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RESEARCH ARTICLE

Genomic and metabolomic profiling reveal *Streptomyces rochei* S32 contributes to plant growth by nitrogen fixation and production of bioactive substances

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Abstract

Aims To unfold the full potential of plant growth-promoting rhizobacteria in crop production it is desirable to explore the mechanisms through which they promote growth. We investigated the potential mechanisms of plant growth promotion by *Streptomyces rochei* S32 in two crops.

Methods The effects of *S. rochei* S32 on plant growth and its antagonistic activity against soil-borne pathogenic fungi were tested. The underlying molecular mechanisms were identified based on whole-genome sequencing and bioinformatics analysis. The results of genomic analysis were verified by widely targeted metabolomics and mechanism studies of plant growth promotion.

Results *S. rochei* S32 significantly improved the growth of wheat and tomato. The shoot length (24.7%) and root length (25.3%) of wheat (400-fold dilution of cell-free fermentation filtrate) were increased, and the root length of tomato (200-fold dilution) was prolonged (40.9%), and the field yield was also increased. *S. rochei* S32 showed antagonistic activity against multiple pathogenic fungi, especially *Macropoma kawatsukai*. The bacterial genome contains an 8,041,158-bp chromosome and two plasmids. A total of 7486 annotated genes were classified into 31 Gene Ontology functional categories. Genomic analysis revealed the potential for the production of indole-3-acetic acid, fungal cell wall hydrolases, antibiotics (e.g., candicidin, streptothricin, borrellin, albaflavenone), and siderophores. Thirty-nine phytohormones and 2205 secondary metabolites were detected, including indole-3-acetic acid, phytosphingosine, acivicin, and corynebactin. Normal bacterial growth occurred on a nitrogen-free medium.

Conclusion *S. rochei* S32 can promote plant growth directly or indirectly through nitrogen fixation and production of phytohormones, extracellular hydrolases, antibiotics, and siderophores.

Keywords Rhizobacteria · Bacterial genome · Secondary metabolites · Crop growth · Molecular mechanisms

Abbreviations

PGPR	Plant growth-promoting rhizobacteria
IAA	Indole-3-acetic acid
TRP	Tryptophan
ACC	1-Aminocyclopropane-1-carboxylic acid
PDA	Potato dextrose agar
GA	Gauze's synthetic medium No. 1

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CAS	Chrome azurol sulfonate
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
CAZy	Carbohydrate-active enzymes
IPA	Indole-3-pyruvate
IAM	Indole-3-acetamide
TAM	Tryptamine
BGCs	Biosynthetic gene clusters
CKs	Cytokinins
JAs	Jasmonates
GAs	Gibberellins
SA	Salicylic acid
SLs	Strigolactones
ABA	Abscisic acid

Introduction

Chemical fertilizers and pesticides are widely used in agricultural production to promote crop growth and control plant diseases. Inappropriate or excessive use of agrochemicals leads to the risk of environmental pollution, disturbs the community structure of beneficial soil microorganisms, and causes food safety problems (Dennis et al. 2018; Nejatzadeh-Barandozi and Gholami-Borujeni 2014; Ye et al. 2020). In contrast with agrochemicals, functional microbial inoculants have the advantages of reduced production costs and environmental impacts, with great potential in improving plant nutrient absorption, growth performance, and disease resistance (Fravel 2005; Safar et al. 2023). In particular, plant growth-promoting rhizobacteria (PGPR) provide alternatives to support the sustainable development of agriculture (Harris et al. 2021; Thilagar et al. 2016).

Streptomyces, a genus of Gram-positive actinobacteria, is ubiquitous in the soil environment and has great potential for agricultural application (Gillon et al. 2023). The filamentous morphology and sporulation ability of *Streptomyces* species allow them to survive in adverse environments and outcompete other species for nutrients and space (Vurukonda et al. 2018). Most known species of *Streptomyces* can enhance plant growth and resistance to biotic and abiotic stresses by producing various phytohormones and antibiotics (Kim et al. 2011). This is exemplified by *Streptomyces rochei* (Zhang et al. 2015), *S. albidoflavus* (Yao et al. 2021), and *S. angustmyceticus* (Luo et al. 2023), which play a role in plant growth promotion and disease control in different crops. Rational application of these *Streptomyces* spp. as PGPR necessitates an in-depth understanding of the molecular mechanisms underlying plant growth promotion.

Phytohormone biosynthesis is considered to be one of the direct mechanisms for PGPR to promote plant growth (Jamil et al. 2022). For example, Liming et al. (2018) demonstrated the potential of a *Streptomyces* isolate to increase plant growth by producing indole-3-acetic acid (IAA). IAA is a plant growth regulator that controls cell elongation and apical dominance (Wang et al. 2001). This phytohormone is commonly used as an indicator to study the mechanisms of plant growth promotion by PGPR. Importantly, IAA is synthesized by PGPR through different pathways—tryptophan (TRP) induction pathway and TRP-dependent pathway (Zhao et al. 2001). In *Streptomyces*, IAA biosynthesis is achieved mainly through the TRP dependent indole-3-acetamide pathway (Manulis et al. 1994). Other direct mechanisms of plant growth promotion by PGPR include the production of 1-aminocyclopropane-1-carboxylic acid (ACC) (Vurukonda et al. 2018) and siderophores (Zhu et al. 2023), as well as nitrogen fixation (Paungfoo-Lonhienne et al. 2014) and organophosphorus solubilization.

PGPR indirectly promote plant growth by inhibiting soil-borne pathogens. Some PGPR strains release extracellular enzymes to alleviate root and stem rot diseases caused by *Fusarium oxyspora* (Compant et al. 2005), and other strains produce antimicrobial metabolites to impair the growth of pathogenic fungi, bacteria, and nematodes (Fu et al. 2016; Singh et al. 2021). *Streptomyces* can synthesize various secondary metabolites with a wide range of biological activities, which confers substantial benefits to plant growth and disease resistance. The most prominent secondary metabolites of *Streptomyces* are antibiotics, such as alkaloids, macrolides, cyclopeptides, terpenoids, and polyketides (Zhang and Qian 2019). Exposure to these antibiotics can hinder the biosynthesis of cell wall components, change the permeability of cell membranes, and inhibit the activity of ribosomes, RNAs, or enzymes in harmful microorganisms (De la Cruz-Rodríguez et al. 2023; Mascher et al. 2003; Siewert and Strominger 1967; Nakamura 1959; Zhang et al. 2023).

The application of *S. rochei* Sr has been shown to improve plant growth and yield in a marine

environment, most likely through the production of IAA and polyamine (Mathew et al. 2020). Field observations in non-marine environments indicate that *S. rochei* S32 also exhibits positive effects on the growth and yield of various crops, such as tomato (*Solanum lycopersicum* L.), watermelon (*Citrullus lanatus* L.), and strawberry (*Fragaria* × *ananassa* Duch.). However, whether *S. rochei* strains adopt the same mechanisms of plant growth promotion in various environments remains unclear. Therefore, this study verified the ability of *S. rochei* S32 as a PGPR strain to promote plant growth and resistance against soil-borne pathogens through petri dish experiments. The potential mechanisms of plant growth promotion and pathogen antagonism were explored based on whole-genome sequencing and biological function analysis. The results of genomic analysis were confirmed by widely targeted metabolomics and mechanism studies of plant growth promotion.

Materials and methods

Plant growth promotion test

The strain of *S. rochei* S32 was isolated from the soil of greenhouse watermelon with continuous cropping obstacle in Yangling (Shaanxi Province, China) and preserved in the Laboratory of Resource Microbiology, Northwest A&F University (Yangling). To verify its effect on plant growth, growth-promoting experiments in dishes and field experiments were carried out.

Petri dish growth test: five treatments were used in the experiment, with five replications per treatment. Four treatment groups received the cell-free fermentation broth of *S. rochei* S32 (i.e., 50-, 100-, 200-, 400-fold diluted), and the control group was added with sterile water. The fermentation broth was prepared in Gauze's synthetic medium No. 1 (GA) at 28 °C, 160 r/min for 8 d and filtered through a 0.22-μm membrane filter to remove cells. Seeds of wheat (*Triticum aestivum* L. cv. Xiaoyan22) and tomato (*Solanum lycopersicum* L. cv. Jinpeng101) were purchased from Jinpeng Seed Co., Ltd. (Xi'an, Shaanxi Province, China). After surface disinfection with sodium hypochlorite (containing 1% chlorine, 15 min), the seeds were washed with sterile water at least five times until odorless. Seeds with full grains were selected and placed uniformly on moistened filter paper in sterile petri dishes (10 seeds per dish). The seeds were cultured in a greenhouse with an average temperature of 28 °C and an average daily illumination of 12 h. Shoot length and root length were measured after 15 d of treatment with the cell-free fermentation broth (10 mL per dish).

The field experiment of wheat (Xiaoyan22) was carried out in Xixiaozhai (107°59′20″E, 34° 14′40″N) Village (Yangling, Shaanxi Province, China), from October 2022 to June 2023. Two treatments were set up in the experiment: S32 treatment (1.0×10^5 cfu/grain) and control without bacterial agent (Ctrl). 750 kg/ha complex fertilizer (15% N, 15% P_2O_5 and 15% K_2O) and 150 kg/ha urea was spread at the sowing stage of wheat growth. For field experiments, experimental plots were 150 m^2 (= 1.5 × 100 m) and 0.02 kg grains were sowing per 1 m^2 using mechanical drilling. The two groups of wheat seeds were sown at the same time, with three replicates for each treatment. After 210 days of sowing, 12 wheat plants were randomly selected to determine the shoot length, shoot dry weight, root dry weight and panicle number. The 1000-grain weight and yield of wheat were measured during the harvest period.

The field experiment of tomato (Provence) greenhouse was carried out in Xixiaozhai (107°59′20″E, 34°14′40″N) Village (Yangling, Shaanxi Province, China), from October 2021 to June 2022. Two treatments were set up in the experiment: S32 treatment (1.0×10^{10} cfu/plant) and control without bacterial agent (Ctrl). The substrate seedling was transplanted when the four foliar, and the cultivation mode was one ridge and two rows. Four replicates/ridges for each treatment (28 plants per ridge). During the whole growth period, 1.5×10^4 kg/ha organic fertilizer was applied, and the total amount of N-P_2O_5-K_2O application was 187.5–213.6-82.4 kg/ha. 12 plants were randomly selected for destructive sampling. The shoot length, root length, stem diameter, shoot dry weight and/or root dry weight were measured after 60 days and 160 days of growth. The total tomato yield of each treatment was counted.

Pathogen antagonism test

The plate standoff method was used to test the antagonistic activity of *S. rochei* S32 against eight plant pathogenic fungi that are commonly found in the

northwest region of China (Soliman et al. 2022). Briefly, *S. rochei* S32 was grown on agar plates of GA medium at 28 °C for 8 d (Xu et al. 2015). The fungal strains (Table 3) were provided by the College of Life Sciences (Northwest A&F University) and cultured on potato dextrose agar (PDA) plates at 30 °C for 5 d (Okull et al. 2003). Agar plugs of *S. rochei* S32 and pathogenic fungi were prepared under aseptic conditions using an 8-mm punch. One plug of *S. rochei* S32 was placed at the center of a fresh PDA plate, with three plugs of a given fungus placed around it in a triangular shape. There were three replicates for each fungus and all plates were incubated in an incubator at 28 °C for 5 d. Colony growth was measured to determine the strength of antagonistic activity. The growth inhibition rate of pathogenic fungi was calculated as follows: inhibition rate (%) = (fungal colony radius in the control – fungal colony radius in the *S. rochei* S32 treatment) / fungal colony radius in the control × 100%.

Whole-genome sequencing, gene annotation, and functional analysis

The whole genome of *S. rochei* S32 was sequenced using a Pacbio sequel II and DNBSEQ platform at Beijing Genomics Institute (Shenzhen, China). Low-quality and short reads were filtered from the sequencing data before downstream analysis. Clean data were assembled to obtain genomic data using Canu v1.5 (https://github.com/marbl/canu/releases) and GATK v1.6–13 (http://www.broadinstitute.org/gatk/). The whole-genome sequence was used to predict coding genes by Glimmer v3.02 (http://www.cbcb.umd.edu/software/glimmer/). Then, the predicted gene sequences were annotated by comparison against available databases (e.g., CAZy, COG, GO, KEGG, antiSMASH). Comparative gene mapping was conducted using Circos v0.69–9 (Krzywinski et al. 2009).

Functional classification of annotated genes was accomplished based on the Gene Ontology (GO) database (http://geneontology.org/). Prediction of secondary metabolite biosynthesis was performed using the antiSMASH v5.2.0 database (https://antismash-db.secondarymetabolites.org/). Prediction of phytohormone and siderophore biosynthesis was carried out based on metabolic pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) v101 database (https://www.genome.jp/kegg/). Prediction of chitinase, cellulase, β-1,3-glucanase, and other enzymes related to plant growth promotion and disease resistance was done using the Carbohydrate-active Enzymes (CAZy) database (http://www.cazy.org/).

Secondary metabolite profiling

S. rochei S32 was inoculated to the GA medium without agar and cultured at 30 °C for 8 d with 180 rpm oscillation. The culture sample was filtered through a 0.22-μm microporous filter membrane (Xiangya Instrument, Shanghai, China), and the filtrate was used for widely targeted metabolomics. First, a high-resolution time-of-flight system (Tremsin et al. 2007) was employed to scan the samples with secondary spectra and extract ion-pair information in the multiple reaction monitoring mode. Then, a specific database of the samples was constructed based on the wide-target library. A quadrupole-linear ion trap mass spectrometer (AB Sciex, Pte. Ltd., Singapore) was used to detect the compounds in the library and given a report. Statistical tools such as ANOVA were used to evaluate and process the data.

Phytohormone assay was also conducted with the culture filtrate samples. A methanol/water/formic acid solution (15:4:1, v/v/v) was used for the original extraction of phytohormones (Floková et al. 2014; Li et al. 2016). The extract was concentrated and reconstituted with an 80% methanol/water solution (4:1, v/v), and then filtered through a 0.22-μm microporous filter membrane. The filtrate was used for the detection of phytohormones and their analogues by ultra-performance liquid chromatography/tandem mass spectrometry (Q-TRAP® 6500, AB Sciex Pte. Ltd.). The peak plots of the downstream data were processed using the Analyst v1.6. software 3 (AB Sciex Pte. Ltd.). Qualitative analysis of the compounds in the samples was carried out based on the retention times and peak shapes of the standards. The relative concentration of each compound was calculated from the peak area, and its absolute concentration was calculated from the concentration of the standard.

Mechanism studies of plant growth promotion

Phosphorus and potassium solubilization. *S. rochei* S32 was inoculated onto agar plates of

phosphorus- and potassium-solubilizing media (Yuan et al. 2015). Colony growth was observed after the inoculated plates were incubated in an incubator at 28 °C for 5–7 d. If a transparent circle (i.e., halo) was formed around the bacterial colony, it indicates that *S. rochei S32* has the ability to solubilize phosphorus or potassium. The cross method was used to measure the colony diameter (d) and halo diameter (D). The D/d ratio was calculated to indicate the phosphorus- and potassium-solubilizing activity. The higher the D/d ratio, the greater the nutrient-solubilizing activity of *S. rochei* S32.

Nitrogen fixation. S. rochei S32 was inoculated onto Ashby's agar plates containing no nitrogen source (Ma et al. 2022). Colony growth was observed and recorded after incubation at 30 °C for 96 h. If colonies formed on the plates, they were transferred to fresh Ashby's agar plates and continuously subcultured three times. If colonies still formed in the third generation, it was considered that *S. rochei* S32 has the ability to fix atmospheric nitrogen.

ACC deaminase production. S. rochei S32 was inoculated onto agar plates of ADF medium, in which ACC was used to replace $(NH4)_2SO_4$ in DF medium (Bai 2017). The inoculated plates were incubated at 28 °C for 7 d before the observation of colony growth. If colonies formed on the plates, they were transferred to fresh ADF agar plates and continuously subcultured three times. If colonies still formed in the third generation, it indicates that *S. rochei* S32 can produce ACC deaminase.

Siderophore production. The ability of *S. rochei* S32 to produce siderophores was determined by chrome azurol sulfonate (CAS) assay (Milagres et al. 1999). It is theorized that when the sample contains siderophores, they can chelate iron ions to form a complex; once the complex is broken, red CAS is released, forming a yellow or orange yellow halo in the solid medium. *S. rochei* S32 was inoculated onto agar plates of GA medium and incubated at 28 °C for 8 d. The CAS detection reagent was sterilized and cooled to 40–50 °C, with a 10-mL aliquot added to each GA agar plate. The change in the color of the plate was observed 1 h later.

Data analysis

Excel v2016 (Microsoft Corp., Redmond, USA) and SPSS v26.0 (International Business Machines Corp., New York, USA) were used to test for the significance of differences between group means. Origin v2022 (OriginLab, Massachusetts, USA) was used for graphing.

Results

Plant growth-promoting effect of *S. rochei* S32 on two crops

Compared with the control group, the addition of 400-fold diluted cell-free fermentation broth significantly promoted the growth of wheat plants (Fig. S1). This effect was manifested by significantly increased shoot length (24.7%) and root length (25.3%) after 15 d of treatment (Fig. 1a). The addition of cell-free fermentation broth also improved the growth of tomato plants mainly in terms of root length (Fig. S2). When treated with 100-, 200-, and 400-fold diluted cell-free fermentation broth, the root length of tomato increased by 9.7%, 40.9%, and 7.8%, respectively, compared with the control group (Fig. 1b).

In the wheat field experiment, the shoot length, shoot dry weight, root dry weight and panicle number increased after 210 days of growth, and the 1000-grain weight and yield increased by 11.8% and 13.2%, respectively (Table 1), compared with the control group.

In the field experiment of tomato greenhouse, the shoot length, root length and stem diameter of tomato increased significantly after 60 days of growth. After 210 days of growth, shoot length, stem diameter, shoot dry weight and root dry weight also increased. In terms of yield, after adding strain S32, the yield increased by 6.2% (Table 2).

Antagonistic activity of *S. rochei* S32 against soil-borne pathogens

S. rochei S32 had no or weak antagonistic effect against *Sclerotinia sclerotiorum*, *F. graminearum*, and *F. oxysporum*. Its inhibitory effect on *Altenaria alternata*, *Macrophoma kawatsukai*, *Rhizoctonia cerealis*, *Colletotrichum orbiculare*, and *F. incarnatum* was striking (Fig. 2). The highest inhibition rate was observed for *M. kawatsukai* (65.0%), followed by *F. incarnatum* (49.8%) and *A. alternate* (48.1%; Table 3).

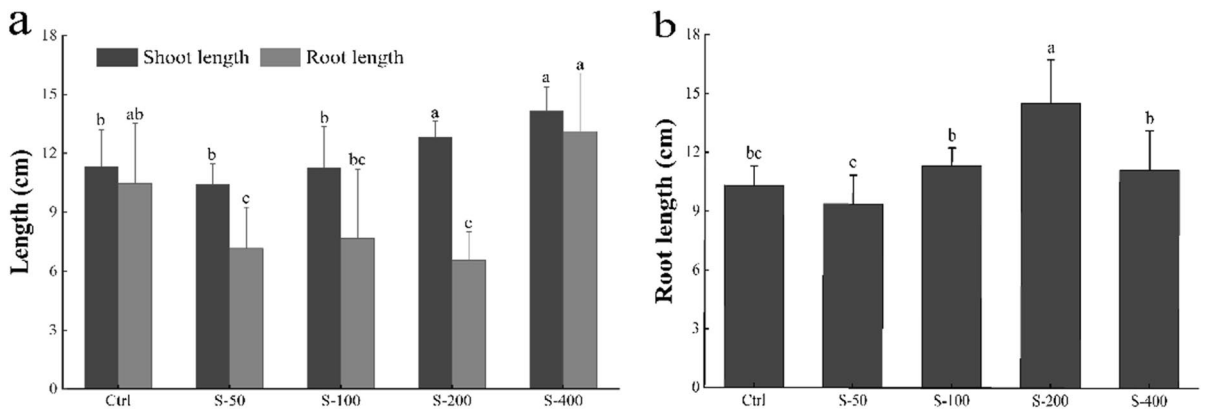

Fig. 1 Effect of *S. rochei* S32 on plant growth of wheat (**a**) and tomato (**b**) after 15 d of treatment. Figure shows changes in the plant height and root length of wheat (a) and root length of tomato (b) treated with 50-, 100-, 200-, and 400-fold diluted cell-free fermentation broth (S-50 to S-400). Data are the means ± standard deviation

Table 1 Effects of *S. rochei* S32 on plant growth and yield of wheat in experimental field

Index	210 d				1000-grain weight (g)	Yield (kg·acre^{-1})
	Shoot length (cm)	Shoot dry weight (g)	Root dry weight (g)	Panicle number		
Ctrl	86.1 ± 4.4	10.3 ± 0.8	2.1 ± 0.1	3.4 ± 1.8	34.0 ± 0.0	374.2 ± 31.1
S32	91.0 ± 3.9*	13.5 ± 1.4*	2.1 ± 0.0	5.2 ± 2.2*	38.0 ± 0.1*	423.6 ± 15.0*

Date are the means ± standard deviation. *, $P < 0.05$

Table 2 Effects of *S. rochei* S32 on plant growth and yield of tomato in Greenhouse experimental field

Index	60 d			210 d				Yield (kg·acre^{-1})
	Shoot length (cm)	Root length (cm)	Stem diameter (mm)	Shoot length (cm)	Stem diameter (mm)	Shoot dry weight (g)	Root dry weight (g)	
Ctrl	22.7 ± 1.5	8.8 ± 0.8	3.2 ± 0.2	203.2 ± 5.7	13.7 ± 0.6	13.1 ± 0.6	4.5 ± 0.3	10,421.6 ± 65.0
S32	29.4 ± 1.4*	10.8 ± 1.7*	3.8 ± 0.1*	234.5 ± 9.8*	16.4 ± 0.6*	18.0 ± 2.1	5.1 ± 0.2*	11,064.1 ± 244.6*

Date are the means ± standard deviation. *, $P < 0.05$

Genomic information of *S. rochei* S32

The whole genome of *S. rochei* S32 contains a linear chromosome (Fig. 3) and two plasmids. The total length of the chromosome is 8,041,158 bp with a GC ratio of 72.5%. The size and GC ratio of plasmid 1 are 171,061 bp and 69.4%, respectively. The size and GC ratio of plasmid 2 are 95,155 bp and 69.4%, respectively. The general characteristics of the bacterial genome are summarized in Table 4.

A total of 7726 coding genes were predicted in the whole genome, and 7486 (96.8%) of them were annotated with specific functions. The number of genes annotated based on different databases was 338 (4.4%) in VFDB, 32 (0.4%) in ARDB, 433 (5.6%) in CAZy, 6133 (79.4%) in IPR, 2364 (30.6%) in Swiss-Prot, 3 (0.0%) in CARD, 7387 (95.6%) in NR, 1459 (18.9%) in T3SS, 5293 (68.5%) in COG, 4208 (54.5%) in GO, and 3866 (50.0%) in KEGG.

In the GO-based gene function classification, there were 6335, 1155, and 5054 genes involved in biological processes, cellular components, and molecular functions, respectively (Fig. 4). The three functional categories were further divided into 17, 3, and 11

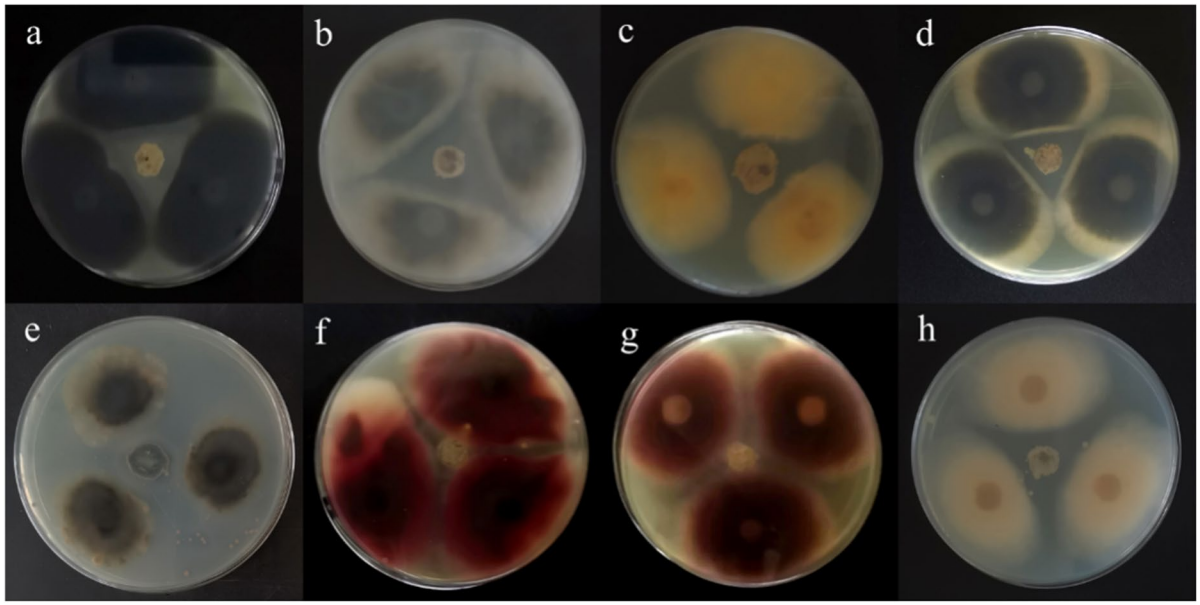

Fig. 2 Antagonistic activity of *S. rochei* S32 against eight pathogenic fungi grown on potato dextrose agar plates. *S. rochei* S32 was inoculated at the center, and a given pathogenic fungus was inoculated around it in a triangle shape. **a** *Altenaria alternata*; **b** *Macrophoma kawatsukai*; **c** *Rhizoctonia cerealis*; **d** *Colletotrichum orbiculare*; **e** *Sclerotinia sclerotiorum*; **f** *Fusarium graminearum*; **g** *Fusarium oxysporum R1*; and (**h**) *Fusarium incarnatum*

Table 3 Growth inhibition rate of pathogenic fungi by *S. rochei* S32 grown on potato dextrose agar plates (28 °C for 5 d)

Soil-borne pathogen	Plant disease	Growth inhibition rate (%)
Altenaria alternata	Tobacco brown spot	48.1 ± 4.0
Macrophoma kawatsukai	Apple ring rot	65.0 ± 2.3
Rhizoctonia cerealis	Wheat sharp eyespot	33.4 ± 5.1
Colletotrichum orbiculare	Cucumber anthracnose	41.3 ± 2.3
Fusarium incarnatum	Muskmelon fruit rot	49.8 ± 4.8

Values are the means ± standard deviation

subcategories, respectively. The main subcategories included "cellular processes" (1959) and "metabolic processes" (2179) in biological processes, as well as "binding" (1837) and "catalytic activity" (2495) in molecular functions.

Prediction of gene functions in plant growth promotion

Phytohormone biosynthesis KEGG pathway enrichment analysis revealed the biosynthesis of L-TRP and indole (two precursors for IAA biosynthesis) via the TRP biosynthesis pathway in *S. rochei S32*. Among the common pathways of IAA biosynthesis with TRP as a substrate, the indole-3-acetamide (IAM) pathway, the indole-3-pyruvate (IPA) pathway, the tryptamine (TAM) pathway, and the indole-3-acetaldehyde oxime (IAOx) pathway were all incomplete in the TRP metabolic pathway (Figs. S3, S4). However, only the *iaam* gene was missing in the IAM pathway (Fig. S3), so IAA was more likely to be synthesized via the IAM pathway at the gene level.

Biosynthesis of fungal cell wall hydrolases The results of CAZy enzyme activity analysis showed the presence of numerous genes encoding fungal cell wall hydrolases in the chromosome of *S. rochei* S32. There were 185 genes for glycoside hydrolases, 113 genes for glycosyl transferases, 30 genes for carbohydrate esterases, 132 genes for carbohydrate-binding modules, 9 genes for auxiliary activities, and 12 genes

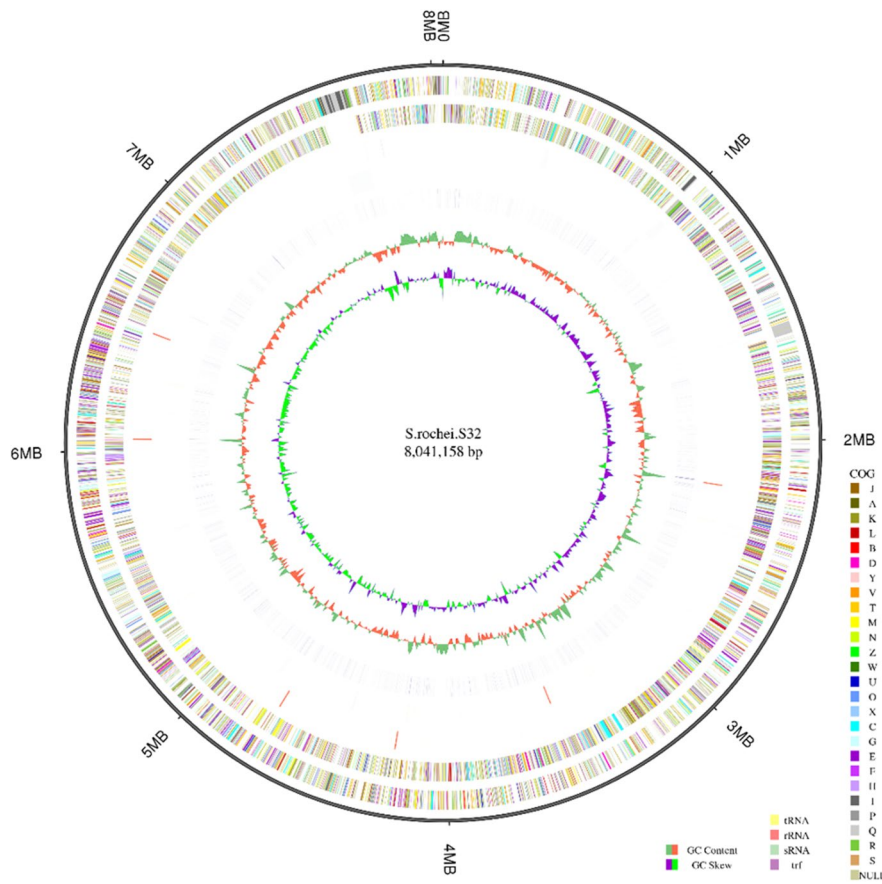

Fig. 3 A comparative gene map of *S. rochei S32* constructed using Circos. The features from outer to inner circle are genome size (circle 1), forward (circle 2) and reverse strand genes (circle 3) colored by cluster of orthologous groups, forward (circle 4) and reverse strand non-coding RNAs (circle 5), repeat (circle 6), GC (circle 7), GC-skew calculated as (nG – nC) / (nG + nC) (circle 8). The green color is skew+, which means that the content of G is greater than that of C; the purple color is skew–, which means that the content of G is less than that of C

Table 4 Genome characteristics of *S. rochei* S32

Characteristic	Chromosome	Plasmid 1	Plasmid 2
Genome size (bp)	8,041,158	171,061	95,155
G + C content (%)	72.5%	69.4%	69.4%
Ribosomal RNAs	18	–	–
Transfer RNAs	66	–	–
Small non-coding RNAs	51	–	–
Coding sequences	7437	180	109
Prophage	8	1	1
CRISPR	5	10	1
GenBank accession No.	CP133098	CP133099	CP133100

for polysaccharide lyases. Genes encoding the main enzymes that hydrolyze fungal cell walls, including chitinase, cellulase, and β-1,3-glucanase, were predicted (Fig. 5).

Secondary metabolite biosynthesis Based on the antiSMASH database, a total of 30 biosynthetic gene clusters (BGCs) were predicted in the chromosome of *S. rochei* S32. Twelve BGCs had >80% similarity to known clusters with high confidence (Table 5). Among them, the products of six BGCs were identified: hopene, geosmin, albaflavenone, ectoine, isorenieratene, and 7-prenylisatin. Despite the unknown products of the remaining BGCs, they showed high similarities to those encoding borrelidin, lipopeptide, streptothricin, candicidin, and desferrioxamine B/E antibiotics. These results indicate that *S. rochei* S32 has the potential to produce antibiotics targeting nucleic acids, macrolides, and peptides, as well as siderophores. Other BGCs were not annotated in available databases or had low similarity to known natural BGCs, suggesting that *S. rochei* S32 is likely to synthesize a wide range of unknown natural products.

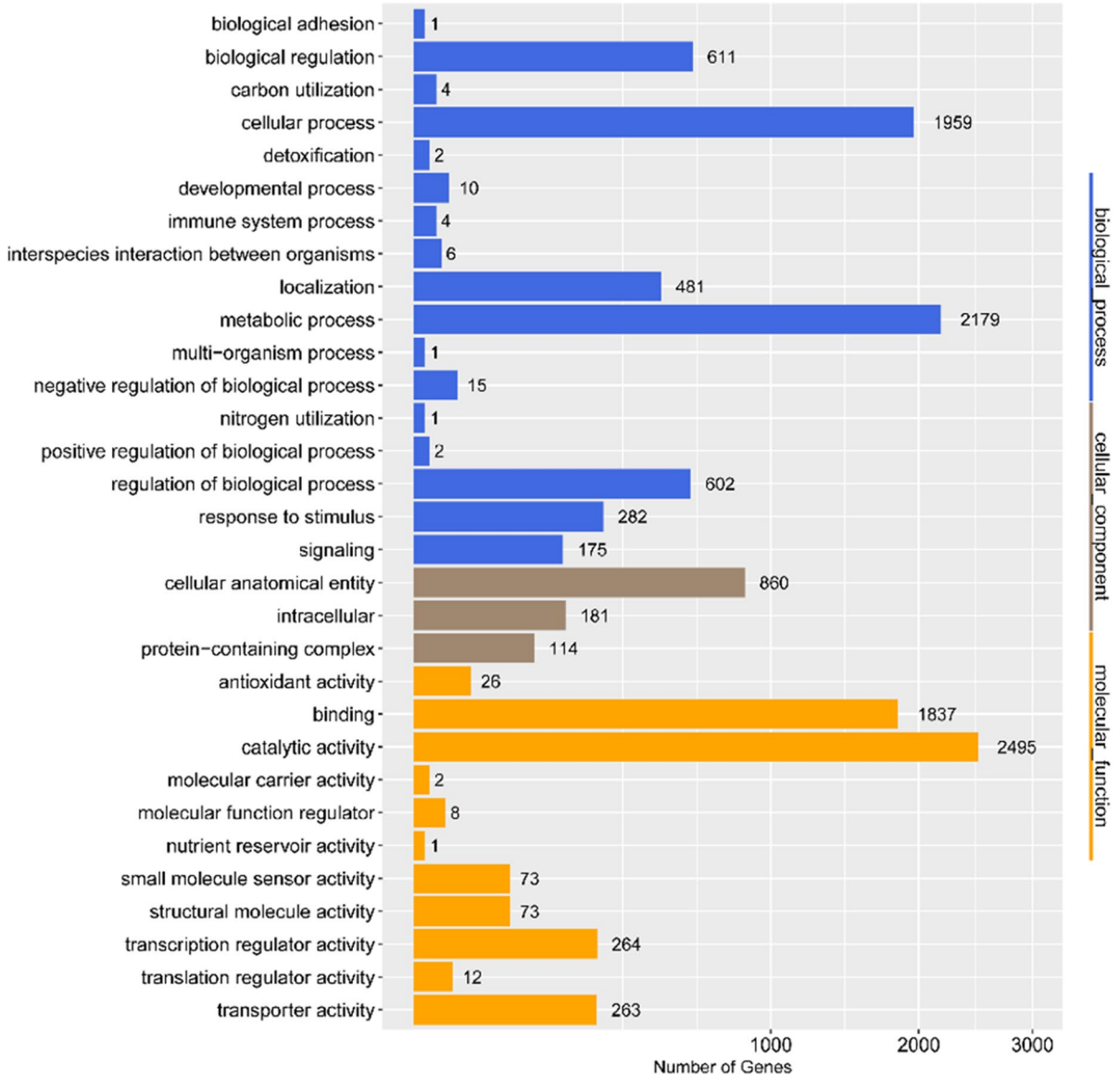

Fig. 4 GO annotation results of the *S. rochei* S32 genome

Phytohormone and metabolome profiles

Thirty-nine phytohormones were detected in the culture filtrate of *S. rochei* S32 (Fig. 6). The phytohormones were classified into seven categories: auxins, cytokinins (CKs), jasmonates (JAs), gibberellins (GAs), salicylic acid (SA), strigolactones (SLs), and abscisic acid (ABA). Additionally, 2205 secondary metabolites were detected. Among the top 200 most frequently detected metabolites (Fig. 7), "benzene and substituted derivatives" (28), "heterocyclic compounds" (25), "organic acid and its derivatives" (22), "small peptides" (19), and "sugars" (15) were relatively abundant. These metabolites included biologically active compounds, such as phytosphingosine, fosfomycin calcium, aspulvinone E, haloprogin, acivicin, nordihydrocapsiate, and corynebactin (Table S1).

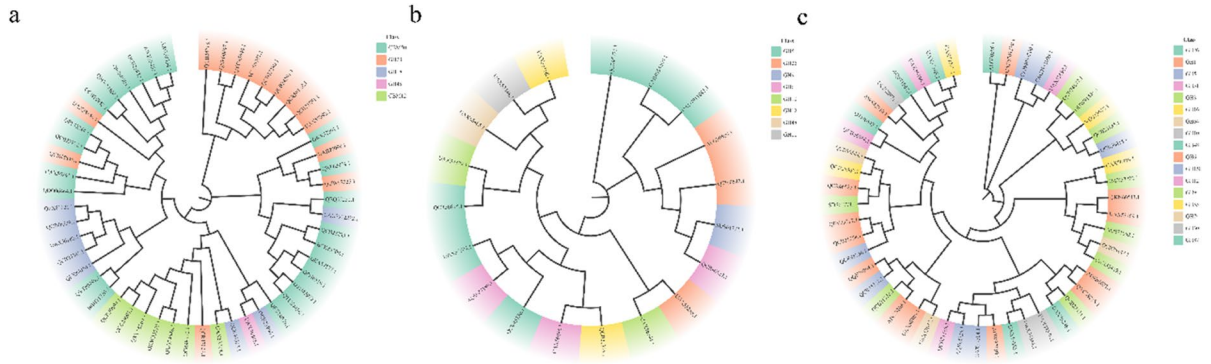

Fig. 5 Phylogenetic trees of fungal cell wall hydrolase gene family members in the genome of *S. rochei* S32. **a** Chitinase; **b** Cellulase; and **c** β-1,3-Glucanase

Table 5 Twelve predicted gene clusters of secondary metabolites in the genome of *S. rochei* S32

Region	Cluster category	Length (bp)	Metabolite type	Metabolite name	Cluster blast similarity (%)
1	Lanthipeptide-class-iii	20,781	Lanthipeptide	SAL-2242	88%
2	T1PKS/NRPS	97,198	Polyketide	Borrelidin	81%
3	Terpene	25,919	Terpene	Hopene	100%
4	NRPS	79,373	Non-ribosomal peptide	Lipopeptide 8D1–1/8D1–2	84%
5	Terpene	19,913	Terpene	Geosmin	100%
6	Terpene	20,630	Terpene	Albaflavenone	100%
7	Siderophore	11,772	Other	Desferrioxamine B/E	83%
8	Ectoine	10,398	Other	Ectoine	100%
9	NRPS-like	41,145	Non-ribosomal peptide	Streptothricin	95%
10	hglE-KS/T1PKS/NRPS-like/NRPS	208,122	Polyketide	Candicidin	95%
11	Terpene	25,581	Terpene	Isorenieratene	100%
12	Indole	21,178	Other	7-Prenylisatin	100%

T1PKS Type I polyketide synthases, *NRPS* Non-ribosomal peptide synthetase, and *KS* Heterocyst glycolipid synthase-like PKS

Mechanisms of plant growth promotion

S. rochei S32 grew normally on the organophosphorus-solubilizing medium, inorganic phosphorus-solubilizing medium, and potassium-solubilizing medium, despite no halos (Fig. 8a). Normal bacterial growth also occurred on the nitrogen-free medium after three subcultures (Fig. 8b). These results indicate that *S. rochei* S32 has the ability to fix atmospheric nitrogen, but not to solubilize phosphorus or potassium. Poor colony growth was observed on the ADF medium with ACC as the sole nitrogen source (Fig. 8c), indicating no production of ACC deaminase. However, siderophores were produced on CAS plates, as indicated by the halo with a distinct orange color around colonies (Fig. 8d).

Discussion

In the present study, we verified the plant growth-promoting effect of *S. rochei* S32 on two different crops and its antagonistic activity against five soil-borne pathogenic fungi through petri dish experiments. We then deciphered the underlying molecular mechanisms based on whole-genome

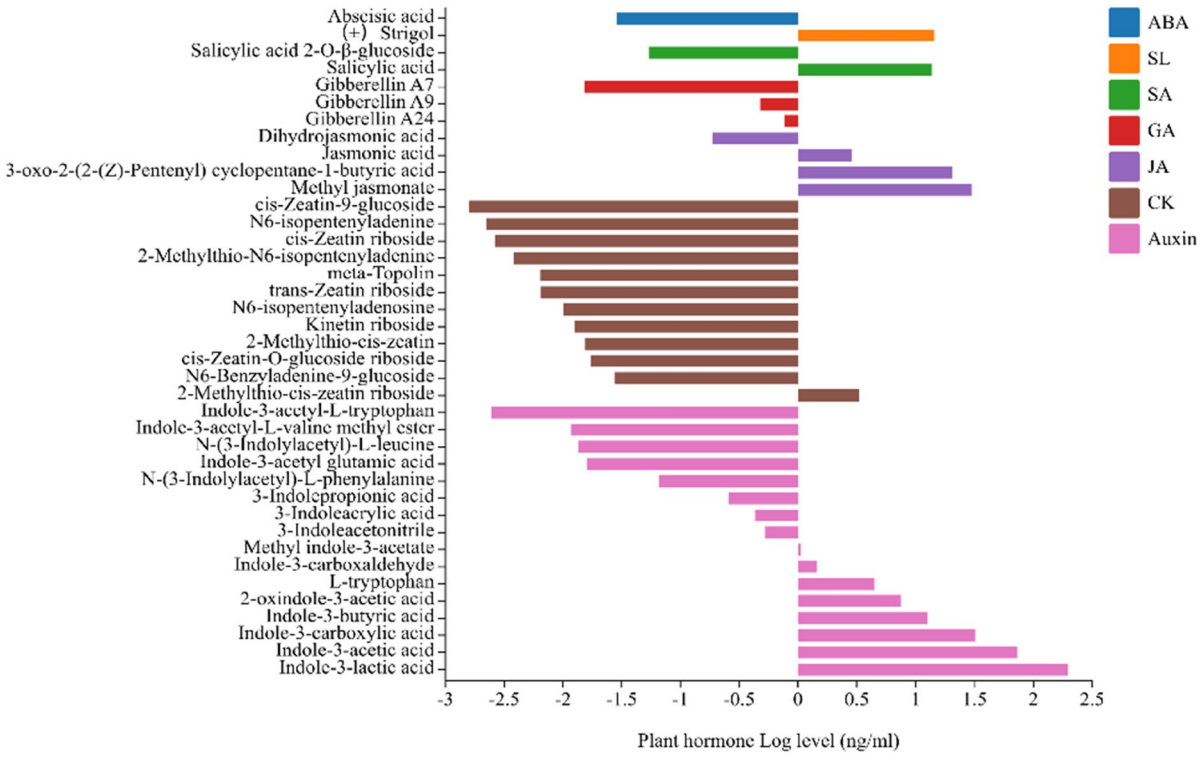

Fig. 6 Seven categories of phytohormones secreted by *S. rochei* S32. CK, cytokinins; JA, jasmonates; GA, gibberellins; SA, salicylic acidl SL, strigolactones; and ABA, abscisic acid

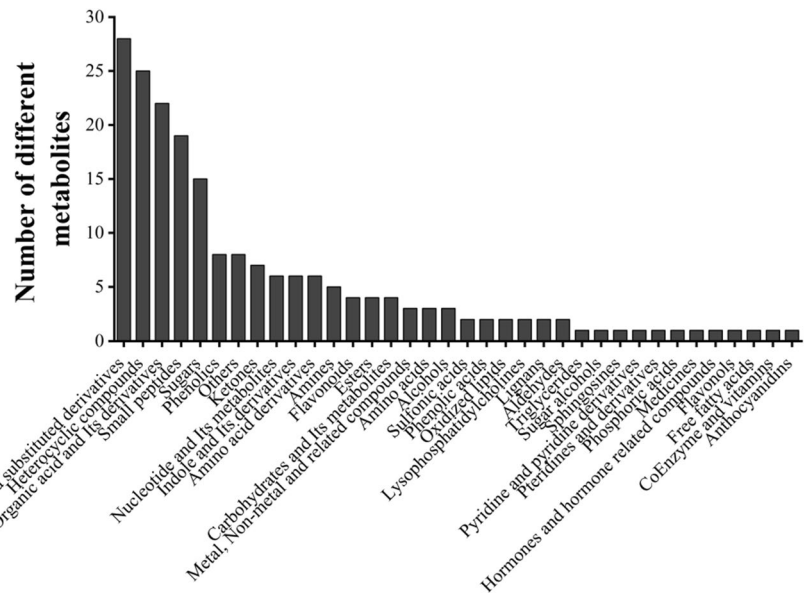

Fig. 7 Secondary classification of the top 200 most frequently detected metabolites from *S. rochei* S32

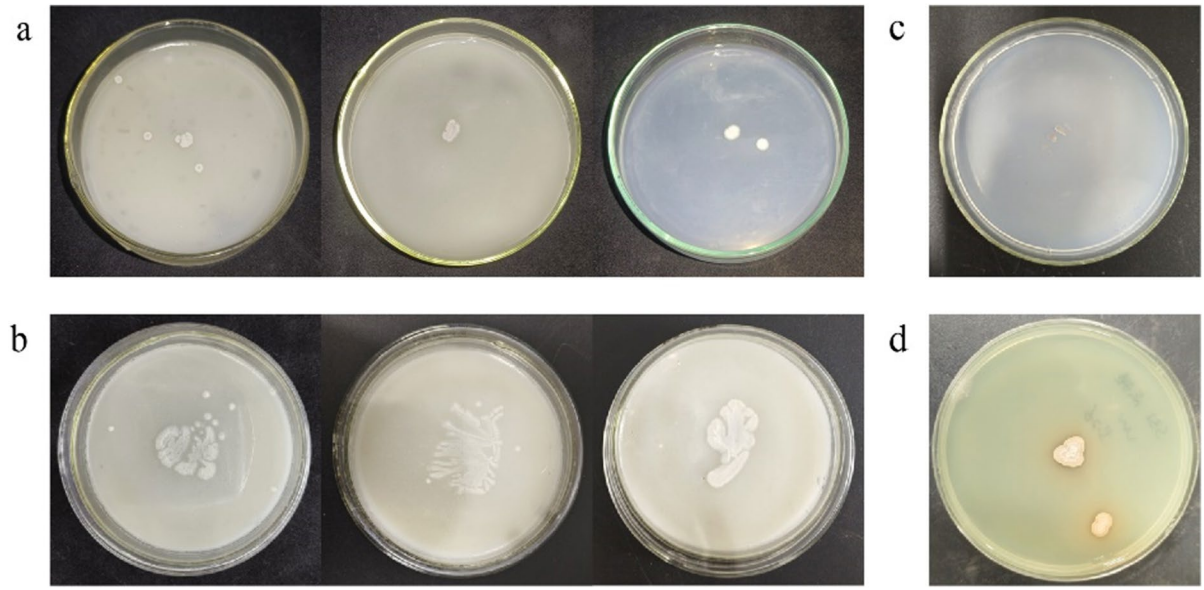

Fig. 8 Colony growth of *S. rochei* S32 on different media. **a** Organophosphorus-solubilizing medium, inorganic phosphorus-solubilizing medium, and potassium-solubilizing medium (from left to right); **b** Nitrogen-free medium, first to third subculture (from left to right); **c** ADF medium; and **d** CAS medium

sequencing, widely targeted metabolomics, and mechanism studies of plant growth promotion. The results indicate that *S. rochei* S32 can promote plant growth through phytohormone biosynthesis (e.g., IAA), nitrogen fixation, and production of extracellular hydrolases, antimicrobial metabolites, and siderophores. Our findings can enable proper application of *S. rochei* S32 as a PGPR strain for sustainable agriculture.

Generally, when the plants were treated with the cell-free fermentation broth of *S. rochei* S32, their growth was inhibited with a low dilution factor. With an increase in the dilution factor, the effect of plant growth promotion was first enhanced and then diminished until it disappeared. The special pattern of wheat root growth (200-fold dilution) is likely due to the influence of external environmental factors (e.g., water). When treated with the highly diluted cell-free fermentation broth, the growth performance of wheat and tomato seedlings in petri dishes was substantially improved in terms of plant height and/or root length. In the field experiment, the growth of wheat and tomato was also improved compared with the control group. This effect can be at least partially attributed to IAA biosynthesis and secretion by *S. rochei* S32 (Alemneh et al. 2022; Sun et al. 2022).

Phytohormones are low-molecular-weight metabolites that play a vital role in regulating plant growth and development. Auxins, mainly represented by IAA, control cell elongation, apical dominance, and the development of main and lateral roots in plants (Sabatini et al. 1999; Wang et al. 2001). IAA also facilitates root exudation by relaxing root cell walls, and as such, improve plant growth (Pang et al. 2022). *S. rochei* S32 has the ability to synthesize L-TRP and indole as precursors for IAA biosynthesis, based on the results of KEGG pathway enrichment analysis. Indole biosynthesis was also predicted in the secondary metabolite gene cluster, providing strong evidence for IAA biosynthesis at the gene level. The predicted results of IAA biosynthesis were confirmed by the detection of L-TRP and IAA in the phytohormone profile of *S. rochei* S32.

As the primary precursor for IAA biosynthesis (Choix et al. 2018), TRP is converted into IAM through TRP monooxygenase encoded by the *iaam* gene. Then, IAM hydrolase encoded by the *iaah* gene, which is the last key enzyme for IAA biosynthesis, catalyzes the conversion of IAM to synthesize IAA (AI-Hosni et al. 2018; Wu et al. 2022). The *S. rochei* S32 genome contains the *iaah* gene (Fig. S3), but lacks the *iaam* gene which was first discovered in

Agrobacterium tumfaciens and *Pseudomonas savastanoi* (Aragón et al. 2014). These findings allow us to posit that *S. rochei* S32 synthesizes IAA mainly through the IAM pathway, and this process is likely to involve interactions with other soil microorganisms.

In addition to auxins, *S. rochei* S32 was found to produce phytohormones of other categories. Among them, CKs and GAs play a role in promoting plant growth (Liu et al. 2023; Rivas et al. 2022; Yamaguchi 2008), whereas JAs, SA, SLs, and ABA mainly mediate biotic and abiotic stresses (Beigi et al. 2023; Keskin et al. 2010; Liu et al. 2023; Ma et al. 2023). *S. rochei* S32 produced various phytohormones without any addtions, providing a possible mechanism for plant growth promotion and disease control.

Nitrogen is essential for plant growth (Fateme et al. 2023), and nitrogen fixation is considered to be one of the direct mechanisms by which *Streptomyces* spp. promote crop growth (Vurukonda et al. 2018). *S. rochei* S32 showed an ability to fix atmospheric nitrogen, based on its normal growth on the nitrogen-free medium for at least three generations. Therefore, *S. rochei* S32 contributes to plant growth by converting atmospheric inorganic nitrogen into organic nitrogen that is available for plant absorption (Fowler et al. 2013). Similarly, a nitrogen-fixing strain of *Burkholderia australis* sp. nov. promotes plant growth of sugarcane by increasing root and shoot biomass (Paungfoo-Lonhienne et al. 2014).

Infection with soil-borne plant pathogens seriously affects crop health and yield (Lamichhane et al. 2023). Under the experimental conditions, inoculation with *S. rochei* S32 hindered the growth of most of the pathogenic fungi tested. This inhibitory effect varied with fungal species and was possibly due to the production of diverse secondary metabolites with biological activities. However, it may also be due to the competition between microorganisms for growth space or different growth rates. Based on the CAZy database, *S. rochei* S32 was predicted to produce chitinase, cellulase, and β-1,3-glucanase. These hydrolases can degrade fungal cell walls and curb hyphal growth (Luo et al. 2023; Woith et al. 2021). Additionally, 12 BGCs with high similarity to known gene clusters that encode antimicrobial substances were predicted in the whole genome of *S. rochei* S32. Antimicrobial substances (e.g., antibiotics) produced by bacteria can inhibit the growth of pathogens (El-Sharkawy and Abdelrazik 2022).

Among the possible products of the BGCs predicted in the *S. rochei* S32 genome, candicidin is a polyene antifungal agent that binds to ergosterol and primarily affects cell membrane permeability and integrity (Barke et al. 2010). Thus, candicidin can serve as a target to screening for biocontrol microorganisms (Yao et al. 2021). Lipopeptide and borrelidin are both antimicrobial peptides synthesized by non-ribosomal peptide synthetase. Lipopeptide is a biosurfactant that exerts a broad-spectrum antimicrobial effect by disrupting the integrity of cell outer and inner membranes (Dasgupta et al. 2023; Zhang et al. 2023). Borrelidin, an inhibitor of threonine transfer RNA synthase (Li et al. 2014), inhibits protein synthesis in bacteria, fungi, and viruses (Shirokikh et al. 2023). Streptothricin also inhibits protein synthesis in bacteria and provides an effective bactericidal antibiotic against Gram-negative bacteria (Morgan et al. 2023). 7-Prenylisatin is a derivative of indole, which has prominent bactericidal activity against *Bacillus subtilis* (Wu et al. 2015). Albaflavenone is a tricyclic sesquiterpene antibiotic with antibacterial effects and a similar aroma to geosmin (Gürtler et al. 1994). Geosmin is a specific characteristic product of *Streptomyces* culture (Becher et al. 2020), with no clear antibacterial effects. However, geosmin can interact with neighboring microorganisms and drive bacterial response or adaptation to environmental changes, affecting interspecific competition and cooperation (Audrain et al. 2015). All these secondary metabolites have been reported to have antibacterial activity, which can enable *S. rochei* S32 to benefit plant growth by antagonizing pathogenic microorganisms.

Unlike the antimicrobial metabolites, desferrioxamine specifically chelates iron in the environment (Mahajan et al. 2021). Desferrioxamine-producing microorganisms mainly compete with harmful microorganisms for the iron element. This competition would hinder the growth of harmful microorganisms and slow down pathogenic infection, thereby promoting plant growth (Jarmusch et al. 2021). Siderophore production is also one of the mechanisms that allow *S. rochei* S32 to promote plant growth. In addition to iron, siderophores chelate other metal cations, reducing their free concentration and toxicity (Schalk et al. 2011). Compared with common metal chelating agents, siderophores are biodegradable and pollution-free in the soil environment (Dimkpa et al. 2009). Among the metabolites of *S. rochei* S32, we detected

the presence of corynebactin, a cyclic catecholate siderophore (Budzikiewicz et al. 2014). Corynebactin has a unique structure with methylation of trilactone ring and glycine spacer, which is more stable than enterobactin and mediates iron transport (Raymond et al. 2003). Despite abundant predictable BGCs at the gene level, only a portion of them can be actually expressed to produce detectable levels of secondary metabolites, depending on the culture conditions and substrates (Nofiani et al. 2023).

Furthermore, we found that the metabolites produced by *S. rochei* S32 included benzene and its derivatives, heterocyclic compounds, organic acids and their derivatives, small peptide and diverse antibiotics (Table S1). The biosynthesis of aromatic volatiles can activate plant growth, defense responses, and abiotic stress resistance (Mares-Rodriguez et al. 2023). Organic acids and their derivatives can penetrate the cell membrane of bacteria and acidify their cytoplasm to exhibit outstanding antibacterial effects (Kovanda et al. 2019). Small peptides can be recognized by plants to regulate immune responses to pathogens (Lyapina et al. 2021). All these metabolites are expected to confer the ability of *S. rochei* S32 to enhance plant growth and resist pathogens.

In summary, when *S. rochei* S32 is applied to crops, it contributes to plant growth and resistance to pathogenic fungi through the production of phytohormones, hydrolases, bioactive metabolites (including antibiotics), and siderophores, as well as nitrogen fixation. This is despite the fact that the relative contributions of various mechanisms cannot be quantified. Based on the functional analysis and verification of genomic analysis results, this study provides novel insights into the known and potential mechanisms of how *S. rochei* S32 promotes plant growth and antagonizes soil-borne pathogens. Our findings offer guidance for the application of *S. rochei* S32 as a PGPR strain in agriculture and further exploration of known and unknown secondary metabolites produced by this bacterial strain. While this study has only looked at the possible effects and mechanisms of *S. rochei* S32 on crop plants, further research is needed to ascertain plant responses to the bacterial strain and its possible interactions with other microorganisms in the soil environment.

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Author contributions All authors contributed to the study conception and design. WM wrote the manuscript and completed data collection and analysis. JMF, NXB, WCC, YXC and LYT performed material preparation and participated in some experiments. WM and WXM reviewed the manuscript.

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Data availability The data sets generated during the current study are available in the NCBI repository, under accession numbers: CP133098, CP133099 and CP133100, for genome, plasmid 1 and plasmid 2, respectively.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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