The Composition Analysis of Endophytic Bacteria Community and Genetic Investigation of Major Strains in Jimai 44 Seeds

Fengning Yang[1*] and Jinpeng Wang[2]

[1*]College of Life Sciences, Shandong Agricultural University, Panhe Street, Taian, China
[2]College of Life Sciences, Shandong Agricultural University, Panhe Street, Taian, China

Abstract. In this research, the community composition and specific strains of endophytic bacteria in the seeds of *Triticum aestivum* L. cv. Jimai 44 were cultured in vitro and initially identified. Sequencing information revealed the main composition of the endophytic bacterial community. The taxonomic status of four target strains was analyzed in detail, and their functions in promoting wheat growth or resisting wheat diseases and pests were discussed and inferred. Additionally, in vitro culture experiments were conducted to verify the capabilities of the endophytic bacterial samples to metabolize major nutrient elements.

1 Introduction

Northern China, especially provinces such as Shandong and Henan, are major grain-producing regions, where the climatic and topographic conditions are ideally suitable for the extensive wheat cultivation. Wheat has long been regarded as one of the most important food crops in China. Ensuring grain yield serves as one of the eternal top priorities. Exploring effective and environmentally friendly cultivation methods has always been an significant issue worthy of great attention and in-depth exploration. The wheat Jimai 44 as semi-winter is a green and strong-gluten wheat variety which was bred through crossbreeding [1]. It is characterized by plump grains, strong tillering ability, ideal and stable yield, as well as certain disease resistance. It has been widely cultivated in northern China, whose planting area in Linyi City, Shandong Province alone exceeded 533 square kilometers in 2020 [2]. The research of endophytic bacteria in wheat seeds provides insights and strategies for effectively and safely increasing grain yield. Numerous studies have been conducted on the community analysis and functional identification of endophytic bacteria in wheat seeds. However, the research in this field focusing on Jimai 44 remains extremely scarce. Focusing on the endophytic bacteria in seeds of Jimai 44, this study systematically explored the endophytic bacterial community and identified specific strains, through preliminary culture, the Oxford Nanopore Technologies (ONT) sequencing technology, constructing phylogenetic trees, and in vitro experiments. The results indicated that the four target strains (named JM-flynny-A/B/C/D in the research) with the highest abundance in the four samples, are inferred to possess great potential as symbiotic beneficial bacteria for promoting wheat growth and controlling diseases and pests. These strains (or their microbial agents) may exhibit promising prospects in the field of agricultural biological control. Petri dish experiments verified that the endophytic bacteria isolated from 44 seeds possess significant nitrogen-fixing, phosphate-solubilizing and potassium-solubilizing metabolic functions.

2 Preliminary isolation (primary screening) of endophytic bacteria

Triticum aestivum L. cv. Jimai 44 seeds were used as test materials, for culturing their endophytic bacteria. Four groups of bacterial samples (I, II, III, IV) which could consist of multiple strains, were picked and obtained from the cultures, for subsequent in-depth investigations.

2.1 Experimental materials

Equipment: Luria-Bertani (LB) bacterial medium (produced by BAIWEI Biotechnology Co., Ltd.), organophosphorus agar medium (with egg yolk lecithin as the phosphorus source, produced by BAIWEI Biotechnology Co., Ltd.), inorganic phosphorus agar medium (with calcium phosphate as the phosphorus source, produced by BAIWEI Biotechnology Co., Ltd.), potassium solubilizing agar medium (produced by Shandong Tuopu Biol-engineering Co., Ltd.), nitrogen-fixing medium (produced by Shandong Tuopu Biol-engineering Co., Ltd.), 9 mm Petri dishes, the autoclave, spreaders, inoculating loops, pipettors, 10 mL culture tubes, etc.

Reagents: 5% sodium hypochlorite solution, 75% ethanol solution, sterile normal saline, etc.

2.2 Experimental procedure

Forty-five normal seeds of Jimai 44 were randomly selected and divided into three parallel groups. Parallel groups were sequentially rinsed three times with sterile water and then treated with 5% sodium hypochlorite solution for 3 minutes and with 75% ethanol solution for 1 minutes. It is finally followed by thorough rinsing with sterile water until no odor remained (> 3 times). After being blotted dry with filter papers, the seeds were set aside for later use.

A total of 1.5 mL sterile normal saline was added to each sterilized mortar. The seeds of three parallel groups were separately placed into mortars and ground into small particles. Subsequently, the ground suspensions were transferred into the 10mL test tubes, adding sterile normal saline to the 8mL mark . After vortexing, the tubes were incubated until large particulate matter settled at the bottom.

A 100 μL aliquot of each suspension was pipetted onto the surface of pre-prepared Luria-Bertani (LB) solid medium and evenly spread with a spreader. Each parallel group had three technical replicates for expansion culture. The nine Petri dishes in total were inverted and incubated at a constant temperature of 28.0 °C in an incubator for 36 hours.

2.3 Sampling

Through the observation of general morphology, color, and texture of the bacterial colonies, four widely distributed and representative colony units (which likely contain multiple overlapping or mixed-growing bacterial species) were selected and named sample I/II/III/IV. Each sample was picked and dissolved in a culture tube containing 10 mL of sterile normal saline, ready for subsequent sequencing analysis.

3 Nucleic acid sequencing and bacterial community analyses

The assembled 16S rRNA gene sequences of four samples were sequenced. The sequencing data were analyzed to determine the composition of the bacterial community. Additionally, diversity levels and the diversity density were evaluated through data analyses.

3.1 Sequencing workflow (technically supported by Youkang Biotechnology Co., Ltd.)

Genomic DNA was extracted from each of the four bacterial samples, followed by amplification of the full-length 16S rRNA gene sequences. Long-read assembly sequencing was performed using the Oxford Nanopore Technology (ONT) platform. After quality control of the raw data, reads around 1500 bp in length were extracted. The results showed that 100% of the clean reads of each sample had a quality higher than Q15, thereby obtaining the sets of assembled 16S rRNA gene sequences within the samples.

3.2 The analysis of endophytic bacterial community composition

Species classification of the four assembled sequences was performed using the program Kraken 2, to generate pie charts of species abundance distribution at the genus level (figure 1, 2, 3, 4). It can be observed that the main endophytic bacterial community composition of Jimai 44 seeds in the tested samples includes *Bacillus*, *Paenibacillus*, *Sphingobacterium* and *Chryseobacterium*. Consistent with previous studies, spore-forming bacilli remain predominant among endophytic bacteria, which is generally considered to be closely associated with the ability of bacterial spores to resist environmental stresses such as high temperature and desiccation. Additionally, spore-forming bacilli often exhibit excellent capability of nutrient metabolism and resisting disease, and also play a crucial plant-growth-promoting role as symbiotic beneficial bacteria by regulating the active content of the plant hormone.

In the following charts of species distribution at the genus level, "Other" (in heavy blue) refers to the collection of genera (or one genus) with a proportion less than 5%, which is presumably composed of a minimal amount of pathogen bacteria in the seed endophytic community, trace contaminating bacteria introduced during the experiment, or the other endophytic bacteria with extremely low abundances. The "Unclassified" fraction refers to the set of sequences (strains) that failed to be successfully assigned to a taxonomic category within the assembled 16S rRNA gene sequence dataset generated by sequencing. Based on the reasonable inference, the "Unclassified" fraction (in pink) may includes unassigned bacterial strains, unstudied novel strains with low abundance and high reproductive capacity, a small number of fungal or actinomycetes which could be cultured theoretically, as well as errors likely occurring during experimental processes such as the sequencing and algorithmic analysis. Additionally, the relative abundance ratios may be influenced by factors such as the growth capacity of bacteria in the culture environment. Thus, the ratios are not considered a direct basis for judging the compositional structure of the endophytic bacterial community.

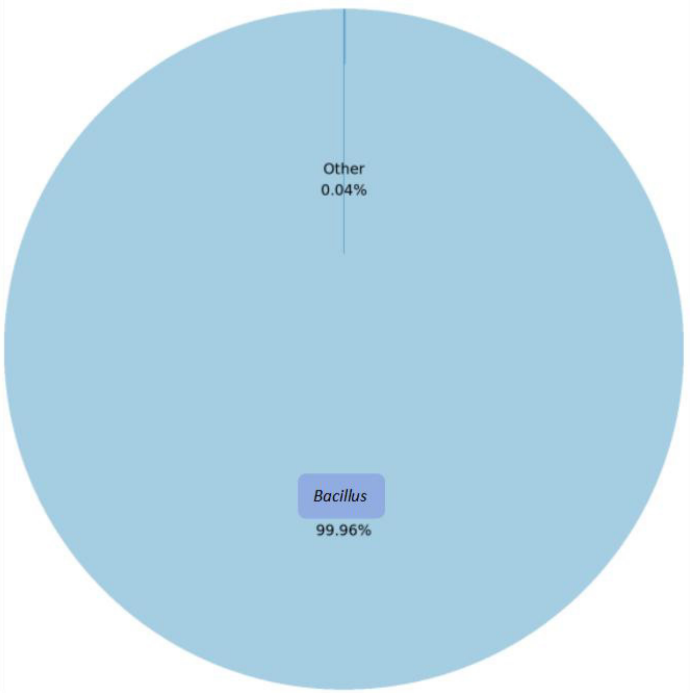


Fig. 1. The genus-level distribution in sample I. *Bacillus* (in light blue) accounts for the absolute majority of sample I.

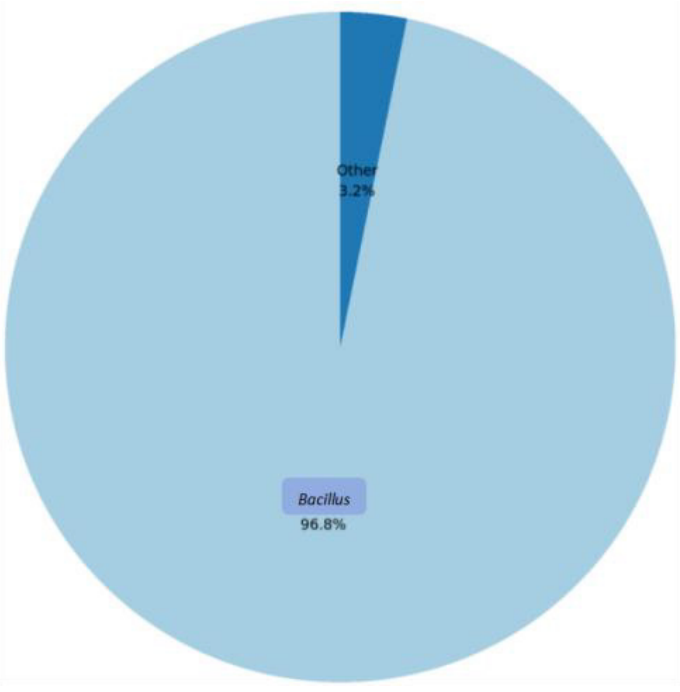


Fig. 2. The genus-level distribution in sample II. *Bacillus* (in light blue) accounts for the absolute majority of sample II.

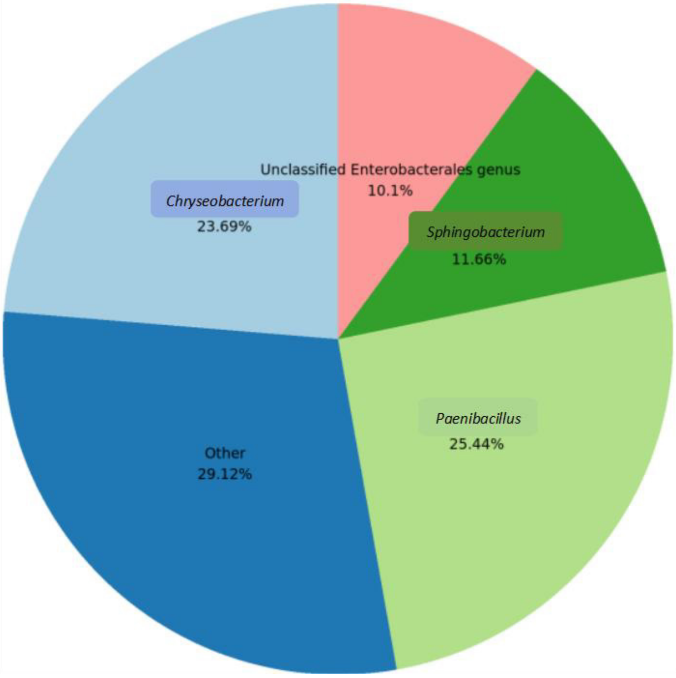


Fig. 3. The genus-level distribution in sample III. *Paenibacillus (in light green)*, *Sphingobacterium* (in heavy green) and *Chryseobacterium* (in light blue) respectively account for 25.44%, 11.66% and 23.69% of sample III.

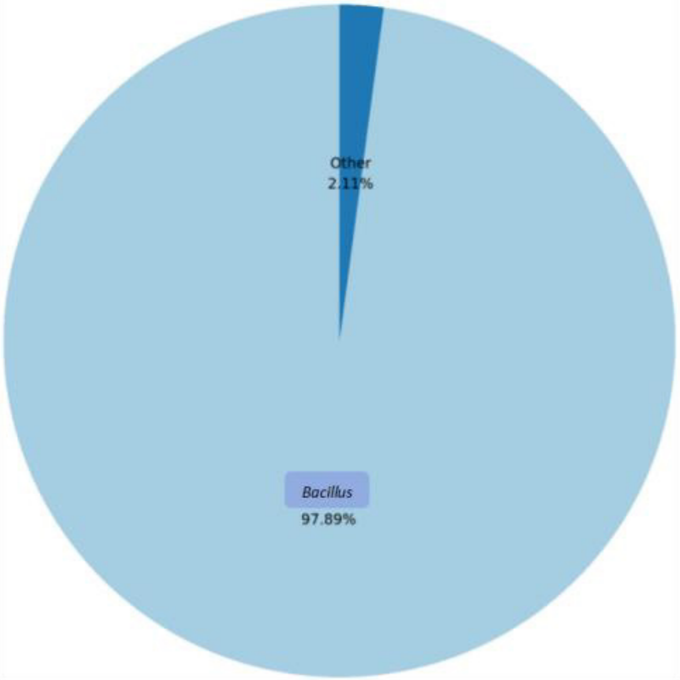


Fig. 4. The genus-level distribution in sample IV. *Bacillus* (in light blue) accounts for the absolute majority of sample IV.

According to the pie charts, sample I, II, and IV can be almost considered to contain bacteria belonging to only one single genus. However, there exists a worth-exploring question which is whether the bacterial diversity in these three samples is mainly at the level of strain diversity or exhibits a certain degree of species diversity. Additionally, the diversity density also serves as an interesting issue. The level of difference in the full-length assembled sequences of bacterial 16S rRNA gene are generally recognized to range from 1% to 5%, with a commonly used threshold of 3%. In further studies, four most abundant 16S rRNA gene assembled sequences of four samples were obtained and aligned with the sequencing datasets of the corresponding samples to yield the proportion of the total length of inconsistent bases (*I*-value) and the average proportion of polymorphic sites (*P*-value).

I = [(the sum of single nucleotide polymorphism (SNP) sites + the sum of insertion-deletion (InDel) polymorphism sites)/ the length of assembled gene sequence] * 100% (1)

P = [(the sum of the proportions of SNP sites + the sum of the proportions of InDel polymorphism sites)/ the total number of polymorphic sites] * 100% (2)

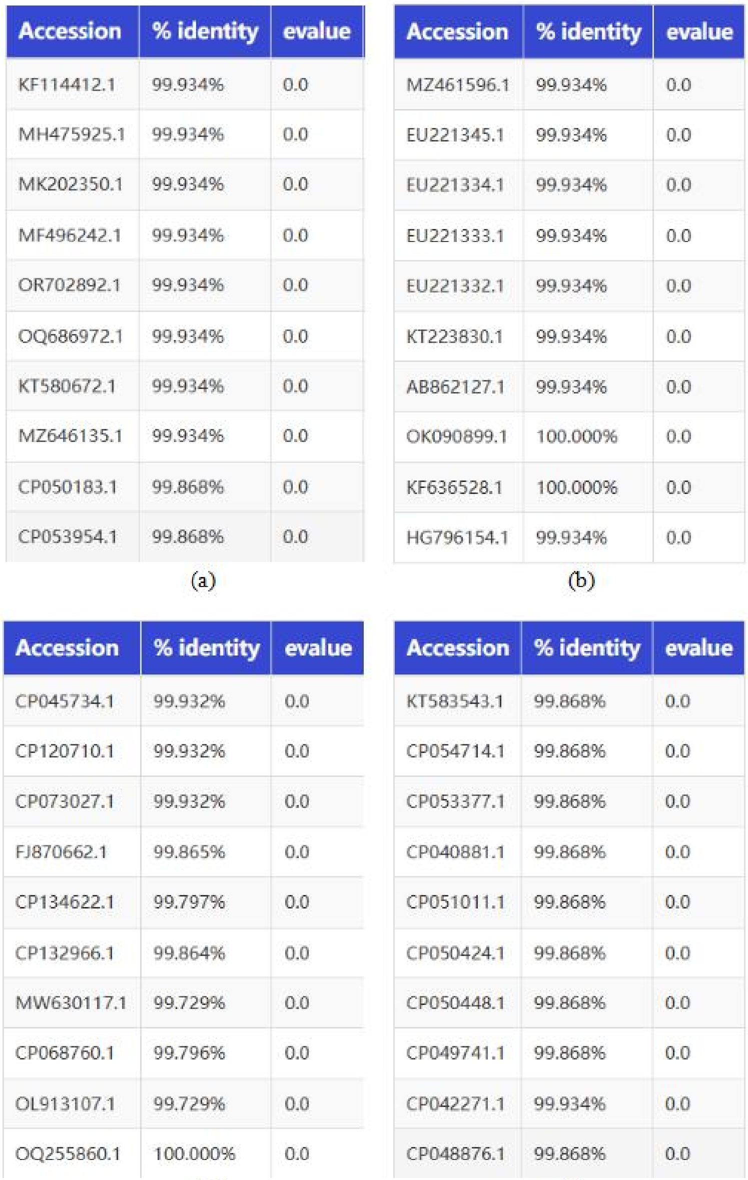
As can be understood from the formula above, *I*-value refers to the percentage of mismatched bases relative to the total length of the assembled gene sequence, reflecting the degree of diversity or "longitudinal depth" on an overall scale. B denotes the average variation density of polymorphic sites, representing the "lateral breadth" of the diversity. The *I*-valueThe *I*-value of sample IV was 0.93%, which indicates the presence of intraspecific variation to a large extent. The *P*-value was 19.53%, which suggests that intraspecific variation is with considerable intensity. The *I*-value of sample I was 6.74% and its *P*-value was 15.05%, which illustrates a certain level of species diversity in theory. The *I*-value of sample II was 1.85% and its *P*-value was 11.09%, which can imply that there exists a certain level of intraspecific diversity.

4 Analyses of taxonomic status and functions of the four strains with the highest abundance

The assembled highest-abundance 16S rRNA gene sequences (corresponding to the strains named JM-flynny-A/B/C/D, with the highest abundance in four samples) from samples (I, II, III, IV), were employed for comprehensive taxonomic status analyses of the corresponding four strains.

4.1 Screening and genetic information alignment of the four strains with the highest abundance

These four assembled sequences obtained were aligned against the database of NT(Non - redundant Nucleotide) Database, using the BLAST program. And four lists of bacterial strains with the highest sequence similarity were obtained (figure 5). According to records of the microbial taxonomy in the NCBI Taxonomy database, the TOP10 strains with the highest similarity to four highest-abundance target strains do not all belong to the same species.



Accession	% identity	evalue
KF114412.1	99.934%	0.0
MH475925.1	99.934%	0.0
MK202350.1	99.934%	0.0
MF496242.1	99.934%	0.0
OR702892.1	99.934%	0.0
OQ686972.1	99.934%	0.0
KT580672.1	99.934%	0.0
MZ646135.1	99.934%	0.0
CP050183.1	99.868%	0.0
CP053954.1	99.868%	0.0

(a)

Accession	% identity	evalue
MZ461596.1	99.934%	0.0
EU221345.1	99.934%	0.0
EU221334.1	99.934%	0.0
EU221333.1	99.934%	0.0
EU221332.1	99.934%	0.0
KT223830.1	99.934%	0.0
AB862127.1	99.934%	0.0
OK090899.1	100.000%	0.0
KF636528.1	100.000%	0.0
HG796154.1	99.934%	0.0

(b)

Accession	% identity	evalue
CP045734.1	99.932%	0.0
CP120710.1	99.932%	0.0
CP073027.1	99.932%	0.0
FJ870662.1	99.865%	0.0
CP134622.1	99.797%	0.0
CP132966.1	99.864%	0.0
MW630117.1	99.729%	0.0
CP068760.1	99.796%	0.0
OL913107.1	99.729%	0.0
OQ255860.1	100.000%	0.0

(c)

Accession	% identity	evalue
KT583543.1	99.868%	0.0
CP054714.1	99.868%	0.0
CP053377.1	99.868%	0.0
CP040881.1	99.868%	0.0
CP051011.1	99.868%	0.0
CP050424.1	99.868%	0.0
CP050448.1	99.868%	0.0
CP049741.1	99.868%	0.0
CP042271.1	99.934%	0.0
CP048876.1	99.868%	0.0

(d)

Fig. 5. It should be noted that due to the combined effects of two factors—differences in the full-length of the queried sequences and the degree of base matching, the identity values are not considered to have absolute validity for judging the genetic relatedness of strains. In principle, the “evalue” of all alignments was 0.0, which indicates that the acceptability of each known strain should be regarded as rank-free. (a). The TOP10 known bacterial species (with the high identity to the most abundant assembled sequence) in sample I. Six of them belong to the species *Bacillus cereus*. The *Bacillus thuringiensis* strain HER1410 also exhibits a 99.868% sequence identity to the target strain JM-flynny-A. *Bacillus*

thuringiensis is generally regarded as an excellent symbiotic beneficial bacterium. (b). The TOP10 known bacterial species in sample II. Nine of them belong to the species *Bacillus subtilis*. (c). The TOP10 known bacterial species in sample III. Six of them belong to the species *Chryseobacterium cucumeris*. (d). The TOP10 known bacterial species in sample IV. Seven of them belong to the species *Bacillus velezensis*. It should be noted that among the TOP10 strains with the high identity in sample I, two accessions refer to the same strain and their full-length 16S rRNA gene sequences are completely identical. This data was redundantly submitted to the NCBI database. Therefore, MH475925.1 was retained while MF496242.1 was excluded during the construction of the phylogenetic tree in the following text.

Existing studies have demonstrated that various *Bacillus cereus* strains exhibit plant growth-promoting and disease-resistant effects on wheat. Among them, *Bacillus cereus* strains SB127 and RC79 have been shown to enhance auxin secretion and the growth of roots, stems and leaves in wheat. In addition, they also possess the capacity of metabolism of phosphorus, to dissolve low-solubility phosphorus-containing compounds (organic or inorganic) [3]. *Bacillus cereus* strain JK14 has been demonstrated to exert a significant inhibitory effect on the pathogenic fungus capable of causing wheat take-all disease. It functions in disrupting the cell wall of the pathogen and leading to hyphal swelling and fragmentation. Pot trials verified that its control efficiency against wheat take-all disease on two wheat cultivars was remarkably superior to that of chemical fungicides [4]. The rhizosphere-colonizing *Bacillus cereus* strain B3-7 has also been demonstrated to possess the ability to control wheat sharp eyespot [5]. *Bacillus cereus* strain 0-9 exerts an inhibitory effect on the spore germination of *Fusarium oxysporum* which serves as the causal agent of wheat root rot. And its antifungal activity against various pathogenic fungi has been verified through the plate assay [6]. *Bacillus subtilis* also serves as a highly effective and promising plant growth-promoting rhizobacterium (PGPR), and has an effect on wheat disease management. Studies have shown that the *Bacillus subtilis* strain 8-32 inoculant exhibits significant effects in increasing the contents of available nutrient element and the accumulation of organic matter in plants. It also functions in the control of wheat scab [7]. Field cultivation experiments have demonstrated that the *Bacillus subtilis* inoculant functions in promoting growth, such as increasing the tiller number and leaf area index (LAI) in the winter wheat cultivar Xinong 979 [8]. Relevant studies have revealed that a strain of *Chryseobacterium cucumeris* (designated as strain C5 in related literature) is capable of producing the plant hormone indole-3-acetic acid (IAA) [9]. Seed soaking experiments with *Bacillus velezensis* strain YB-1465 have shown that the crown rot caused by *Fusarium pseudograminearum was* effectively inhibited. And the strain YB-1465 significantly promotes the development of stems and roots [10].

Notably, although the existing enormous research results have confirmed the roles of these bacterial species above-mentioned in growth promotion and disease resistance, the potential of four target strains to exert the aforementioned multiple beneficial effects can only be inferred based on their 16S rRNA gene sequences.

4.2 Phylogenetic tree analyses of the four major bacterial strains

To further explore and analyze the phylogenetic relationships between each target strain and a series of corresponding known strains with the high identity, the phylogenetic tree was constructed and analyzed in this study. The full-length 16S rRNA gene sequences (or whole-genome sequences) of the known strains were retrieved from the NCBI database. And the phylogenetic tree were constructed using the MEGA 12 software. In addition, for certain strains with only whole-genome information recorded in the database, the RNAmmer program was used to accurately predict their full-length rRNA gene sequences. The 16S rRNA gene sequences were subsequently extracted. The hyper-variable regions and conserved regions among the full-length 16S rRNA gene sequences were selected and imported into the MEGA 12 for subsequent analyses.

4.2.1 Analysis methods and workflow

The ClustalW analysis was performed by importing the assembled full-length 16S rRNA gene sequence of each target strain with those of the corresponding series of known strains with the high identity, which is followed by extraction and alignment of the hyper-variable regions and conserved regions. Subsequently, phylogenetic analyses was conducted. And the phylogenetic tree was constructed using the neighbor-joining (NJ) method. Five parallel phylogenetic tree constructions were performed for each target strain. The most representative phylogenetic tree corresponding to each target strain was selected after comprehensive evaluation.

4.2.2 The analysis of the phylogenetic tree

Four bootstrap consensus trees regarding four target strains are shown beneath (figure 6, 7, 8, 9). Phylogenetic trees (bootstrap consensus trees) of the groups consisting of a target strain and its corresponding TOP10 strains with the high identity. They are representative and typical trees selected from the results of five parallel constructions. Each bootstrap consensus tree above is derived through the further analyses and optimization of the corresponding original tree. It distills and integrates the consistent topological structures of multiple trees. Thereby, bootstrap consensus trees has significantly reducing random errors, while retaining and labeling node parameters with a support value of no less than 50%.

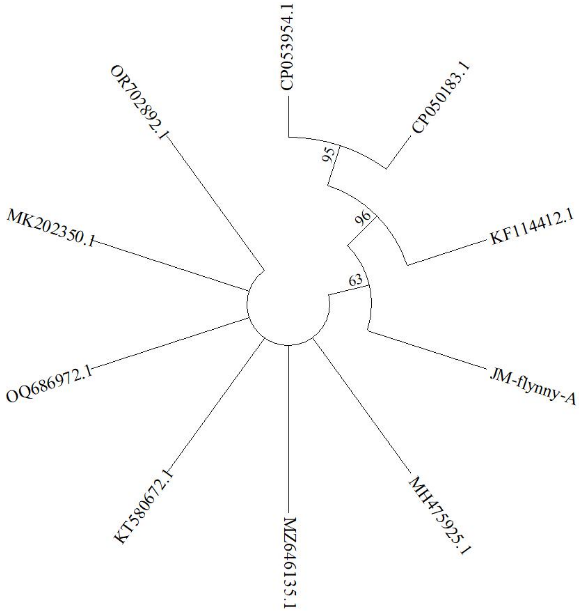


Fig. 6. *The consensus tree of strain JM-flynny-A, constructed with 3999 bootstrap replicates.*

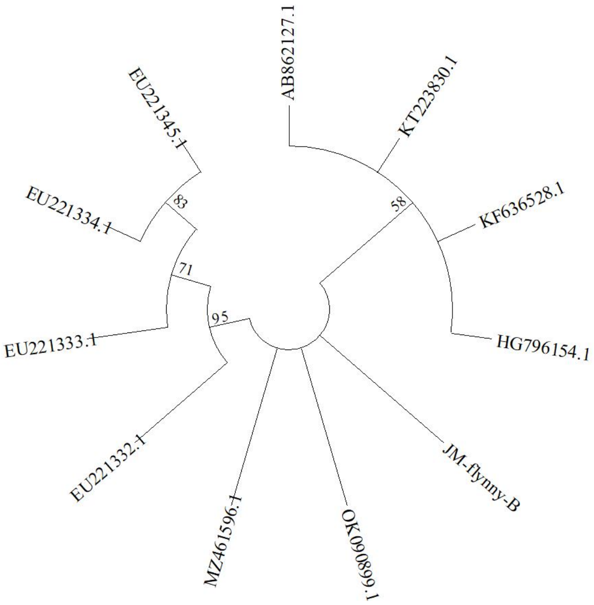


Fig. 7. *The consensus tree of JM-flynny-B, constructed with 9999 replicates (distinct differences were observed among the five repetitions while setting 3999 replicates).*

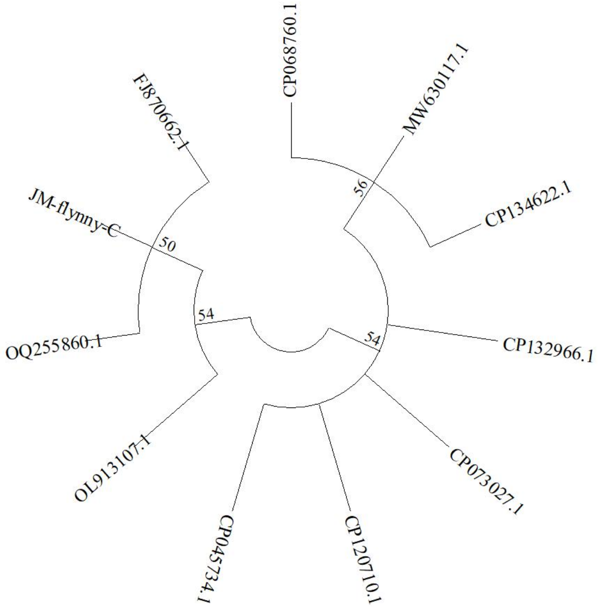


Fig. 8. *The consensus tree of JM-flynny-C, constructed with 3999 replicates.*

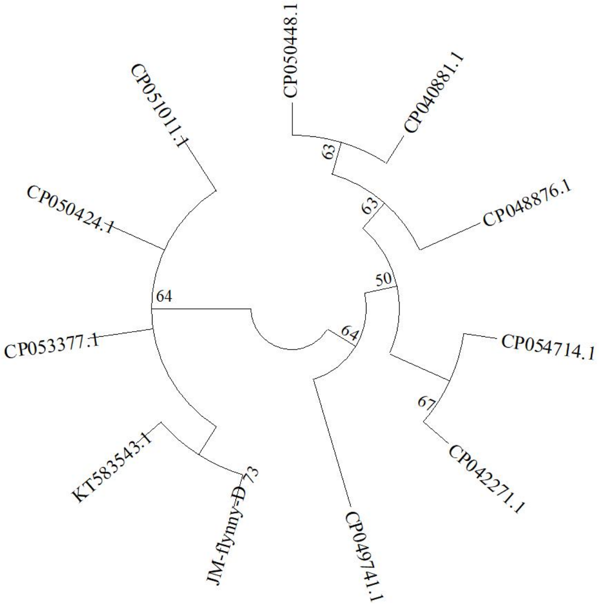


Fig. 9. *The consensus tree of JM-flynny-D, constructed with 3999 replicates.*

The phylogenetic tree showed that the JM-flynny-A exhibits close phylogenetic relationship with KF114412.1 (*bacterium A15(2013)*), CP050183.1 (*Bacillus thuringiensis)* and CP053954.1 (*Bacillus cereus*) compared to other known strains. At this point, it is necessary to supplement existing research findings regarding the plant growth-promoting or disease-suppressive functions of *Bacillus thuringiensis.* Existing studies have shown that multiple strains of *Bacillus thuringiensis* can produce effective 1-aminocyclopropane-1-carboxylate (ACC) deaminase and exhibit the capacity of solubilizing mineral phosphate. More than ten strains possess comprehensive plant growth-promoting (PGP) capabilities [11]. The genomic analysis and functional gene mining of *Bacillus thuringiensis* strain BT1012 revealed that it contains multiple insecticidal genes encoding dozens of virulence factors, as well as several key gene clusters associated with plant growth promotion (including siderophore production) [12].

The JM-flynny-C shares the closest phylogenetic relationship with strains FJ870662.1 (*Chryseobacterium sp. pp2f*) and OQ255860.1 (*Chryseobacterium cucumeris*), which is indicated by the consensus tree. However, the original tree suggests a probable closer phylogenetic relationship between it and FJ870662.1.

The JM-flynny-D exhibits the closest phylogenetic relationship with KT583543.1 (*Bacillus sp. C-3-19*, rather than *Bacillus velezensis*).

The phylogenetic data analysis failed to further identify the strains with which the JM-flynny-B shares a closer phylogenetic relationship.

5 In vitro validation of the metabolic functions of three primary nutrient elements

In this study, in vitro culture experiments were also extensively performed to evaluate the metabolic roles of nutrient elements, for four bacterial samples. Experiments include biochemical assays for nitrogen fixation, phosphate solubilization (targeting both organic and inorganic phosphorus) and potassium solubilization, using specific solid media. The results demonstrated that all four bacterial samples exhibited varying degrees of metabolic capacity of utilizing important element in forms with low solubility. Whereas, no bacterial growth was observed in the control group.

Notably, the bacterial growth of four samples on different biochemical media is highly likely to be selective, suggesting that only a subset of the strains possesses the corresponding metabolic functions. Combined with the sequencing results of the four most abundant strains JM-flynny-A/B/C/D and the existing experimental conclusions of TOP10 known species in each group, it can be inferred that these four target strains play significant roles and exhibit considerable abundance, among the cultures grown on these selective media. Further purification and validation are still required, including in vitro culture verification and auxiliary validation via whole-genome sequencing and functional prediction.

6 Conclusion

This study used Jimai 44 seeds as materials, to analyze the general community composition of their endophytic bacteria. Taxonomic status analysis and functional prediction were performed in detail regarding four selected typical target strains (JM-flynny-A/B/C/D). During the process, the ONT platform was employed for sequencing the full-length assembled sequences of 16S rRNA genes. And the MEGA 12 program was used to construct phylogenetic trees. This study also verified the metabolic functions of major nutrient elements regarding four endophytic bacterial samples through in vitro biochemical experiments, which is followed by a series of reasonable analyses and inferences. Notably, the colonization process of endophytic bacteria in seeds involves two pathways: horizontal transmission and vertical inheritance [13]. The compositions of the endophytic bacterial communities of seeds from different environmental conditions exhibit diversity to a certain extent. Therefore, the exploration of endophytic bacteria in wheat seeds still holds great potential and significant importance nowadays.

References

1. Xin, L. (2025). Varietal characteristics and supporting cultivation key points of Jimai 44. *Agricultural Engineering Technology*, 45(04), 92-93. https://doi.org/10.16815/j.cnki.11-5436/s.2025.04.038.
2. Wang, L., Li, Q., Lin, W., Zhang, B., & Yu, X. (2022). Growth performance and high-yield & high-quality cultivation techniques of strong-gluten wheat Jimai 44 in Linyi. *China Agricultural Technology Extension*, 38(06), 40-42.
3. Pang, F., Du, R., Wang, T., & Huang, S. (2016). Screening of plant growth-promoting endophytic bacteria strains in wheat and correlation analysis of factors affecting wheat growth. *Journal of China Agricultural University*, 21(01), 8-21.
4. Han, Y., Hu, B., & Wang, G. (2008). Control of wheat take-all disease by *Bacillus cereus* strain JK14 and its disease-suppressive mechanism. *Agricultural Science & Technology*, (01), 70-74. https://doi.org/10.16175/j.cnki.1009-4229.2008.01.007.
5. Huang, Q., Zhang, Y., Liu, F., Wang, M., & Wang, G. (2014). Colonization dynamics of *Bacillus cereus* strain B3-7 in roots of field-grown wheat and its control efficacy against wheat sharp eyespot. *Acta Ecologica Sinica*, 34(10), 2559-2566.
6. Huang, Q., Han, X., & Liu, F. (2020). Antibacterial activity of *Bacillus cereus* strain 0-9 against wheat root rot pathogens. *Journal of Anhui Agricultural Sciences*, 48(12), 135-138.
7. Yang, Q., Li, X., Peng, Z., Li, X., Ge, S., Gao, Z., ... & Li, Y. (2025). Effects of *Bacillus subtilis* strain 8-32 application rate on winter wheat growth and development, yield and quality, and soil chemical properties. *Jiangsu Agricultural Sciences*, 53(14), 78-85. https://doi.org/10.15889/j.issn.1002-1302.2025.14.010.
8. Yang, L., Zhou, B., Hou, Y., Wang, Q., & Chem, X. (2021). Effects of *Bacillus subtilis* inoculant on winter wheat growth and soil water and nitrogen distribution under salt stress. *Journal of Drainage and Irrigation Machinery Engineering*, 39(05), 517-524.
9. Cao, K. (2021). Study on the efficacy of high siderophore-producing rhizosphere plant growth-promoting bacteria in controlling tomato bacterial wilt (Master's thesis, Nanjing Agricultural University). https://doi.org/10.27244/d.cnki.gnjnu.2021.002181.
10. Xu, W., Xie, X., Li, P., Dong, Q., Sun, R., Zhang, J., ... & Yang, L. (2025). Analysis of biocontrol characteristics of *Bacillus velezensis* strain YB-1465 and its biocontrol effect against wheat crown rot. *Chinese Journal of Biological Control*, 41(04), 877-886. https://doi.org/10.16409/j.cnki.2095-039x.2025.02.041.
11. Raddadi Noura, Cherif Ameur, Boudabous Abdellatif, & Daffonchio Daniele. (2008). Screening of plant growth promoting traits ofBacillus thuringiensis. *Annals of Microbiology*, 58(1), 47-52. http://dx.chinadoi.cn/10.1007/BF03179444.
12. Cao, B., Wang, K., Sun, X., Shu, C., & Zhang, J. (2025). Genomic functional analysis of the first highly effective Bacillus thuringiensis strain targeting hemipteran pests reveals its plant growth-promoting potential. *Pest management science*, *81*(7), 4085–4096. https://doi.org/10.1002/ps.8774
13. Soluch, R., Hülter, N. F., Romero Picazo, D., Özkurt, E., Stukenbrock, E. H., & Dagan, T. (2021). Colonization dynamics of Pantoea agglomerans in the wheat root habitat. *Environmental microbiology*, *23*(4), 2260–2273. https://doi.org/10.1111/1462-2920.15430

14. Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, *4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
15. Felsenstein J. (1985). CONFIDENCE LIMITS ON PHYLOGENIES: AN APPROACH USING THE BOOTSTRAP. *Evolution; international journal of organic evolution*, *39*(4), 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x
16. Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(30), 11030–11035. https://doi.org/10.1073/pnas.0404206101
17. Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: Molecular Evolutionary Genetic Analysis Version 12 for Adaptive and Green Computing. *Molecular biology and evolution*, *41*(12), msae263. https://doi.org/10.1093/molbev/msae263