quality filtering steps were carried out with mothur as described for the 16S rRNA gene amplicons. Subsequently, ITS2 region was extracted from the sequences using the ITSx 1.1-beta software (Bengtsson-Palme et al., 2013) based on the findings of Nilsson et al. (2010). Taxonomic assignment was carried out with mothur's classify.seqs(cutoff = 80) using the UNITE v8.3 database (Abarenkov et al., 2020) as reference. Reads not assigned to any fungal phyla were removed from the read set using the remove.lineage(taxon=k_Fungi_unclassified) command of mothur. ITS2 reads were clustered to OTUs with VSEARCH using a 97 % similarity threshold.

2.4. Exploratory data analyses

Differences in richness estimators and diversity indices between non-treated and *Bacillus paralicheniformis* 2R5 treated plants from 0 to 44 days after bacterization were tested with *t-test* for equal means in Past v 3.08. For non-normally distributed data, a test for equal medians - Mann-Whitney was performed. ANOVA analysis following the Tuckey post hoc test was performed to determine OTU richness, Shannon H′, and Inv Simpson indices changes over time of rhizospheres of the treated and non-treated canola plants. The Shapiro-Wilk test was used to check the assumptions of normality, while Levenés test was used to assess the homogeneity of variances. The Principle Component Analysis (PCoA), ANOSIM analysis, and rarefaction curves were performed in R v 4.0.3. A linear discriminant analysis (LDA) effect size (LEfSe) of 100 most abundant OTUs was used to determine the significant differential abundance ($p < 0.05$) of bacterial and fungal taxonomy groups between the rhizospheres of *Bacillus paralicheniformis* 2R5-treated and untreated canola plants from 0 to 44 days after bacterization (https://huttenhower.sph.harvard.edu/galaxy/, Segata et al., 2011).

2.5. Data availability

The Illumina MiSeq sequences of bacterial 16S rRNA and fungal ITS genes were submitted to GenBank-SRA under Bioproject PRJNA876229.

3. Results

3.1. Sequencing results

Sequencing resulted in 2,687,943 and 2,453,619 high-quality sequence reads (clustered into 16,443 and 2760 OTUs) for bacteria and fungi, respectively. The rarefaction curves showed a high coverage of bacterial and fungal communities in all rhizosphere samples (Fig. S1).

3.2. Changes in bacterial and fungal diversity

The alpha diversity indices of bacterial and fungal communities in the groups of samples were compared in two different ways: i) according to the time (Table S1); ii) between treated with *B. paralicheniformis* 2R5 (Bp) and untreated control (C) samples (Table S2).

Fungal diversity increased with time and significant differences were observed in OTU richness for samples CT0, CT22, CT44. Significant differences were detected in bacterial OTU richness and in Shannon diversity for samples CT0, CT22, CT44, and BpT0, BpT22, BpT44. The Simpson index (1-D) showed no significant differences for CT0, CT22, and CT44, while did show significant differences for BpT0, BpT22, and BpT44. The bacterial communities' mean values in each index were the highest on the 44th day both for the treated and untreated samples (Table S1).

Significant differences were not detected in the fungal indices

between the C samples and samples treated with Bp at T0, T22, and T44. However, bacterial alpha-diversity based on observed OTU richness at T22 and T44, and the Shannon index at T22 showed significant differences. The mean values were significantly lower in Bp samples than in the control at T22, while at T44 mean values of Bp samples were higher (Table S2).

The Principal Coordinate Analysis (PCoA) of bacterial (Fig. 1A) and fungal communities (Fig. 1B) showed a clear separation of C samples and samples treated with Bp at T0, T22, and T44 indicating different microbial community structures, which were also confirmed by ANOSIM (Table S3).

3.3. Changes in the bacterial and fungal community structure

The top 10 bacterial and fungal phyla detected with the highest relative abundance are presented in Fig. 2. The most dominant bacterial phyla were *Proteobacteria*, *Actinobacteriota*, *Acidobacteriota*, and *Bacteroidota*. Whereas, the most abundant fungal phyla were *Ascomycota*, *Mortierellomycota*, *Basidiomycota,* and *Chytridiomycota*. LefSe analysis showed significant differences with time and treatment in bacterial (Fig. 3) and fungal (Fig. 4) communities.

Regarding the bacterial communities, 4 phylotypes were enriched in C and 15 in Bp in the T0 time point. Among cultured representatives of bacteria or bacterial sequences of established taxonomy at the genus level, *Kribbella* and *Nitrosospira* were more abundant in C compared to Bp. For the T22 time point, 25 phylotypes were enriched in C, and 30 were more abundant in Bp treatment. Among cultured bacterial organisms, representatives of *Bradyrhizobium*, *Marmoricola*, *Adhaeribacter*, *Nocardioides*, and *Pseudoxanthomonas* prevailed in C, while *Nitrospira*, *Sphingomonas*, *Terrimonas*, *Massilia,* and *Ramlibacter* were more abundant in the PGPR treated samples. For the T44 time point, 12 phylotypes were enriched in C and 14 in Bp. Among cultured bacterial representatives, *Nocardioides*, *Nakamurella,* and *Microvirga* were the most abundant in C, while *Dongia* prevailed in Bp samples (Fig. 3 and Fig. S2).

Based on ITS2 amplicon sequencing, 11 fungal phylotypes were enriched in C, and 12 were more abundant in Bp treatment in the T0 time point. Among fungal reads of established taxonomy at the species level representatives of *Cladorrhinum foecundissimum*, *Cladorrhinum bulbillosum*, and *Heydenia* sp. prevailed in C, while *Humicola grisea* and *Solicoccozyma terricola* were the most abundant in Bp (Fig. 4, Fig. S3). For the T22 time point, 11 phylotypes were more abundant in Bp treatment. *Solicoccozyma aeria* and *Fusicolla septimanifiniscientiae* were more abundant in Bp compared to C (Fig. 4, Fig. S4). For the T44 time point, 13 phylotypes were enriched in C and 11 in Bp. Fungi *Trichobolus zukalii*, *Heydenia* sp., and *Cephalotrichum stemonitis* were detected with the highest relative abundance in the control, while *Schizothecium* sp., *Plectosphaerella cucumerina*, and *Cyphellophora vermispora* were more abundant in Bp treated samples (Fig. 4, Fig. S5).

4. Discussion

The microbial diversity of a rhizosphere is an important ecological bioindicator in maintaining healthy plants growth (Wang et al., 2022). In the present study, the changes in the bacterial and fungal diversity metrics confirmed the microbiota variability depended on the growth of the plant. As indicated by the Shannon H′ index, the highest bacterial diversity was noted after 44 days in the rhizosphere of both untreated and treated plants. Moreover, bacterial and fungal observed OTU richness was the highest after 44 days in untreated plants. Similarly, Wang et al. (2017) indicated that rhizosphere microbiome structure varied during plant development due to changes in root exudates. Plant roots can produce secondary metabolites such as terpenoids, polyphenols, and nitrogen-containing compounds, which do not directly contribute to growth and development but play a significant role in shaping the structure of the rhizosphere microbiome (Mishra et al., 2022). However, the effect of inoculated PGPR on the rhizosphere microbiome is still underexplored. Our results showed initially decreased OTU bacterial richness in samples treated with *B. paralicheniformis* 2R5 compared to the control, while after 44 days of treatment the alpha diversity index increased. In other research, bacterial inoculation caused changes in *Brassica juncea* rhizosphere microbiome after 8 weeks, suggesting that it takes some time for the inoculated PGPR to survive, actively proliferate, and function (Jeong et al., 2013).

Moreover, there is still limited information on how changes in the rhizosphere microbial communities contribute to improving plant growth and development. It is generally believed that a high abundance of beneficial microorganisms indicates better plant growth, lower plant disease, and higher nutrient and enzyme activities (Wang et al., 2017). Our results showed that the *B. paralicheniformis* 2R5 possibly affected the soil microbial community structure by increasing the abundance of beneficial microorganisms for plants. For example, *Nitrospira* and *Ramlibacter* were more abundant in the *B. paralicheniformis* 2R5 treated soil. Some representatives of *Nitrospira* belong to completely nitrifying 'comammox' bacteria (Daims et al., 2016), while *Ramlibacter* is involved

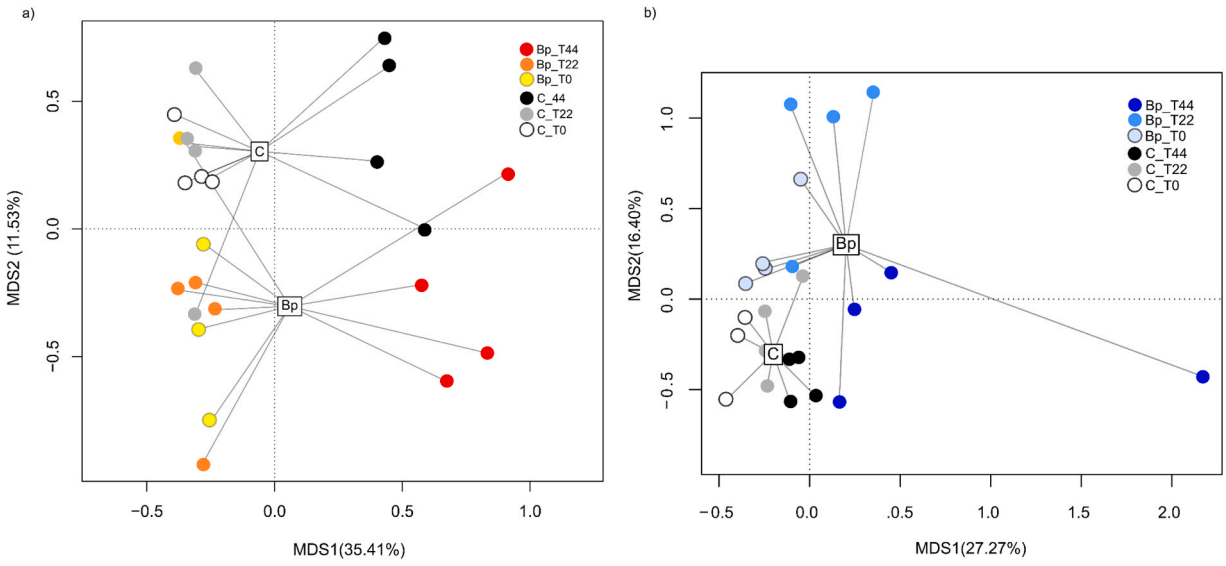

Fig. 1. Principal coordinate analysis (PCoA) of the a) bacterial and b) fungal communities in the rhizospheres of *Bacillus paralicheniformis* 2R5-treated (Bp) and untreated (C) canola plants (n = 4). T0, T22, and T44 – time after bacterization in days.

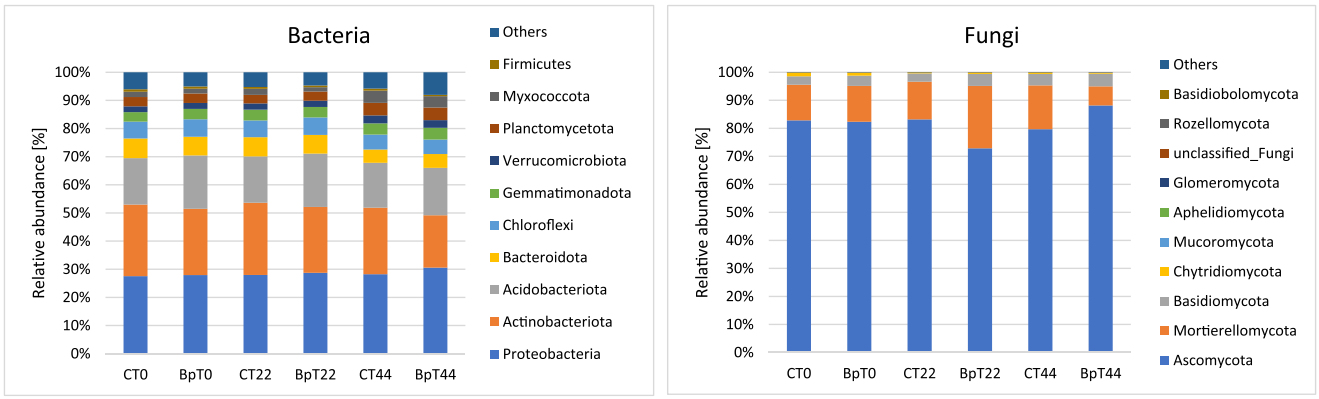

Fig. 2. Mean relative abundance (n = 4) of top 10 most abundant bacterial and fungal phyla in the rhizospheres of *Bacillus paralicheniformis* 2R5-treated (Bp) and untreated (C) canola plants. T0, T22, and T44 – time after bacterization in days.

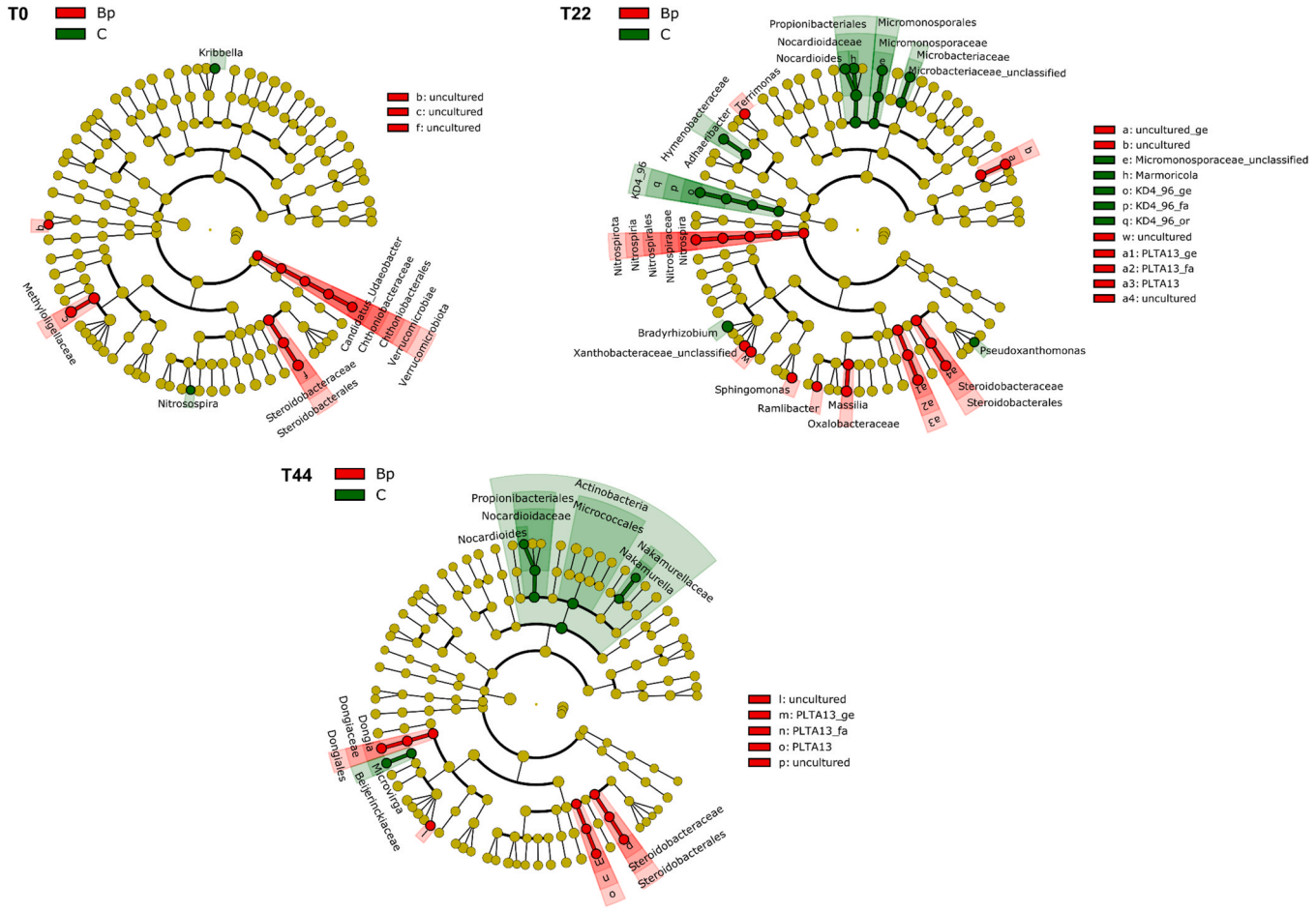

Fig. 3. Cladograms of bacterial top 100 groups in the rhizospheres of *Bacillus paralicheniformis* 2R5-treated (Bp) and untreated (C) canola plants (n = 4). T0, T22, and T44 – time after bacterization in days.

in N-cycling (Milkereit et al., 2021). The increasing abundance of these genera might confirm our earlier results, where *B. paralicheniformis* 2R5 through interactions with soil microbiota increased the number of microorganisms associated with the nitrogen cycle, simultaneously promoting canola growth (Świątczak et al., unpublished). Moreover, relative abundance of other beneficial microorganisms was increased in *B. paralicheniformis* 2R5 treated samples. It was reported that the family of *Xanthobacteraceae* and *Steroidobacteraceae* play the most important role to survive under drought stress conditions in rice (Jang et al., 2020), while the genus *Sphingomonas* have the ability to increase *Solanum pimpinellifolium* growth under salinity stress (Khan et al., 2017). The family of *Gemmatimonadaceae* is known as beneficial bacteria which have a positive effect on plant growth when it is inoculated into the soil (Zhao et al., 2020). Members of the genus *Massilia* can promote plants growth through their ability to solubilize phosphorus and nitrogen fixation (Guo et al., 2019) or through their positive effect on root colonization by arbuscular mycorrhizal fungi (Krishnamoorthy et al., 2016). Genus *Terrimonas* was positively linked with the non-ribosomal peptide synthetase (NRPS) gene – COG 1020 involved in biocontrol of soil-borne plant pathogen *Ralstonia solanacearum* (Michelsen et al., 2015; Wei

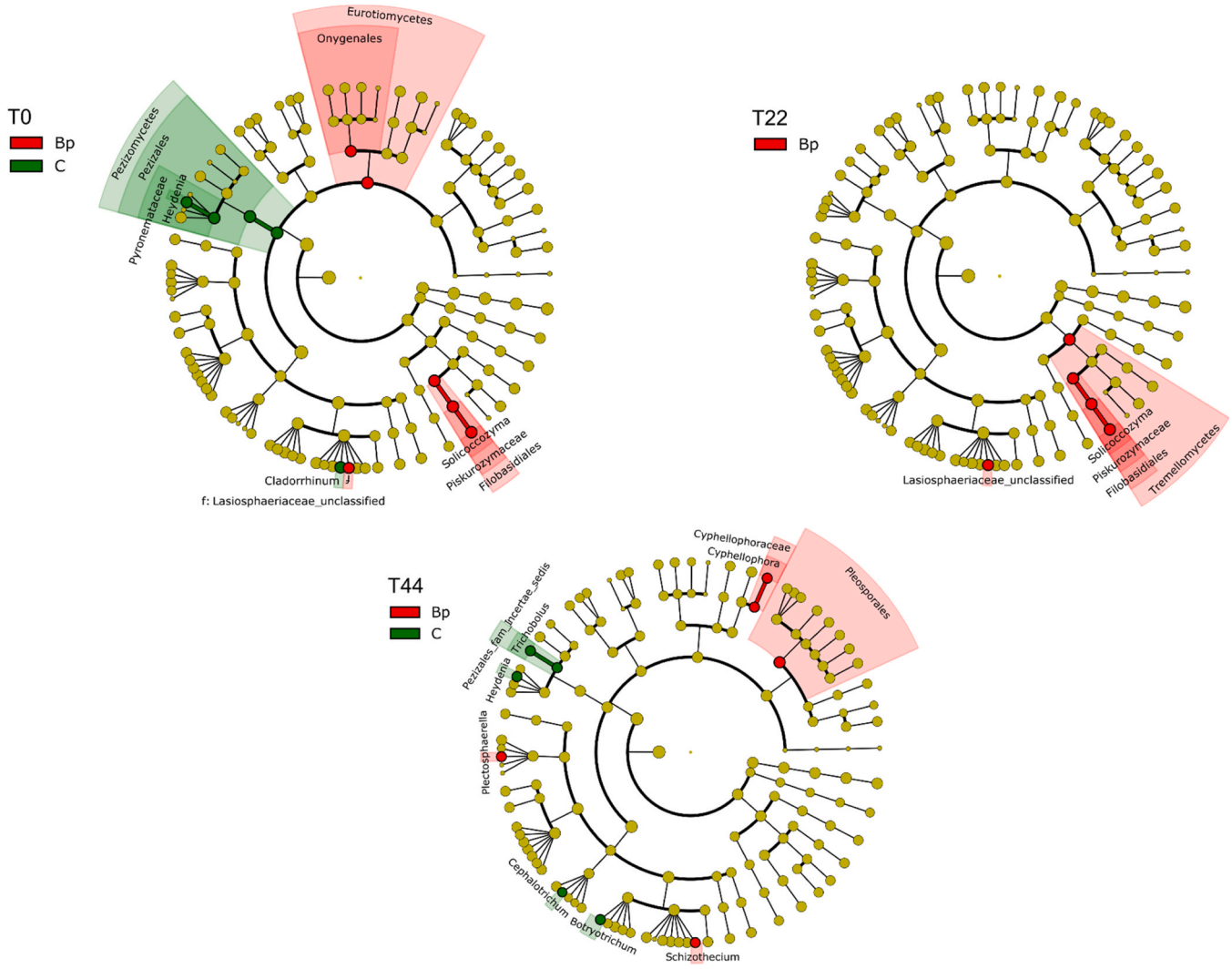

Fig. 4. Cladograms of fungal top 100 groups in the rhizospheres of *Bacillus paralicheniformis* 2R5-treated (Bp) and untreated (C) canola plants (n = 4). T0, T22, and T44 – time after bacterization in days.

et al., 2019). Whereas, "*Candidatus* Udaeobacter" was reported to exhibit multidrug resistance and have the ability to release antibiotics in the soil (Willms et al., 2020). Members of the family *Dongiaceae*, and *Vicinamibacterales* were found to be associated with the roots (Syranidou et al., 2018; Barreto and Alonso, 2021), while families of PLTA13 and *Methyloligellaceae* were previously found in soils, but not much is known about the function of these groups in this environment (Ceja-Navarro et al., 2010; Köberl et al., 2017; Rummel et al., 2020).

Regarding the fungal communities, *Solicoccozyma*, *Schizothecium*, *Cyphellophora*, *Fusicolla,* and *Humicola* were more abundant in the treated rhizosphere. It can have a positive effect on plants because *Solicoccozyma* has the ability to produce indole-3-acetic acid (IAA), which is the most common phytohormone regulating plant development (Nicola et al., 2021). Whereas, *Schizothecium* sp. is well known plant root-associated colonizer (Hugoni et al., 2018) which has antifungal activity towards a number of plant pathogens (Narisawa, 2018; Tymon et al., 2020). Moreover, *C. vermispora* is a saprophyte, isolated from decaying roots, stems, and leaves of plants (Gao et al., 2015), while *F. septimanifiniscientiae* and *H. grisea* are known as soil-inhabiting fungi (White and Downing, 1953; Crous et al., 2021). It should be also noted that the species *Plectosphaerella cucumerina* – a pathogen of several plant species such as radish (Miao et al., 2018), cucurbits, tomatoes, and bell pepper (Carlucci et al., 2012), was more abundant in samples treated with *B. paralicheniformis* 2R5. However, there is no information about *P. cucumerina* as a pathogen causing canola diseases.

Increase in relative abundance of above bacterial and fungal groups could indicate beneficial effect of *B. paralicheniformis* 2R5 treatment on canola plants. However, these increased groups of microorganisms should be further isolated to confirm their ability to promote canola growth as it is presented in Jin et al. (2023) study. The authors found that biochar amendments increased the relative abundance of potential PGPR, e.g. *Sphingomonas*, *Lysobacter* and *Pseudomonas* spp. in tomato rhizosphere. These representative microorganisms were further isolated and characterized to validate their function. A pot experiment showed that specially culturable bacteria i.e. *Sphingomonas* sp. S21, *Lysobacter* sp. L08 and *Pseudomonas* sp. P13 promoted tomato growth and mitigated Cd toxicity (Jin et al., 2023).

5. Conclusion

Bacillus paralicheniformis 2R5 seems to be a promising alternative to chemical pesticides and can be considered for practical application in the field. Its potential ability to alter the rhizosphere microbiome by increasing the diversity and composition of bacterial communities and increasing plants' beneficial groups of fungi appears to be important in terms of improving canola development. However, further studies on these increased microbial taxa are necessary to confirm their function in promoting canola growth.

CRediT authorship contribution statement

JŚ wrote the original draft, made greenhouse experiment and sampling, made isolation of DNA, conducted statistical analyses, and prepared the manuscript editorially. AK made rarefaction curves, LEfSe PCoA, and ANOSIM analysis, coordinated the study, and checked the validity of the original draft. AS carried out the sequence analysis. MS designed and coordinated the study and checked the validity of the original draft.

Declaration of Competing Interest

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

Data Availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2023.127448.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2020. UNITE QIIME release for Fungi [Data set]. UNITE Community.

Barreto, R., Alonso, J., 2021. Elucidation of the plant growth–promoting effect of *Hartmannibacter diazotrophicus* on tolerance of barley to salt stress.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., De Sousa, F., Amend, A., 2013. Improved software detection and extraction of ITS1 and ITS 2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4, 914–919.

Bhattacharyya, D., Duta, S., Yu, S.M., Jeong, S.C., Lee, Y.H., 2018. Taxonomic and functional changes of bacterial communities in the rhizosphere of kimchi cabbage after seed bacterization with *Proteus vulgaris* JBLS202. Plant Pathol. J. 34, 286.

Carlucci, A., Raimondo, M.L., Santos, J., Phillips, A.J.L., 2012. *Plectosphaerella* species associated with root and collar rots of horticultural crops in southern Italy. Pers: Mol. Phylogeny Evol. Fungi 28, 34–48.

Ceja-Navarro, J.A., Rivera-Orduna, F.N., Patino-Zúniga, L., Vila-Sanjurjo, A., Crossa, J., Govaerts, B., Dendooven, L., 2010. Phylogenetic and multivariate analyses to determine the effects of different tillage and residue management practices on soil bacterial communities. Appl. Environ. Microbiol. 76, 3685–3691.

Ciftci, C., Tekdal, D., Cetiner, S., 2021. The importance of plant growth–promoting rhizobacteria for plant productivity. Abiotic Stress Legumes 69–80.

Crous, P.W., Lombard, L., Sandoval-Denis, M., Seifert, K.A., Schroers, H.J., Chaverri, P., et al., 2021. Fusarium: more than a node or a foot-shaped basal cell. Stud. Mycol. 98, 100116.

Daims, H., Lücker, S., Wagner, M., 2016. A new perspective on microbes formerly known as nitrite-oxidizing bacteria. Trends Microbiol. 24, 699–712.

Gao, L., Ma, Y., Zhao, W., Wei, Z., Gleason, M.L., Chen, H., Hao, L., Sun, G., Zhang, R., 2015. Three new species of *Cyphellophora* (*Chaetothyriales*) associated with sooty blotch and flyspeck. PLoS One 10, e0136857.

Guo, Y.Z., Ding, X.M., Yao, L., Xu, D.M., Zhao, Y.J., Feng, F.Y., Meng, J.Y., 2019. Isolation and identification of *Massilia* sp. B260 and its effect on seedling raising. Biotechnol. Bull. 35, 144–149.

Hashmi, I., Paul, C., Al-Dourobi, A., Sandoz, F., Deschamps, P., Junier, T., Junier, P., Bindschedler, S., 2019. Comparison of the plant growth-promotion performance of a consortium of *Bacilli* inoculated as endospores or as vegetative cells. FEMS Microbiol. Ecol. 95, fiz147.

Hugoni, M., Luis, P., Guyonnet, J., Haichar, F.E.Z., 2018. Plant host habitat and root exudates shape fungal diversity. Mycorrhiza 28, 451–463.

Jang, S.W., Yoou, M.H., Hong, W.J., Kim, Y.J., Lee, E.J., Jung, K.H., 2020. Re-analysis of 16S amplicon sequencing data reveals soil microbial population shifts in rice fields under drought condition. Rice 13, 1–7.

Jeong, S., Moon, H.S., Shin, D., Nam, K., 2013. Survival of introduced phosphate-solubilizing bacteria (PSB) and their impact on microbial community structure during the phytoextraction of cd-contaminated soil. J. Hazard. Mater. 263, 441–449.

Jin, X., u Rahman, M.K., Ma, C., Zheng, X., Wu, F., Zhou, X., 2023. Silicon modification improves biochar's ability to mitigate cadmium toxicity in tomato by enhancing root colonization of plant-beneficial bacteria. Ecotoxicol. Environ. Saf. 249, 114407.

Khan, A.L., Waqas, M., Asaf, S., Kamran, M., Shahzad, R., Bilal, S., Khan, M.A., Kang, S., Kim, Y., Yun, B., Al-rawahi, A., Al-harrasi, A., Lee, I.J., 2017. Plant growth-promoting endophyte *Sphingomonas* sp. LK11 alleviates salinity stress in *Solanum pimpinellifolium*. Environ. Exp. Bot. 133, 58–69.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1.

Köberl, M., Dita, M., Martinuz, A., Staver, C., Berg, G., 2017. Members of *Gammaproteobacteria* as indicator species of healthy banana plants on *Fusarium* wilt-infested fields in Central America. Sci. Rep. 7, 1–9.

Kong, Z., Liu, H., 2022. Modification of rhizosphere microbial communities: a possible mechanism of plant growth promoting rhizobacteria enhancing plant growth and fitness. Front. Plant. Sci. 13.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112–5120.

Krishnamoorthy, R., Kim, K., Subramanian, P., Senthilkumar, M., Anandham, R., Sa, T., 2016. Arbuscular mycorrhizal fungi and associated bacteria isolated from salt-affected soil enhances the tolerance of maize to salinity in coastal reclamation soil. Agric. Ecosyst. Environ. 231, 233–239.

Kunin, V., Engelbrektson, A., Ochman, H., Hugenholtz, P., 2010. Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ. Microbiol. 12, 118–123.

Miao, Z., He, S., Zhang, X., Wen, Z., Bai, B., Kong, X., 2018. Pathogens of root internal discoloration (black heart disease) in radish in Xining City of Qinghai Province, China. Mycosystema 37, 444–455.

Michelsen, C.F., Watrous, J., Glaring, M.A., Kersten, R., Koyama, N., Dorrestein, P.C., Stougaard, P., 2015. Nonribosomal peptides, key biocontrol components for *Pseudomonas fluorescens* In5, isolated from a Greenlandic suppressive soil. MBio 6 e00079–15.

Milkereit, J., Geisseler, D., Lazicki, P., Settles, M.L., Durbin-Johnson, B.P., Hodson, A., 2021. Interactions between nitrogen availability, bacterial communities, and nematode indicators of soil food web function in response to organic amendments. Appl. Soil Ecol. 157, 103767.

Mishra, A.K., Sudalaimuthuasari, N., Hazzouri, K.M., Saeed, E.E., Shah, I., Amiri, K.M., 2022. Tapping into plant–microbiome interactions through the lens of multi-omics techniques. Cells 11, 3254.

Narisawa, K., 2018. The inhibitory role of dark septate endophytic fungus *Phialocephala fortinii* against *Fusarium* disease on the Asparagus officinalis growth in organic source conditions. Biol. Control 121, 159–167.

Nicola, L., Landinez-Torres, A.Y., Zambuto, F., Capelli, E., Tossi, S., 2021. The mycobiota of high altitude pear orchards soil in Colombia. Biology 10, 1002.

Nilsson, R.H., Veldre, V., Hartmann, M., Unterseher, M., Amend, A., Bergsten, J., Kristiansson, E., Ryberg, M., Jumpponen, A., Abarenkov, K., 2010. An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. Fungal Ecol. 3, 284–287.

Pacheco da Silva, M.L., Moen, F.S., Liles, M.R., Feng, Y., Sanz-Saez, A., 2022. The response to inoculation with PGPR plus orange peel amendment on Soybean is cultivar and environment dependent. Plants 11, 1138.

Patel, J.S., Kumar, G., Bajpai, R., Teli, B., Rashid, M., Sarma, B.K., 2021. PGPR formulations and application in the management of pulse crop health. In: Biofertilizers. Woodhead Publishing, pp. 239–251.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596.

Rudolph, N., Labuschagne, N., Aveling, T.A.S., 2015. The effect of plant growth promoting rhizobacteria on seed germination and seedling growth of maize. Seed Sci. Technol. 43, 507–518.

Rummel, P.S., Pfeiffer, B., Pausch, J., Well, R., Schneider, D., Dittert, K., 2020. Maize root and shoot litter quality controls short-term CO_2 and N2O emissions and bacterial community structure of arable soil. Biogeosciences 17, 1181–1198.

Santos, L.F., Olivares, F.L., 2021. Plant microbiome structure and benefits for sustainable agriculture. Curr. Plant Biol. 26, 100198.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, 1–18.

Syranidou, E., Thijs, S., Avramidou, M., Weyens, N., Venieri, D., Pintelon, I., Vangronsveld, J., Kalogerakis, N., 2018. Responses of the endophytic bacterial communities of *Juncus acutus* to pollution with metals, emerging organic pollutants and to bioaugmentation with indigenous strains. Front. Plant Sci. 9, 1526.

Tymon, L.S., Morgan, P., Gundersen, B., Inglis, D.A., 2020. Potential of endophytic fungi collected from Cucurbita pepo roots grown under three different agricultural mulches as antagonistic endophytes to *Verticillium dahliae* in western Washington. Microbiol. Res. 240, 126535.

Wang, R., Zhang, H., Sun, L., Qi, G., Chen, S., Zhao, X., 2017. Microbial community composition is related to soil biological and chemical properties and bacterial wilt outbreak. Sci. Rep. 7, 1–10.

Wang, T., Yang, K., Ma, Q., Jiang, X., Zhou, Y., Kong, D., Wang, Z., Parales, R.E., Li, L., Zhao, X., Ruan, Z., 2022. Rhizosphere microbial community diversity and function analysis of cut Chrysanthemum during continuous monocropping. Front. Microbiol. 13.

Wei, Z., Gu, Y., Friman, V.P., Kowalchuk, G.A., Xu, Y., Shen, Q., Jousset, A., 2019. Initial soil microbiome composition and functioning predetermine future plant health. Sci. Adv. 5, eaaw0759.

White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc.: Guide Methods Appl. 18, 315–322.

White, W.L., Downing, M.H., 1953. *Humicola grisea*, a soil-inhabiting, cellulolytic hyphomycete. Mycologia 45, 951–963.

Willms, I.M., Rudolph, A.Y., Göschel, I., Bolz, S.H., Schneider, D., Penone, C., Poehlein, A., Schöning, I., Nacke, H., 2020. Globally abundant "Candidatus Udaeobacter" benefits from release of antibiotics in soil and potentially performs trace gas scavenging. mSphere 5 e00186–20.

Zhao, X.L., Lu, S.J., He, S.L., Luo, L.L., Zhou, L.N., Zhou, Y.F., 2020. Bacterial diversity of tea rhizosphere soil based on high-throughput sequencing technology and the effect of soil beneficial microorganisms on the occurrence of tea anthracnose. Tea N. 194, 49–55.