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Genomic characteristics of a potentially novel phosphate-solubilizing Phyllobacterium sp. YNK-FB0058 with plant growth-promoting functions

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Abstract

Background

Phosphate-solubilizing bacteria are soil-dwelling probiotics that promote plant growth and convert unavailable phosphorus into absorbable forms for plants. A potentially novel *Phyllobacterium* strain YNK-FB0058 isolated from the rhizosphere of safflower in tobacco-safflower rotation fields was used as the research material in this study. Whole-genome sequencing and in vitro verification tests were conducted to explore its genetic basis and functional traits.

Results

Phylogenetic analysis based on 16S rRNA and 31 housekeeping genes, combined with genomic comparisons of ANI and dDDH, confirmed that strain YNK-FB0058 represents a potential novel species of the *Phyllobacterium*. Its complete genome size is 4,694,147 bp with a GC content of 61.14%, and a total of 4,459 CDSs were annotated. The genome harbors gene clusters involved in inorganic phosphorus solubilization, organic phosphorus mineralization, phosphorus transport and regulation. It also carries growth-promoting genes related to IAA synthesis, nitrogen fixation, siderophore production and zinc solubilization. The strain exhibited high efficiency in solubilizing organic and inorganic phosphorus, with solubilization capacities of 566.3 mg/L and 474.46 mg/L respectively, and secreted IAA at 19.319 μg/mL. It also showed activities of nitrogen fixation, zinc solubilization and siderophore synthesis. Inoculation with this strain significantly increased plant height, stem diameter, leaf area and biomass of flue-cured tobacco, elevated nitrogen and phosphorus contents and enzyme activities in rhizosphere soil, and promoted phosphorus accumulation in tobacco plants.

Conclusions

YNK-FB0058 is a potentially novel species of the genus *Phyllobacterium*, with highly efficient phosphate-solubilizing capability and multiple plant growth-promoting traits. This strain provides a high-quality microbial resource and theoretical basis for reducing chemical phosphorus fertilizer application and developing high-efficiency microbial inoculants in green agriculture.

1. Background

Phosphorus (P) is one of the indispensable macronutrients for plant growth and development. It participates in a series of key biological processes including DNA synthesis, cell membrane formation, energy metabolism, and regulation of enzyme activity, which directly affect crop growth, yield, and quality formation[1, 2]. In agricultural production, application of phosphate fertilizer is an effective measure to improve crop yield and quality to meet the demand for higher productivity and product quality. However, due to the strong fixation and poor mobility of phosphorus in soil, long-term application of phosphate

fertilizers easily leads to the formation of insoluble phosphates, which are rapidly adsorbed and fixed by soil minerals or immobilized by microorganisms. As a result, the utilization efficiency of phosphate fertilizers by crops is very low, causing large amounts of phosphorus to accumulate in soil or enter water bodies. This increases the risk of phosphorus loss and water eutrophication, which is inconsistent with the goals of green and sustainable agricultural development[3–5].

Phosphate-solubilizing microorganisms (PSMs) convert phosphorus forms that are unavailable for plant uptake into available forms. PSMs not only mobilize immobilized phosphorus in soil but also regulate root exudates, promote the uptake of nutrients including K, Ca, Mg, Fe, and Zn, and enhance plant adaptability to adverse environments[6, 7]. To date, reported PSMs cover more than 20 genera of bacteria, fungi,and actinomycetes, and are widely distributed in diverse ecosystems[8–10]. PSMs primarily transform insoluble soil phosphorus into plant-available forms via acidolysis[11–13] and enzymatic hydrolysis[14]. Inoculation with PSMs represents an eco-friendly approach to increase available phosphorus in soil and reduce reliance on chemical phosphorus fertilizers, playing a critical role in the soil phosphorus cycle[15]. Therefore, inoculating soils with PSMs can not only reduce fertilizer dependence and improve phosphorus use efficiency but also simultaneously increase crop yield and strengthen plant tolerance to various stresses. Clearly, PSMs are of great importance in regulating the soil phosphorus cycle and enhancing phosphorus availability in agroecosystems.

The precise exploration of the functional potential of rhizosphere microorganisms faces dual challenges. First, the interaction mechanisms between microorganisms and plants involve multidimensional networks such as signal recognition and metabolic regulation, which have not been fully elucidated. Second, conventional phenotypic screening fails to reveal the intrinsic correlations between functional genes and metabolic pathways, thereby restricting the targeted optimization and application of microbial strains. In recent years, whole-genome sequencing has emerged as a core approach to decipher microbial functions. Through genome sequencing and functional annotation, gene clusters related to plant growth promotion and stress resistance can be systematically identified, and key functional modules can be screened, pioneering a novel avenue for exploring the physiological and ecological functions of rhizosphere bacteria[16–19]. For instance, Soni et al[20] identified phosphorus-solubilizing genes in a phosphate-solubilizing bacterium via genomic analysis, verifying its growth-promoting and biocontrol capacities; Xu et al[21] uncovered the genetic characteristics associated with plant growth of functional strains through genome annotation, providing theoretical support for the application optimization of phosphate-solubilizing bacteria. Therefore, integrated whole-genome sequencing and functional annotation technologies can effectively break through the limitations of traditional phenotypic screening, systematically clarify the functional genes and metabolic basis of rhizosphere microorganisms, and supply critical technical support and theoretical foundations for the precise exploration of their application potential and the targeted functional improvement of microbial strains.

This study focused on *Phyllobacterium* sp. YNK-FB0058, a phosphate-solubilizing bacterial strain isolated from the rhizosphere soil of Carthamus tinctorius in a tobacco-safflower rotation field. To clarify its molecular mechanism of phosphorus solubilization and explore its plant growth-promoting potential,

whole-genome sequencing was performed on the strain in this study. Functional annotation was conducted across multiple databases, including COG, KEGG, and GO, to systematically identify key phosphorus-solubilizing genes involved in organic acid synthesis, phospholipase production, and phosphorus transport systems, as well as growth-promoting gene clusters related to nitrogen fixation, siderophore synthesis, and phytohormone biosynthesis. Furthermore, pot experiments were carried out to verify its effects on phosphorus nutrient uptake and growth promotion in flue-cured tobacco. The findings of this study enrich the resource pool of rhizosphere phosphate-solubilizing microorganisms, elucidate the molecular basis of phosphorus solubilization and growth-promoting characteristics of strain YNK-FB0058, and provide a theoretical foundation and high-quality strain resources for the development and application of high-efficiency phosphate-solubilizing and plant growth-promoting microbial inoculants.

2. Materials and Methods

2.1 Culture media

The formulas of the culture media used in this study are described below: Organic phosphorus bacterial medium: Glucose 10 g, ammonium sulfate 0.5 g, sodium chloride 0.3 g, potassium chloride 0.3 g, magnesium sulfate heptahydrate 0.3 g, ferrous sulfate heptahydrate 0.03 g, manganese sulfate tetrahydrate 0.03 g, lecithin 0.2 g, calcium carbonate 5.0 g, agar 15 g, distilled water 1000 mL, pH 7.0 ± 0.5.Solid inorganic phosphorus bacterial medium: Glucose 10 g, ammonium sulfate 0.5 g, sodium chloride 0.3 g, potassium chloride 0.3 g, magnesium sulfate heptahydrate 0.3 g, ferrous sulfate heptahydrate 0.03 g, manganese sulfate tetrahydrate 0.03 g, calcium phosphate 5.0 g, agar 15 g, distilled water 1000 mL, pH 7.0 ± 0.5.Nutrient Broth (NB) medium: Peptone 10 g, beef extract 3 g, sodium chloride 5 g, distilled water 1000 mL, pH 7.0.Nutrient Agar (NA) medium: The formula was consistent with NB medium, with an additional 18 g of agar per liter, pH 7.0. All the above media were sterilized by high-pressure steam autoclaving at 121°C for 30 min.

2.2 Isolation and 16S rRNA-based phylogenetic identification of tested strain

Phyllobacterium sp. YNK-FB0058 was isolated from the rhizosphere soil surrounding Carthamus tinctorius in a tobacco and safflower rotation field (26°28'46" N, 100°61'31" E) with clay loam soil in Lijiang, China. The strain was isolated on April 28, 2025, and subsequently deposited in the China Center for Type Culture Collection (CCTCC) under the preservation number CCTCC M 20251973(GenBank accession No.: PX312842).

The bacterial 16S rRNA universal primers (27F: AGAGTTTGATCCTGGCTCAG; 1492R: GGTTACCTTGTTACGACTT) were used for PCR amplification.. High-quality PCR products were recovered and sent to Wuhan Tsingke Biotechnology Co, Ltd. for sequencing. The obtained sequences were

subjected to BLAST homology alignment in the NCBI database, and the phylogenetic tree was constructed using MEGA 7.0 software based on the alignment results.

Comparative analysis of the 16S rRNA gene sequences revealed closely related type strains. The complete genome sequences of these strains were obtained from the NCBI Genome database (https://www.ncbi.nlm.nih.gov/genome/) for further comparison with the genome sequence of strain YNK-FB0058.

2.3 DNA extraction, identification, and sequencing of strain YNK-FB0058

The procedures for cultivation, DNA extraction, library construction and genome sequencing of strain YNK-FB0058 were as follows. Firstly, the strain was inoculated into NB medium and incubated at 28°C with shaking at 180 r/min for 24 h. After cultivation, bacterial cells were collected by centrifugation at 12,000 r/min for 15 min. Genomic DNA was extracted using a bacterial genomic DNA extraction kit from Thermo Fisher Scientific (Waltham, MA, USA), following the manufacturer's standard protocol. Subsequently, the extracted genomic DNA was fragmented into approximately 400 bp segments using a Covaris M220 focused-ultrasonic disruptor manufactured by the same company, followed by sequencing library construction. The qualified libraries were finally sent to Shanghai MajorBio Bio-technology Co., Ltd. for paired-end (PE150) sequencing on the Illumina platform.

2.4 Genome assembly and annotation of strain YNK-FB0058

Genome assembly and annotation were performed according to the method described in Reference [23], with the specific procedures as follows: The trimmed sequencing data were subjected to genome assembly using SOAPdenovo v2.04. Subsequently, plasmid sequences in the assembled contigs were identified with PLASMe v1.1, and the obtained plasmid sequences were further annotated by alignment against the PLSDB database (v202106) using BLAST v2.3.0. To improve prediction accuracy, coding sequence (CDS) prediction was performed using three tools in combination: Glimmer v3.02, GeneMarkS v4.3, and Prodigal v2.6.3. Prediction of tRNA and rRNA was conducted using tRNAscan-SE v2.0 and Barrnap v0.9, respectively. For functional characterization of the predicted CDSs, sequence alignment and functional annotation were carried out against major databases, including the Non-Redundant Protein Database (NR), Swiss-Prot, Pfam, Clusters of Orthologous Groups of Proteins (COG), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG). In addition, the Carbohydrate-Active Enzymes database (CAZy) was used to predict carbohydrate-active enzymes (CAZymes) of the strain.

2.5 Multilocus phylogenetic analysis and ANI comparison

Average nucleotide identity (ANI) is recognized as a robust genome-based criterion for determining the species identity of genetically related microorganisms[22]. ANIm values were calculated using JSpeciesWS (https://jspecies.ribohost.com/jspeciesws/), and DNA–DNA hybridization (DDH) values

were calculated using the Genome-to-Genome Distance Calculator 3.0 (https://ggdc.dsmz.de/ggdc.php) to further determine the differences between YNK-FB0058 and the reference genomes of the type strains.

Based on 31 housekeeping genes (*dnaG*, *frr*, *infC*, *nusA*, *pgk*, *pyrG*, *rplA*, *rplB*, *rplC*, *rplD*, *rplE*, *rplF*, *rplK*, *rplL*, *rplM*, *rplN*, *rplP*, *rplS*, *rplT*, *rpmA*, *rpoB*, *rpsB*, *rpsC*, *rpsE*, *rpsI*, *rpsJ*, *rpsK*, *rpsM*, *rpsS*, *smpB*, *tsf*), 19 bacterial strains with the closest phylogenetic relationships at the genus and species levels were selected. The phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with MEGA 6.0 software.

2.6 Qualitative and quantitative analysis of organic and inorganic phosphorus solubilization by strain YNK-FB0058

Qualitative analysis of phosphate solubilization: The strain was inoculated onto plates of Mengjinna solid medium for inorganic phosphorus solubilization and organic phosphorus solubilization, respectively. All plates were incubated in a constant-temperature incubator at 28°C for approximately 48 h. Colonies forming phosphate-solubilizing halos were observed. The phosphate-solubilizing ability of the strain was preliminarily evaluated based on the presence or absence of transparent halos and the calculated solubilization index (solubilization index = transparent halo diameter / colony diameter). Briefly, 100 mL of Mengjinna inorganic and organic phosphorus liquid media were transferred into 250 mL Erlenmeyer flasks and sterilized by autoclaving at 121°C for 30 min. Bacterial cells cultured to the logarithmic phase (OD_{600} = 1.0) in NB liquid medium were collected to prepare a bacterial suspension. The suspension was inoculated into the sterilized media at an inoculum size of 1%. Uninoculated media served as the control, with three replicates for each treatment. All samples were incubated on a shaker at 28°C and 180 r/min for 5 days.A standard curve was established using potassium dihydrogen phosphate as the standard ($y = 0.4946x + 0.125$, $r^2 = 0.9926$). Samples were collected every 24 h, and the content of soluble phosphorus in the culture supernatant was determined by the molybdenum-antimony-ascorbic acid colorimetric method. In addition, the pH value of the culture solution was measured when the phosphate-solubilizing capacity reached the maximum[24].

2.7 Other bioactivity assays of strain YNK-FB0058

The nitrogen-fixing capacity of the strain was determined using Ashby nitrogen-free solid medium. Strain YNK-FB0058 was inoculated onto the medium and incubated at 28°C for 5–7 d in a constant-temperature incubator. The nitrogen-fixing ability was evaluated by observing bacterial growth and the formation of transparent halos around colonies[25]. Zinc-solubilizing activity was qualitatively assessed on zinc-containing solid medium[26]. Siderophore production was detected via the Chrome Azurol S (CAS) colorimetric method. After inoculation, plates were cultured at 28°C for 5–7 d, and the colony growth as well as the formation of orange halos were observed daily[27]. The indole-3-acetic acid (IAA) secretion capacity was measured using the Salkowski colorimetric method[28]. Strain YNK-FB0058 was pre-cultured overnight in NB medium for activation. The bacterial suspension was inoculated at 1% inoculum size into fresh NB medium supplemented with 100 mg/L L-tryptophan, and incubated with shaking at 30°C and 200 r/min for 5 d. The culture was centrifuged daily at 10,000 r/min for 5 min to

collect the supernatant. Subsequently, 4 mL of supernatant was mixed with an equal volume of Salkowski reagent, and the mixture was incubated in darkness for 30 min. The absorbance was measured at 535 nm. A standard curve was established with gradient IAA concentrations to calculate the IAA content in the culture supernatant[29].

2.8 Evaluation of the growth-promoting effect of strain YNK-FB0058 in flue-cured tobacco pot experiments

The tested soil was red loam continuously cropped with flue-cured tobacco for three consecutive years. The physicochemical properties of the soil were as follows: pH 6.1, organic matter 28.3 g/kg, total nitrogen 1.40 g/kg, total phosphorus 0.552 g/kg, total potassium 16.32 g/kg, hydrolyzable nitrogen 117 mg/kg, available phosphorus 27.1 mg/kg, and rapidly available potassium 296.9 mg/kg.

The soil was thoroughly mixed and sieved, with each plastic pot filled with 4 kg of the tested soil. Uniform and healthy flue-cured tobacco seedlings were transplanted into the pots, one seedling per pot. Root irrigation with bacterial solution was carried out 7 days after transplanting. Seedlings in the treatment group (T1) were irrigated with 200 mL of phosphate-solubilizing bacterial suspension at a concentration of 1×10^{7}–1×10^{10} CFU/mL. Seedlings irrigated with an equal volume of deionized water were set as control CK1, and those treated with sterile NB liquid medium as control CK2. Each treatment was established with six replicates.All pots were basal-fertilized with 20 g of special compound fertilizer for flue-cured tobacco. The potted plants were cultivated in a greenhouse at 25°C, and watered once a week to maintain soil moisture. The tobacco plants were harvested 60 days after transplanting for the determination of various physiological and growth indices.

Agronomic traits and biomass were determined in accordance with *Investigation and Measurement Methods for Agronomic Traits of Flue-cured Tobacco* (YC/T142-2010). The determined indices included plant height, stem diameter, leaf length, leaf width, effective leaf number and root length of flue-cured tobacco. The maximum leaf area was calculated using the formula: Leaf area (cm²) = Leaf length (cm) × Leaf width (cm) × 0.6345.

The fresh weights of stem and leaf, as well as root fresh weight of flue-cured tobacco were measured. The samples were killed at 105 ℃ for 30 min, then dried to constant weight at 75 ℃, followed by determination of stem-leaf dry weight and root dry weight. The SPAD value of chlorophyll in functional leaves of flue-cured tobacco was determined using a SPAD-502 chlorophyll meter[30].

2.9 Effects of strain YNK-FB0058 on soil physicochemical properties, enzyme activities and phosphorus nutrient utilization of flue-cured tobacco

Rhizosphere soil samples were collected from the potted plants. After removing impurities, the soil samples were dried at 60 ℃, sieved, and stored in a desiccator for subsequent use.Total nitrogen content was determined by the Kjeldahl method, and hydrolyzable nitrogen was measured by titration[31]. Total phosphorus and available phosphorus contents were determined using the

molybdenum-antimony anti-colorimetric method[32]. Soil activities of phytase, acid phosphatase, and alkaline phosphatase were determined by visible spectrophotometry using commercial assay kits (Addison, Jiangsu)[33].

Flue-cured tobacco root, stem and leaf samples were collected, rinsed, fixed at high temperature, dried and crushed. The samples were digested by the H_2SO_4-H_2O_2 digestion method. The phosphorus contents in roots, stems and leaves were determined via the molybdenum blue colorimetric method, and the total phosphorus accumulation amount of the whole plant was calculated[34].

2.10 Data Analysis

Experimental data were organized and summarized using Microsoft Excel 2021. Statistical analysis was performed with R software (version 4.5.1). Independent-sample t-test were conducted to compare the differences between the inoculated treatment group (T1) and the two control groups (CK1, CK2), respectively. All tests were two-tailed, and $P < 0.05$ was considered statistically significant. The significance levels in the figures were annotated as follows: * indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$.

3. Results

3.1 Morphological and molecular identification of strain YNK-FB0058

Strain YNK-FB0058 formed round, convex, glossy, pale yellow colonies with a smooth, viscous surface and no pigment production (Fig. 1A). Observation under an electron microscope revealed that the cells were rod-shaped (Fig. 1B). The 16S rRNA gene sequence of the strain was amplified by PCR and sequenced. The obtained sequence was subjected to BLAST homology alignment in the NCBI database. The results showed that strain YNK-FB0058 shared the highest sequence similarity of only 98.16% with the type strain *Phyllobacterium myrsinacearum* IAM 13584 (accession No. D12789), which was lower than the 98.7% threshold for bacterial species delineation. A phylogenetic tree was constructed using the Neighbor-Joining method via MEGA 7.0 software. The results indicated that strain YNK-FB0058 clustered in the same branch as *Phyllobacterium pellucidum* BT25 (accession No. MN658537) with a bootstrap value of 98%, but did not form a tight cluster with known species (Fig. 1C). Therefore, strain YNK-FB0058 is likely a potential novel species of the *Phyllobacterium*.

3.2 Basic genomic characteristics of strain YNK-FB0058

Whole-genome sequencing was performed on strain YNK-FB0058 to clarify its taxonomic status and potential functions. Whole-genome sequencing resuls revealed that the chromosome size of *Phyllobacterium* sp. YNK-FB0058 was 4 ,694, 147 bp with a GC content of 61.14%. A total of 4459 protein-coding sequences (CDS) were annotated in this study, and 3 ribosomal RNA (rRNA) genes as well as 44 transfer RNA (tRNA) genes were predicted. Genomic island and prophage region prediction

were performed using IslandViewer and Phage Finder, identifying 9 genomic islands and 3 prophage regions. The basic genomic characteristics of strain YNK-FB0058 are shown in Table 1, and the circular genome map representing the whole-genome assembly results is presented(Fig. 2A).

Based on 31 housekeeping genes. The results showed that strain YNK-FB0058 formed an independent clade in the phylogenetic tree (Fig. 2B), exhibiting a distinctly different branching structure from all reference strains and failing to cluster with any known species. Furthermore, The results of ANI revealed that the ANI values between this strain and all reference strains were lower than 77.05%, while the dDDH values were all below 20.1%, which were distinctly lower than the species delineation thresholds for bacteria, namely ANI ≥ 95% and dDDH ≥ 70% (Supplementary Table S1). Genomic divergence confirmed the genetic differentiation between strain YNK-FB0058 and known validly published *Phyllobacterium* species, thereby verifying its taxonomic status as a potentially novel species at the whole-genome level.

Table 1
The genome characterization of *Phyllobacterium* sp. YNK-FB0058.

The characterization	Value
Assembly information	
Sequence length (bp)	4694147
Total scaffold No	8
Scaffold N50(bp)	3291538
Contigs	10
G + C content(%)	61.14
Contings N50(bp)	1055532
Annotation information	
Number of tRNAs	44
Number of rRNAs	3
CDSs (total)	4459
Mobile Genetic Element (MGE)	
Gene islands	9
Prophage regions	3
CRISPR-Cass	3
Insertion sequence	0
Tns	6
NCBI	
BioProject ID	PRJNA1437519
BioSample ID	SAMN56513470
GeneBank accession number	JBWDIU000000000

3.3 Gene annotation results of YNK-FB0058

A total of 4459 functional annotated genes were obtained in YNK-FB0058, including 4405 from the NR database, 3374 from SwissProt, 3874 from Pfam, 3777 from COG, 1595 from GO, and 3406 from KEGG (Fig. 3A).

KEGG annotation assigned genes to six major categories, with Metabolism being the most prominent. Key pathways included carbohydrate metabolism (346 genes), biosynthesis of other secondary metabolites (319 genes), energy metabolism (211 genes), and lipid metabolism (243 genes). In Environmental Information Processing, membrane transport accounted for 341 genes, highlighting efficient material exchange with the rhizosphere (Fig. 3B). CAZyme analysis identified 140 carbohydrate-active enzyme genes, dominated by glycosyl transferases (GT, 48 genes) and glycoside hydrolases (GH, 47 genes), followed by carbohydrate esterases (CE, 24 genes), auxiliary activities (AA, 18 genes), and carbohydrate-binding modules (CBM, 2 genes). This indicates a robust capacity for carbohydrate synthesis and degradation, supporting efficient utilization of rhizosphere carbon sources (Fig. 3C). COG classification showed that Category E (amino acid transport and metabolism) was the most enriched (463 genes), followed by Category G (carbohydrate transport and metabolism, 440 genes) and Category K (transcription, 363 genes), reflecting the strain's strong capacity for nutrient metabolism and genetic information processing (Fig. 3D). GO analysis revealed enrichment in biological processes such as transmembrane transport (61 genes) and proteolysis (29 genes); cellular components including plasma membrane (279 genes) and cytoplasm (214 genes); and molecular functions dominated by ATP hydrolase activity (171 genes) and transmembrane transporter activity (111 genes) (Fig. 3E).

3.4 Phosphorus nutrient transformation capacity

KEGG pathway annotation revealed abundant phosphorus-solubilizing genes of pentose phosphate pathway in strain YNK-FB0058, forming an integrated functional system covering inorganic phosphorus dissolution, organic phosphorus mineralization, hierarchical transportation and metabolic regulation.

Four functional modules jointly mediate efficient phosphorus solubilization. The *gcd* and *pqq* gene clusters drive gluconic acid synthesis to acidify surroundings and activate insoluble inorganic phosphorus. Intact phn cluster and hydrolytic enzyme genes degrade diverse organic phosphorus substrates via C-P bond cleavage and cascade hydrolysis. Multiple transporters including *TC.PIT*, *Pst ABC*, *phn* and *ugp* systems adapt to phosphorus uptake under varied phosphorus conditions. The *pho* family genes build a signal perception-regulation-response loop to stabilize phosphorus metabolism. All phosphorus-metabolizing genes are summarized in Supplementary Table S2.

Strain YNK-FB0058 was inoculated onto Mengjinna solid media containing inorganic phosphorus and organic phosphorus, respectively, and incubated inversely at a constant temperature of 28°C for 3 days. Obvious transparent phosphorus-solubilizing halos were observed on both media (Fig. 4AB), indicating that this strain possesses the capacity to solubilize both inorganic and organic phosphorus.

The molybdenum-antimony anti-spectrophotometric method was further adopted to quantitatively determine its phosphorus-solubilizing ability. The results revealed that the content of soluble inorganic phosphorus in the fermentation broth of strain YNK-FB0058 reached a peak value of 474.46 mg/L on the 3rd day of cultivation, while the maximum content of soluble organic phosphorus was 566.3 mg/L on the 2nd day (Fig. 4H).

3.5 Analysis of Indole-3-Acetic Acid (IAA) production and other nutrient transformation capabilities of strain YNK-FB0058

KEGG pathway analysis indicated that the genome of YNK-FB0058 harbors multiple genes associated with nutrient transformation. These include nitrogen fixation-related functional genes *iscU*, *glnA*, *glnB*, *cynT*, and *npd*(Supplementary Table S3); key genes involved in siderophore biosynthesis(Supplementary Table S5), such as *dhbF*, *efeB*, *efeU*, *efeO*, *ftrA*, *fepD*, *fepG*, *fepC*, *afuA*, *afuB*, *afuC*, and *furB*; as well as the zinc-solubilization-related gene *zur*(Supplementary Table S4). Combined with KEGG database alignment and genome annotation results, the YNK-FB0058 genome also contains a series of genes involved in IAA biosynthesis, including *aroF*, *aroB*, *aroQ*, *aroE*, *aroK*, *aroA*, and *aroC*, which provide genetic basis for its capability of IAA secretion. Detailed information on these relevant genes is listed in Supplementary Table S6.

Furthermore, Functional screening assays showed that distinct transparent halos were formed when YNK-FB0058 was cultured on nitrogen-fixing medium, zinc-solubilizing medium and chrome azurol S (CAS) medium, confirming that this strain possessed the capacities of nitrogen fixation, zinc solubilization and siderophore production (Fig. 4CDE). Qualitative experiments revealed that strain YNK-FB0058 could secrete indole-3-acetic acid (IAA) (Fig. 4F), and its maximum IAA secretion content reached 19.319 μg/mL on the 4th day of incubation (Fig. 4G).

3.6 Effects of strain YNK-FB0058 on the growth promotion of flue-cured tobacco seedlings

A pot experiment was conducted to explore the growth-promoting effect of strain YNK-FB0058 on flue-cured tobacco seedlings, and relevant growth and physiological indices were determined 60 days after transplanting. Comprehensive analysis revealed that flue-cured tobacco seedlings under T1 treatment exhibited superior performance in key indicators including plant height, stem diameter, leaf area, biomass and root morphology compared with the control groups, indicating that strain YNK-FB0058 exerted comprehensive and remarkable growth-promoting effects on flue-cured tobacco seedlings($P < 0.05$). Compared with CK1 and CK2, seedling plant height in T1 increased by 6.12% and 31.34%, stem diameter rose by 23.90% and 9.13%, maximum leaf area increased by 53.41% and 29.77%, and leaf relative SPAD chlorophyll value elevated by 16.10% and 3.09%(Fig. 5ACDE), respectively. Additionally, the above-ground fresh weight increased by 141.81% and 40.30%, and above-ground dry matter accumulation increased by 257.87% and 46.16%s(Fig. 5GH). In parallel,the total taproot length of seedlings in T1 was increased by 55.52% and 30.61% relative to CK1 and CK2. The underground root fresh weight was enhanced by 232.52% and 50.89%, and root dry matter accumulation increased by 234.17% and 39.68%(Fig. 5BIJ). Meanwhile, the number of effective root tips was substantially increased (Fig. 5F).

Note

(A) Plant height. (B) Root length. (C) Stem diameter. (D) SPAD. (E) Maximum leaf area. (F) Root tip number. (G) Aboveground fresh weight;(H) Aboveground dry weight. (I) Underground fresh weight. (J) Underground dry weight.

3.7 Effects of strain YNK-FB0058 on soil physicochemical properties and enzyme activities in flue-cured tobacco planting soil

Nitrogen and phosphorus are essential for soil nutrient cycling, ecological functions and plant growth and development. Pot experiment results showed that compared with the control groups (CK1 and CK2), inoculation with strain YNK-FB0058 (T1) significantly improved nitrogen and phosphorus nutrient supply in flue-cured tobacco rhizosphere soil and increased the activities of enzymes related to phosphorus transformation ($P < 0.05$). The total phosphorus content in T1 soil increased by 7.83% and 7.75%, and available phosphorus content rose significantly by 18.93% and 20.29%, reaching 342.83 mg/kg (Fig. 5BD). Meanwhile, the total nitrogen content was elevated by 13.11% and 13.28%, and hydrolyzable nitrogen content increased markedly by 11.54% and 11.92%, with a final value of 428.67 mg/kg (Fig. 5AC).

For soil phosphataseThe results demonstrated that the activities of the three enzymes in the treatment group were significantly higher than those in the control groups ($P < 0.05$). The activity of acid phosphatase reached 1605.07 nmol/h/g, increasing by 54.88% and 52.15% compared with the controls (Fig. 5E). Alkaline phosphatase activity was 552.23 nmol/h/g, with increases of 48.69% and 56.35%, showing the most prominent elevation (Fig. 5F). Phytase activity peaked at 5.04 μmol/h/g, rising by 54.60% and 57.65% respectively (Fig. 5G).

For plant phosphatase, application of YNK-FB0058 inoculant significantly promoted phosphorus accumulation in flue-cured tobacco compared with the control groups (CK1 and CK2) ($P < 0.05$). As shown in (Fig. 6H), the above-ground phosphorus accumulation was the highest under T1 treatment, reaching 55.56 mg, which increased by 36.55% and 26.54% relative to the controls. The underground phosphorus accumulation presented a similar variation trend, with the value of 5.13 mg in T1 treatment, showing significant increases of 50.05% and 54.30%. Similarly, the total plant phosphorus accumulation was maximized in T1 group at 60.68 mg, rising by 37.59% and 28.50% compared with the two controls.

Figure 6 Effects of Strain YNK-FB0058 on Soil Nutrients of Flue-Cured Tobacco. Note: (A) Total nitrogen; (B) Total phosphorus; (C) Hydrolyzable nitrogen; (D) Available phosphorus; (E) Acid phosphatase; (F) Alkaline phosphatase; (G) Phytase; (H) Phosphorus accumulation in plants.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The same below.

4. Discussion

In this study, strain YNK-FB0058 belonging to the genus *Phyllobacterium* was isolated from the rhizosphere soil of safflower in Lijiang, Yunnan. Polyphasic taxonomic identification confirmed that it

represents a potentially novel species of *Phyllobacterium*. Whole-genome sequencing and functional verification were performed to systematically elucidate the molecular mechanisms underlying phosphate solubilization and plant growth promotion, as well as its field application potential. This study provides theoretical basis and strain resources for the exploitation of rhizosphere plant growth-promoting microbial resources and the development of green phosphorus reduction in agriculture.

Sequence alignment of the 16S rRNA gene revealed that strain YNK-FB0058 shared only 98.16% similarity with the type strain *Phyllobacterium myrsinacearum* IAM 13584, which was lower than the bacterial species delineation threshold of 98.7%. The phylogenetic tree constructed based on 31 housekeeping genes showed that this strain formed an independent clade and did not cluster with any known species of the genus *Phyllobacterium*.Further genomic analysis indicated that the average nucleotide identity (ANI) values between YNK-FB0058 and all reference strains were below 77.05%, while digital DNA–DNA hybridization (dDDH) values were lower than 20.1%. Both values were far below the commonly accepted species delineation thresholds of ANI ≥ 95% and dDDH ≥ 70% for bacterial species classification[35–36]. Combined phylogenetic and genomic analyses confirmed that strain YNK-FB0058 represents a potentially novel species within the genus *Phyllobacterium*, which enriches the species resources and genetic diversity of this genus. At present, further taxonomic research on the biochemical and physiological characteristics of this strain is ongoing.

Genome mining results revealed that strain YNK-FB0058 possesses genes related to phosphate mineralization, inorganic phosphorus solubilization, phosphate transport, and the regulation of phosphorus starvation response.The strain harbors the *gcd* gene and the *pqq* gene cluster, which can secrete organic acids to acidify the surrounding environment and solubilize insoluble inorganic phosphorus, thereby reducing environmental pH. Meanwhile, it carries the *phn* gene cluster, as well as genes encoding phosphodiesterase and pyrophosphatase, enabling efficient mineralization of organic phosphorus. In addition, it is equipped with the high-affinity Pst transport system and the constitutive Pit transport system, allowing efficient phosphorus absorption under varying phosphorus concentrations in the rhizosphere. The regulatory modules *phoR*, *phoU*, and *phoH* further maintain the homeostasis of intracellular phosphorus metabolism.Compared with the reported phosphate-solubilizing endophytic strain Q2H1, YNK-FB0058 possesses a more complete phosphorus-solubilization system[37]. When compared with the functionally well-characterized strain *Bacillus bombysepticus* X42, YNK-FB0058 contains a greater number of genes associated with phosphorus activation[38]. Phenotypic verification confirmed that YNK-FB0058 exhibits high-efficiency solubilizing capacity for both inorganic and organic phosphorus. Its inorganic phosphorus solubilization capacity reached 474.46 mg/L, and organic phosphorus solubilization capacity reached 566.3 mg/L, which were significantly higher than those of most reported high-efficiency phosphate-solubilizing strains. The strain also shows a broad phosphorus-solubilizing spectrum and high metabolic efficiency, which is highly consistent with the genomic functional prediction results[39–40].

In addition to its core phosphorus-solubilizing function, strain YNK-FB0058 exhibits multiple plant growth-promoting traits, including IAA secretion, siderophore synthesis, nitrogen fixation, and zinc

activation. It forms a compound growth-promoting system dominated by phosphorus activation, coordinated multi-nutrient supply, and synergistic regulation of plant hormones. The comprehensive growth-promoting capacity of this strain is comparable to or even superior to that of previously reported high-efficiency phosphate-solubilizing strains[41–45].

Whole-genome functional annotation indicated that YNK-FB0058 contains key genes involved in the shikimate and tryptophan pathways, such as *aroF/B/Q/E/K/A/C* and *trpE/G/D/C/F/B*, which support the efficient synthesis of indole hormones including IAA. The IAA secretion capacity reaches 19.319 μg/mL, which can significantly promote root elongation and root tip proliferation of flue-cured tobacco[46]. The strain carries core nitrogen metabolism genes including *iscU*, *glnA*, *glnB*, *cynT*, and *npd*, which are involved in iron–sulfur cluster assembly, glutamine synthesis, and nitrogen regulation, endowing the strain with nitrogen fixation ability and synergistically improving soil nitrogen supply[47]. Genes related to siderophore synthesis and transport, such as *dhbF*, *efeB/U/O*, *ftrA*, *fepD/G/C*, *afuA/B/C*, and *furB*, were annotated, enabling efficient chelation of rhizosphere iron and improvement of plant iron nutrition[48]. Meanwhile, the zinc regulatory gene *zur* was identified, which participates in soil zinc activation and enhances the availability of trace elements[49]. The synergistic action of multiple growth-promoting genes enables YNK-FB0058 to break the functional limitations of traditional single-function phosphate-solubilizing strains, and establishes an efficient growth-promoting mode integrating nutrient activation and growth regulation.

Pot experiment results further confirmed that the phosphate-solubilizing strain YNK-FB0058 exerted a significant growth-promoting effect on flue-cured tobacco, which was consistent with findings of previous studies[50–53]. Inoculation with phosphate-solubilizing microorganisms (PSM) can effectively increase soil available phosphorus content, promote plant absorption of nitrogen and phosphorus nutrients, and enhance plant height, leaf area, stem diameter and biomass of crops.The present study demonstrated that inoculation with YNK-FB0058 not only increased the biomass of stems, leaves and roots of flue-cured tobacco seedlings, but also promoted chlorophyll synthesis and improved photosynthetic efficiency. The underlying mechanism may be that the phosphate-solubilizing strain secretes plant hormones such as indole-3-acetic acid (IAA) and cytokinins, which facilitate chlorophyll synthesis and enhance root activity, thereby improving the absorption and utilization of soil nutrients and water by plants[54].

5. Conclusions

YNK-FB0058 as a potential novel *Phyllobacterium* species. The genome size is 4,694,147 bp with a GC content of 61.14%, containing 4459 CDSs. Gene clusters related to phosphate solubilization, IAA biosynthesis, nitrogen fixation, siderophore production and zinc solubilization were annotated. Phenotypic verification verified its high capability to dissolve organic and inorganic phosphorus, as well as activities of nitrogen fixation, zinc solubilization, siderophore secretion and IAA synthesis. Pot experiments revealed that inoculation with YNK-FB0058 markedly increased soil nitrogen and phosphorus contents, enhanced phosphatase and phytase activities, and accelerated tobacco growth,

biomass accumulation and phosphorus absorption. Collectively, YNK-FB0058 is a superior rhizosphere probiotic with strong phosphate-solubilizing and diverse growth-promoting functions. It provides valuable strain resources and theoretical reference for chemical fertilizer reduction and development of efficient microbial inoculants.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors approved the manuscript to be published.

Availability of data and materials

The whole genome sequence of strain YNK-FB0058 has been submitted to the NCBI database. The BioProject ID is PRJNA1437519; the BioSample ID is SAMN56513470; the GenBank accession number is JBWDIU000000000. This strain has been deposited in the China Center for Type Culture Collection (CCTCC) with the deposit number CCTCC M 20251973.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Yu Wang , Jianpeng Jia , Runrun Du , Xuewei Li and Peiwen Yang contributed equally to this study, participating in the conception and design of the research paper, data acquisition and analysis, manuscript writing, review, and editing; Jianpeng Jia , Te Pu , Zilin Li , Ming Ni :literature review, manuscript writing; Yongqin Liao , You Luo , Xiao Yao , Xun He : data collection and organization, figure and table preparation; Xin He , Jing Li , Xuewei Li and Peiwen Yang: research conception, manuscript review and revision, funding acquisition. All authors reviewed and approved the final version for publication.

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Figures

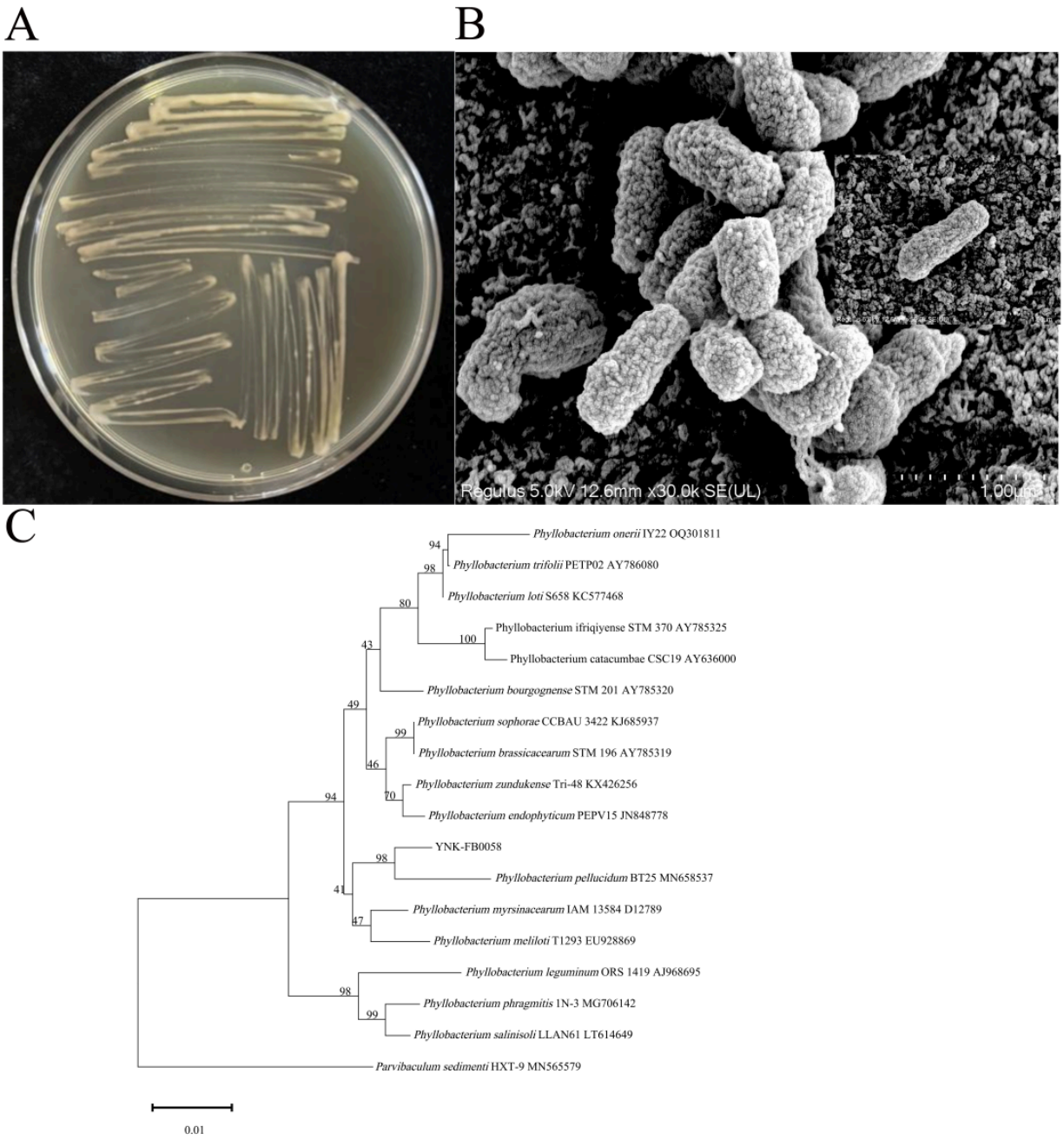


Figure 1

Morphological characteristics and phylogenetic trees of strain YNK-FB0058. Note: (A) Colony morphology on NA medium.(B) Scanning electron micrograph of bacterial cells at 30 000 magnification. (C) Phylogenetic tree constructed based on 16S rDNA gene sequences.

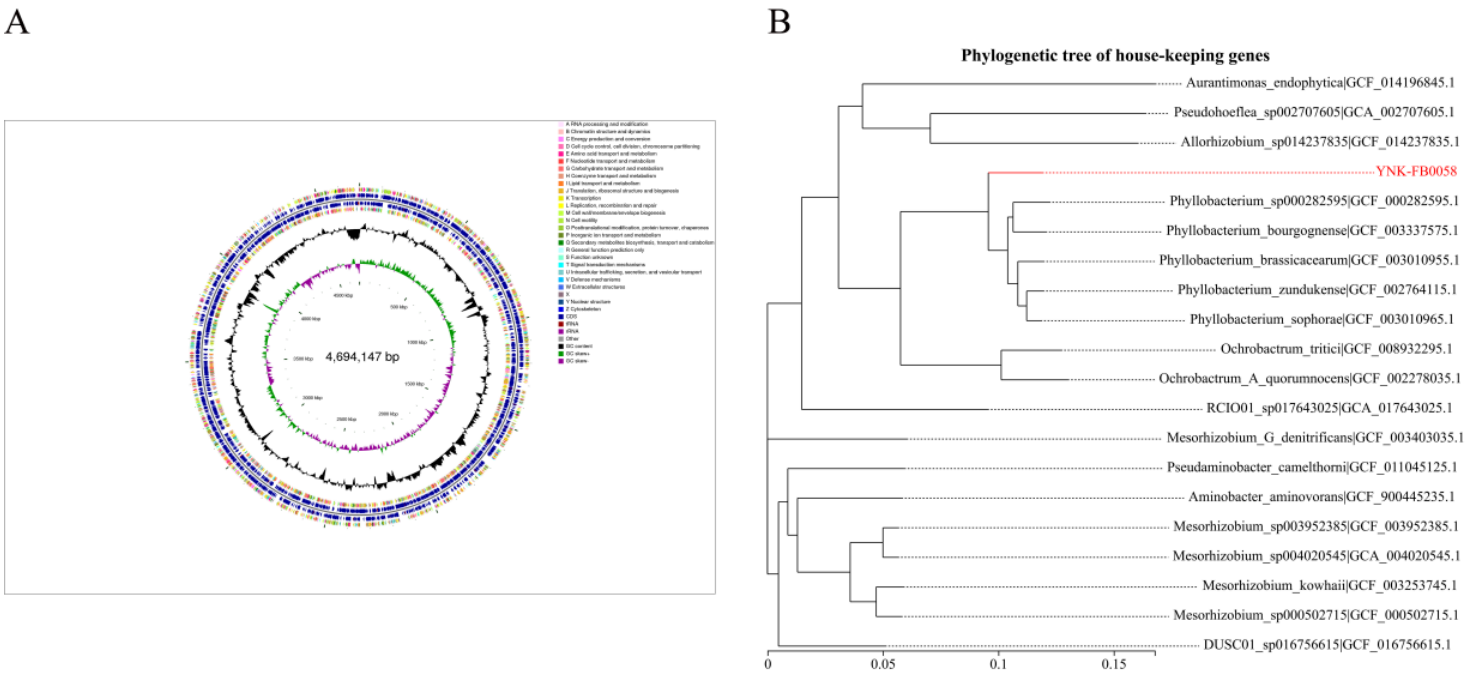


Figure 2

(A) Genome circle map of *Phyllobacterium* sp.YNK-FB0058. (B) Phylogenetic tree of strain YNK-FB0058 constructed based on 31 housekeeping genes. Note: From the inside to the outside, the first circle represents the scale; the second lap represents GC skew; the third circle represents the GC content; the fourth and seventh circles represent the COG to which each CDS belongs; the fifth and sixth circles represent the position of CDS, tRNA, and RNA on the genome.

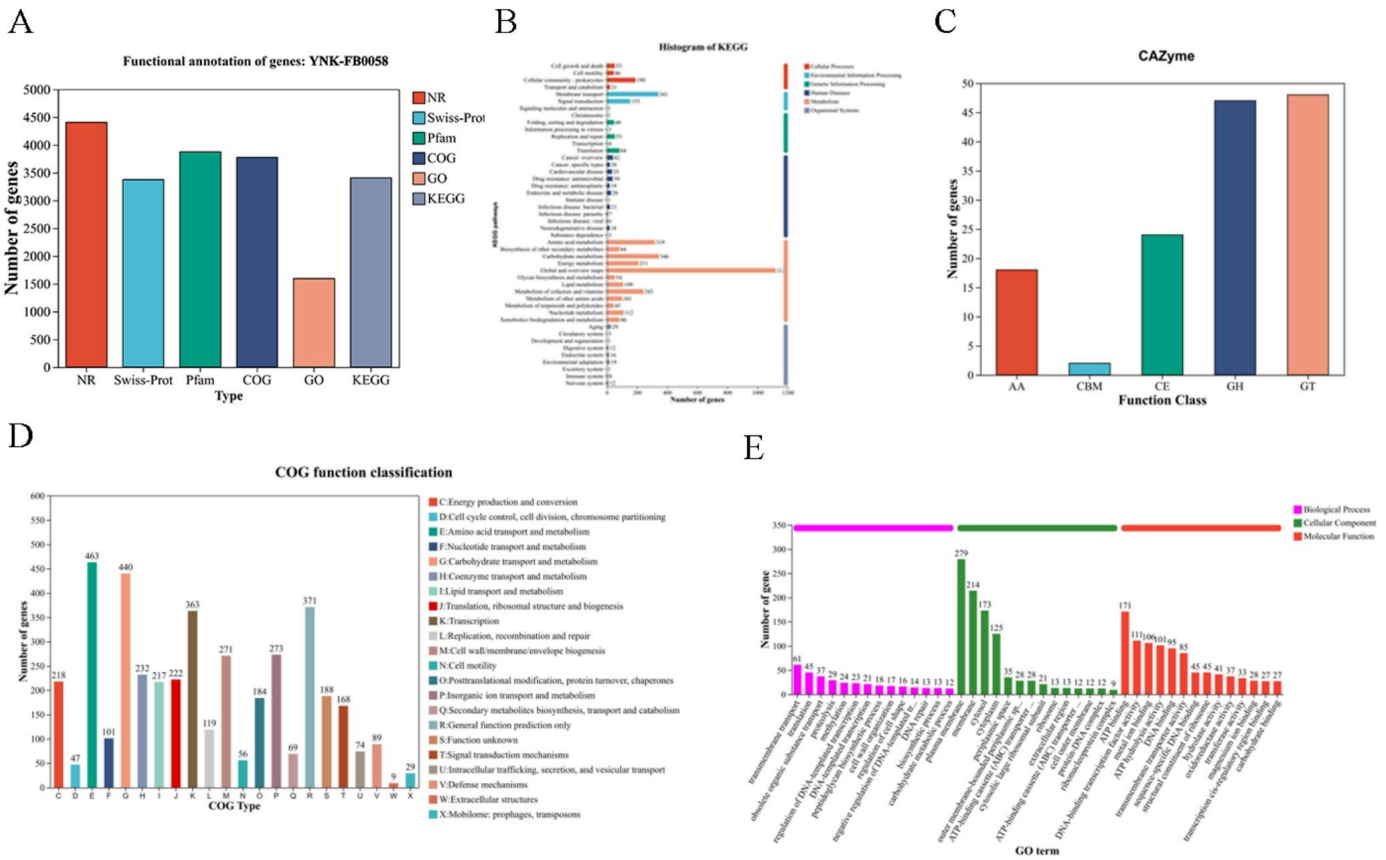


Figure 3

Distribution of gene function annotations of *Phyllobacterium*sp.YNK-FB0058 across different databases. Note: (A) Distribution of gene function annotations of Phyllobacterium sp. YNK-FB0058 in different databases. (B) Genome functional annotation based on KEGG database. (C) Genome functional annotation based on CAZy database. (D) Genome functional annotation based on COG database. (E) Genome functional annotation based on GO database.

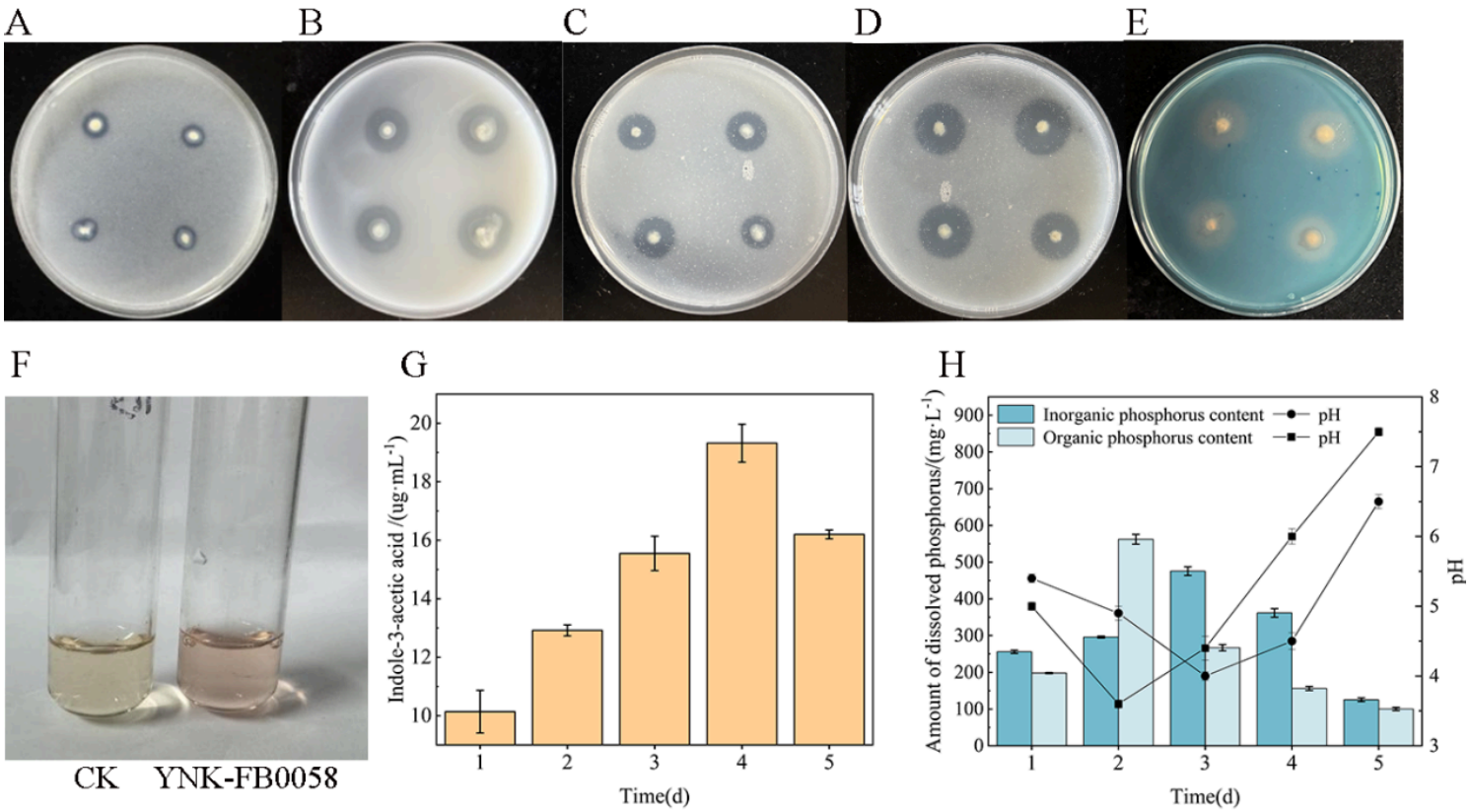


Figure 4

Analysis of Phosphorus-Solubilizing Capacity and Other Functional Activities of Strain YNK-FB0058. Note: (A) Inorganic phosphorus-solubilizing. (B) Organic phosphorus-solubilizing. (C) Nitrogen fixation. (D) Zinc solubilization. (E) Siderophore production. (F) IAA production. (G) Quantitative determination of IAA production. (H) Phosphorus-solubilization capacity and pH of the culture medium.

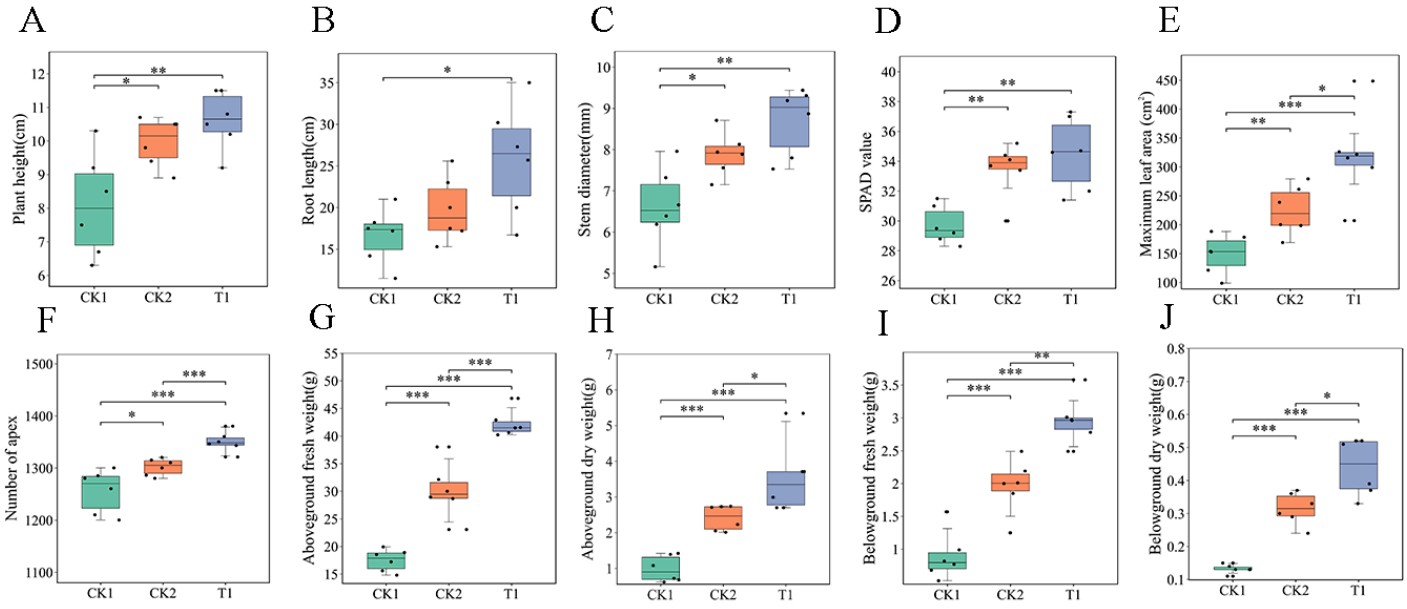


Figure 5

Growth-promoting effect of strain YNK-FB0058 on flue-cured tobacco seedlings.

Note: (A) Plant height. (B) Root length. (C) Stem diameter. (D) SPAD. (E) Maximum leaf area. (F) Root tip number. (G) Aboveground fresh weight;(H) Aboveground dry weight. (I) Underground fresh weight. (J) Underground dry weight.

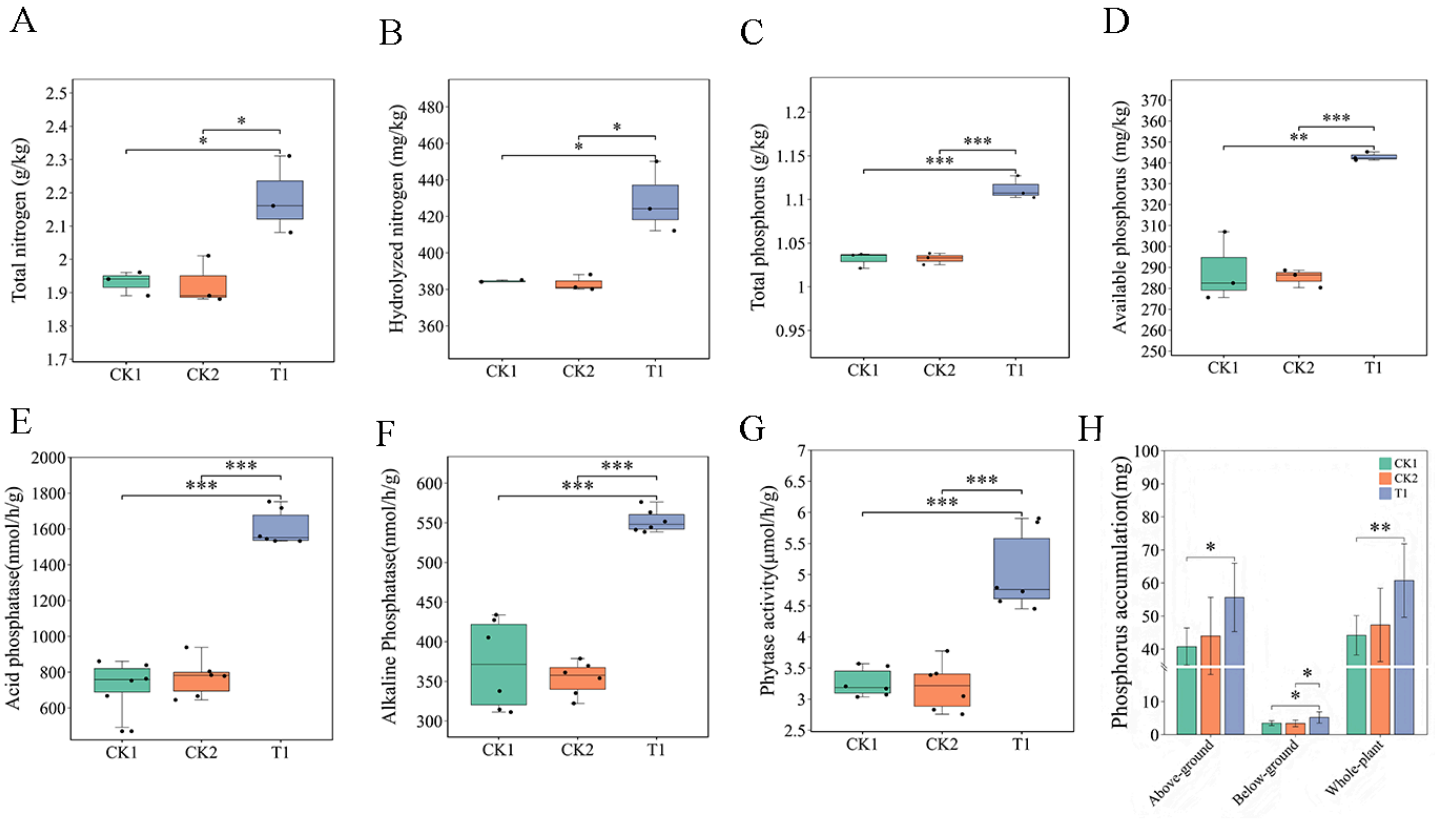


Figure 6

Effects of Strain YNK-FB0058 on Soil Nutrients of Flue-Cured Tobacco. Note: (A) Total nitrogen; (B) Total phosphorus; (C) Hydrolyzable nitrogen; (D) Available phosphorus; (E) Acid phosphatase; (F) Alkaline phosphatase; (G) Phytase; (H) Phosphorus accumulation in plants.

* *P*< 0.05, ** *P*< 0.01, *** *P*< 0.001. The same below.

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