Interactional mechanisms of *Paenibacillus polymyxa* SC2 and pepper (*Capsicum annuum* L.) suggested by transcriptomics

Hu Liu, Yufei Li, Ke Ge, Binghai Du, Kai Liu, Chengqiang Wang* and Yanqin Ding*

**Abstract**

**Background:** *Paenibacillus polymyxa* SC2, a bacterium isolated from the rhizosphere soil of pepper (*Capsicum annuum* L.), promotes growth and biocontrol of pepper. However, the mechanisms of interaction between *P. polymyxa* SC2 and pepper have not yet been elucidated. This study aimed to investigate the interactional relationship of *P. polymyxa* SC2 and pepper using transcriptomics.

**Results:** *P. polymyxa* SC2 promotes growth of pepper stems and leaves in pot experiments in the greenhouse. Under interaction conditions, peppers stimulate the expression of genes related to quorum sensing, chemotaxis, and biofilm formation in *P. polymyxa* SC2. Peppers induced the expression of polymyxin and fusaricidin biosynthesis genes in *P. polymyxa* SC2, and these genes were up-regulated 2.93- to 6.13-fold and 2.77- to 7.88-fold, respectively. Under the stimulation of medium which has been used to culture pepper, the bacteriostatic diameter of *P. polymyxa* SC2 against *Xanthomonas citri* increased significantly. Concurrently, under the stimulation of *P. polymyxa* SC2, expression of transcription factor genes *WRKY2* and *WRKY40* in pepper was up-regulated 1.17-fold and 3.5-fold, respectively.

**Conclusions:** Through the interaction with pepper, the ability of *P. polymyxa* SC2 to inhibit pathogens was enhanced. *P. polymyxa* SC2 also induces systemic resistance in pepper by stimulating expression of corresponding transcription regulators. Furthermore, pepper has effects on chemotaxis and biofilm formation of *P. polymyxa* SC2. This study provides a basis for studying interactional mechanisms of *P. polymyxa* SC2 and pepper.

**Keywords:** *Paenibacillus polymyxa*, *Capsicum annuum*, PGPR, Interaction mechanisms, RNA-seq
growth of \textit{Fusarium oxysporum} f. sp. \textit{Niveum} by producing volatile organic compounds [16]. \textit{P. polymyxa} CR1 enhanced growth of maize, potato, cucumber, \textit{Arabidopsis}, and tomato plants through direct mechanisms such as phosphate solubilization and production of indole-3-acetic acid (IAA) [17]. \textit{P. polymyxa} BFKC01 promoted growth of \textit{Arabidopsis} by secreting IAA and promoting iron acquisition [18]. \textit{P. polymyxa} P2b-2R, an endophytic diazotroph of pine, might facilitate regeneration and growth of western red cedar at nitrogen-poor sites [19]. \textit{P. polymyxa} B2 promoted growth of winter wheat by increasing the available phosphorus in the soil [20]. \textit{P. polymyxa} CF05 promoted growth of tomato seedlings in the greenhouse [21], and \textit{P. polymyxa} SC2 was reported as a plant growth-promoting rhizobacterium isolated from the rhizosphere soil of pepper in Guizhou, China [13]. \textit{P. polymyxa} SC2 has a wide antimicrobial spectrum and antagonistic effects on various plant pathogens [22], including \textit{Fusarium vasinfectum} Atk., \textit{F. oxysporum} f. sp. \textit{cucumerinum}, \textit{Pseudoperonospora cubensis}, \textit{Botrytis cinerea} Pers, and \textit{Botrytis cinerea}. \textit{P. polymyxa} SC2 could promote pepper growth, but the molecular mechanisms underlying the interaction between \textit{P. polymyxa} SC2 and pepper remain unclear.

The development of omics technologies has led to interest in the interaction between PGPR and plants. A metabolomics study showed that \textit{Pseudomonas fluorescens} induced root formation in \textit{Sedum alfredii} by increasing the concentration of IAA and reducing the contents of abscisic acid, brassinolide, trans zeatin, ethylene, and jasmonic acid [23]. Transcriptome analysis of \textit{Arabidopsis thaliana} revealed that aluminum-activated malate transporter (ALMT1) plays an important role in \textit{Bacillus subtilis} FB17 colonization [24]. In response to rice seedlings, 43 genes related to metabolism or transport of carbohydrates or amino acids were significantly expressed in \textit{B. subtilis} OKB105 [25]. Singh et al. reported that \textit{Enterobacter cloacae} SBP-8 increased the tolerance of wheat to salinity stress through regulation of transcription factors, proteins of the Ninja family, and other defense-related enzymes and proteins [26]. These studies demonstrate that by using omics technologies some progress has been made in elucidating the interactions between \textit{Bacillus}, \textit{Enterobacter}, \textit{Pseudomonas}, and plants. However, studies on the interaction between \textit{P. polymyxa} and plants are limited. Kwon et al. reported that \textit{P. polymyxa} E681 increased the concentrations of tryptophan, indole-3-acetonitrile (IAN), IAA, and camalexin in the treated plants, and also activated defense-related proteins against fungal pathogens in plants [27]. Our group previously found that \textit{P. polymyxa} YCO136 promoted the growth of tobacco (\textit{Nicotiana tabacum} L.) by inducing hormone-related genes and systemic resistance genes in tobacco [28]. The present study aimed to understand the molecular mechanisms involved in the interaction between \textit{P. polymyxa} SC2 and pepper by conducting transcriptomic sequencing of co-cultured \textit{P. polymyxa} SC2-pepper samples.

**Results**

**Growth promotion characteristics of \textit{P. polymyxa} SC2 on peppers**

To identify growth promotion characteristics of \textit{P. polymyxa} SC2 on pepper, pot experiments in healthy soil and continuous cropping soil were performed in the greenhouse. In healthy soil, there were distinct differences in pepper growth between the \textit{P. polymyxa} SC2-treated group and the control group. At 30- and 40-days post-inoculation (dpi), stem diameters of peppers inoculated with \textit{P. polymyxa} SC2 were significantly thicker than those of the control group, with increases of 5.26 and 5.7%, respectively (\( P < 0.05 \); Fig. 1a). At 50 dpi, there was an extremely significant difference (\( P < 0.01 \)) in the stem diameter of peppers, with a 6.52% increase in the \textit{P. polymyxa} SC2-treated group compared with the control group. The growth status of pepper treated with \textit{P. polymyxa} SC2 in healthy soil at 40 dpi was shown in Fig. 1b. At 40 dpi, the indices of pepper leaves were evaluated (Table 1). The width and length of leaves in the \textit{P. polymyxa} SC2-treated group were 6.1 and 4.51% larger, respectively, than those of the control group. There was a significant difference (\( P < 0.05 \)) in chlorophyll content between the two treatment groups. Chlorophyll content in the \textit{P. polymyxa} SC2-treated group increased by 14% compared with the control group. These results indicated that \textit{P. polymyxa} SC2 promoted the growth of pepper in healthy soil.

In the continuous cropping soil, \textit{P. polymyxa} SC2 also promoted the growth of pepper, as evidenced by increases in the diameters of pepper stems at different harvest intervals (Fig. 1a). At 30 dpi, stem diameters in the \textit{P. polymyxa} SC2-treated group were 6.4% thicker than the control group (\( P < 0.01 \)), and at 40 and 50 dpi, the differences in pepper stem diameters between the two treatment groups were significant at the level of \( P < 0.05 \).

** Transcriptome analysis of co-cultured \textit{P. polymyxa} SC2 and pepper**

To study the molecular mechanisms involved in the interaction of \textit{P. polymyxa} SC2 and pepper, RNA-seq of \textit{P. polymyxa} SC2 and pepper co-cultured under a sterile environment was performed. After sequencing, about 156,499,912 and 158,836,654 raw reads were obtained in the pepper control group (marked as P) and pepper treated group (marked as PH) respectively. And about 72,827,434 and 92,447,218 raw reads were generated in the strain SC2 control group (labeled as S) and strain SC2 treated group (labeled as SH). For \textit{P. polymyxa} SC2
and pepper, 151,205,362 and 308,431,888 high-quality sequences were generated, respectively. Mapping of the transcriptome sequences with the whole-genome sequence of *P. polymyxa* SC2 indicated that the mapped percentage of each sample was more than 90% (the mapping proportion statistics was shown in Additional File 1: Table S1). In *P. polymyxa* SC2, 5014 genes mapped with the reference genome. The percentage of mapped genes in each pepper sample was higher than 85% (the mapping proportion statistics were shown in Additional File 1: Table S2). We also carried PCA analysis, and the results were shown in Additional File 2: Fig. S1. All results met the requirements for subsequent analyses.

Differentially expressed genes (DEGs) of *P. polymyxa* SC2 were detected based on the criterion of *p*-value < 0.05 and |log 2FC| > 2, while DEGs of pepper were detected according to *p*-value < 0.05 and |log 2FC| > 4. Genes with significantly up-regulated and down-regulated expression are shown in Fig. 2. In *P. polymyxa* SC2, there were 812 DEGs, of which 465 were up-regulated and 347 were down-regulated (Fig. 2a). Annotation information for these DEGs is displayed in Additional File 3: Table S3. The most significantly up-regulated genes of *P. polymyxa* SC2 were involved in polymyxin biosynthesis, fusaricidin biosynthesis, phosphatase/MFS transporter, acetolactate synthase, and 3-hydroxydecanoyl dehydratase, etc. The down-regulated significant genes were related to D-ribose transport subunit RbsB, Ribose ABC transporter, membrane protein, oxido-reductase and stress protein, etc. In pepper (*Capsicum annuum* L.), there were 758 DEGs, of which 573 were up-regulated and 185 were down-regulated (Fig. 2b). Annotation information for the DEGs in pepper is displayed in Additional File 4: Table S4. The most significantly up-regulated genes in pepper were involved in laccase, transcription regulator, protease inhibitors, proline dehydrogenase, reticulase, glutathione transferase, chaperones, and, etc. The most significantly down-regulated genes were involved in expansin, WAT1 related protein, bidirectional sugar transporter, vacuolar iron transporter homologue, and carbohydrate esterase, etc.

Verification of selected DEGs using RT-qPCR
To verify the accuracy of the RNA-seq data, selected genes in *P. polymyxa* SC2 and pepper were subjected to RT-qPCR amplification.

In RNA-seq, the genes (*pmxA, pmxB, pmxC, pmxD, and pmxE*) related to polymyxin synthesis in *P. polymyxa* SC2 were up-regulated by 2.93-, 4.95-, 5.13-, 6.13-, and 4.93-fold, respectively. In the RT-qPCR, compared with the control group, the expression of *pmxA/B/C/D/E* genes

![Fig. 1](image_url)

**Fig. 1** Interaction effects of *P. polymyxa* SC2 on pepper seedlings. In pot experiments, a pepper seedling was irrigated with 5 mL *P. polymyxa* SC2 cells (1 × 10^8^ CFU/mL) and diluted with water to 200 mL. Control plant was irrigated with 5 mL sterilized LB medium diluted with water to 200 mL. At 30, 40, and 50 dpi, stem diameters (diameter at the ground base) were investigated. Panel (a) shows pepper stem diameters in healthy (H) soil and continuous cropping (C) soil. Values indicate means ± SD (*n* = 9; *P* < 0.05, **P** < 0.01, Student’s *t*-test). Panel (b) is a representative image of the status of pepper in healthy soil at 40 dpi.

### Table 1

| Treatment  | Leaf length /cm | Leaf width /cm | Chlorophyll content / mg·g⁻¹ |
|------------|----------------|----------------|-------------------------------|
| Control    | 10 ± 0.67 a     | 4.45 ± 0.31 a   | 2.0277 ± 0.13178 a            |
| Strain SC2 | 10.61 ± 0.85 b  | 4.66 ± 0.33 a   | 2.3115 ± 0.09624 b            |

Note: Values indicate mean ± SD (*n* = 5). The different lower-case letters (a, b) indicate differences at the level of *P* < 0.05 in Student’s *t*-test.
were also up-regulated in the treated group (Fig. 3a). A gene cluster involved in fusaricidin biosynthesis was also detected in *P. polymyxa* SC2. In the RT-qPCR, relative expression levels of genes in this cluster (fusA, fusB, fusC, fusD, fusE, fusF, and fusG) were significantly higher in the treatment group than in the control group (Fig. 3b), and in RNA-seq of *P. polymyxa* SC2, these genes were up-regulated 7.5-, 7.81-, 7.74-, 7.88-, 6.65-, 2.77-, and 4.73-fold, respectively. In the RNA-seq of pepper, some genes encoding transcription factors and genes related to disease resistance were changed in varying degrees. RT-qPCR results of pepper treated with *P. polymyxa* SC2 revealed that expression of the genes wrky2, wrky3, wrky27, wrky40, and pti5 was up-regulated (Fig. 3c), congruent with the RNA-seq results. Overall, the expression trend of the selected genes in RT-qPCR was consistent with that in RNA-seq, indicating that the RNA-seq data were reliable.

**Overall analysis of DEGs in *P. polymyxa* SC2**

DEGs of *P. polymyxa* SC2 were mainly distributed in 36 sub-classes of three major categories in the GO database (Fig. 4a). Many genes were classified into cellular process, metabolic process, single-organism process, binding, and catalytic activity classes. Up-regulated genes were predominantly in the classes of enzyme regulator activity, biological adhesion, and multi-organism process, while down-regulated genes were associated with negative regulation of the biological process, antioxidant activity, and structural molecule activity. About 65 genes were significantly enriched into molecular function term. And there were 12, 12, 16, 13, and 12 genes enriched into phosphopantetheine binding term, modified amino acid binding term, amino acid binding term, vitamin binding term, and amide binding term, respectively. There were 61 genes enriched into biological process term. About 21, 18, 11, and 11 genes were significantly enriched into tetrapyrrole metabolic process, tetrapyrrole biosynthetic process, cobalamin biosynthetic process, cobalamin metabolic process, respectively.

DEGs were also enriched according to the KEGG database. Results for *P. polymyxa* SC2 are shown in Fig. 5a. Numerous genes were enriched in various categories connected to metabolism, including 60 genes in the metabolism of amino acids and other
amino acid classes of the metabolism category; 91 genes in carbon metabolism; 28 in energy metabolism; 25 associated with lipid metabolism; and 43 genes in the metabolism of cofactors and vitamins. A total of 63 genes were significantly enriched into the metabolism pathway and genetic information processing pathway. There were 19, 14, 12, 5, 5 genes enriched into porphyrin and chlorophyll metabolism pathway, fatty acid metabolism pathway, fatty acid biosynthesis pathway, fatty acid degradation pathway, and tryptophan metabolism pathway, respectively. Meanwhile, eight genes were enriched into sulfur relay system pathway which belongs to genetic information processing. Many genes were not enriched significantly. Transport genes were also up-regulated in *P. polymyxa* SC2. These included genes related to sulfate (*cysW*, *cysT5*, *cysT3*), molybdate (*modA1*, *modA3*), glycine (*proV*), and betaine (*PPSC2_06215*) transport in the mineral and organic ion transport classes. Up-regulation of genes associated with the transport of inorganic salt ions and minerals is beneficial for the absorption of inorganic salt ions and minerals in *P. polymyxa* SC2. A total of 43 genes related to ABC transport were detected in *P. polymyxa* SC2. ABC transport system genes associated with phosphate and amino acid transport (*glnP1*, *glnP3*, *occM3*, *hisP*, *phnE*, *ptxC*, and *pstB5*) were significantly up-regulated. Genes related to glutamine-transport (*glnP1*, *glnP3*) and cystine-transport (*occM3*, *hisP*) were up-regulated, as well as genes related to iron complexes, zinc/manganese/iron, and biotin transport in the metal cations, siderophores, and vitamin B12 transport category. Iron complex transport-related genes (*fluC1*, *fluD1*, *fluG7*, *fluC3*, *yclP*, *yceB11*, *yfmD*, and *cbrA1*) were also up-regulated in varying degrees. Up-regulation of all these genes enhances the ability of *P. polymyxa* SC2 to transport metal ions. Metal ions have significant roles in the function of enzymes,
which will be involved in many biological processes. Thus, the ability of *P. polymyxa* SC2 to transport ions will be beneficial to its growth.

**Overall analysis of DEGs in peppers**

Inoculation of pepper with *P. polymyxa* SC2 led to some changes in gene expression in pepper. DEGs in pepper were mainly distributed in 44 sub-categories of three main categories in the GO database (Fig. 4b). There were abundant genes distributed in the classes of metabolic process, catalytic activity, binding, single-organism process, and cellular process. About 1110, 35, and 1069 genes were significantly enriched into biological process, cellular component, molecular function terms, respectively. A total of 276 DEGs of pepper were enriched by KEGG analysis (Fig. 5b). A total of 105 genes were significantly enriched into five pathways, such as organismal systems (12 genes), metabolism (55 genes), human diseases (19 genes), genetic information processing (13 genes), environmental information processing (6 genes). These included 15 genes involved in the mitogen-activated protein kinase (MAPK) signaling pathway (6 genes) and plant hormone signal transduction (9 genes); 153 genes involved in metabolism; 12 genes involved in sesquiterpene and triterpenoid biosynthesis; and 11 genes related to phenylpropanoid biosynthesis.

**Correlation between functional genes of *P. polymyxa* SC2 and pepper**

*Mutual recognition, chemotaxis, and colonization ability of *P. polymyxa* SC2 with pepper*

Under the stimulation of pepper, a total of 19 genes related to the quorum sensing in *P. polymyxa* SC2 were up-regulated (Table 2). Up-regulated expression of genes related to quorum sensing could help *P. polymyxa* SC2 perceive environment changes. Expression of the gene `PPS2C_08335`, encoding chemotactic protein AER, was up-regulated by 3.25-fold, and this is likely to benefit *P. polymyxa* SC2 in receiving external signals and responding to environmental changes. Correlation analysis revealed that genes involved in histidine metabolism (`c66011_g2`), glutamic acid...
metabolism (c39553_g2), phenylalanine/tyrosine/tryptophan biosynthesis (c119522_g1), amino sugar/nucleotide glycogen metabolism (c48054_g1), alpha-linolenic acid metabolism gene (c79159_g1) in pepper associated with the gene encoding AER protein in P. polymyxa SC2. Pepper not only stimulated the expression of chemoreceptors but also affected the expression of specific chemotaxis genes in P. polymyxa SC2. These included genes such as cheA, cheW, cheY, cheD, and cheC, which were up-regulated by 1.49-to 2.11-fold. Meanwhile, two genes (fliM and fliN) encoding flagellar motor switch proteins were both up-regulated by 2.16-fold. This indicated that in the presence of pepper, the motility of P. polymyxa SC2 was enhanced. This would be conducive to the colonization of P. polymyxa SC2 in the pepper rhizosphere.

After interacting with peppers, genes related to biofilm formation of P. polymyxa SC2 were changed in varying degrees. Expression of kinA, epsB, and epsE was up-regulated by 2.34-to 6.69-fold, while expression of sinR and abrB was down-regulated by 1.21-fold and 3.06-fold, respectively. The genes (degU and abh) related to biofilm formation were up-regulated by 2.02- and 2.74-fold. They may be indicated to promote biofilm formation for P. polymyxa SC2. In summary, pepper stimulated biofilm formation of P. polymyxa SC2, which would be conducive for colonization in the pepper rhizosphere.

Growth-promoting analysis of potential nutrient supply between P. polymyxa SC2 and pepper
After interacting with peppers, expression of gene encoding phytase (PPSC2_05715) in P. polymyxa SC2 was
up-regulated by 2.65-fold. Phytase can hydrolyze phosphate residues from phytic acid. The up-regulated expression of phytase may increase the concentration of inorganic phosphorous in the medium and promote the growth of peppers. In the RNA-seq of *P. polymyxa* SC2 interacted with pepper, some genes related to carbon metabolism and amino acid metabolism were detected in *P. polymyxa* SC2. Most of them were up-regulated (data are shown in Table 3). It seems clear that peppers provide some nutrients for the growth of *P. polymyxa* SC2.

**Defense mechanisms between *P. polymyxa* SC2 and pepper**

Polymyxin and fusaricidin are important secondary metabolites of *P. polymyxa* SC2, and inhibit the

### Table 2 DEGs related to quorum sensing, chemotaxis, and biofilm formation in *P. polymyxa* SC2

| Gene      | Product                                      | name                  | log$_2$FC(SH/S) |
|-----------|----------------------------------------------|-----------------------|----------------|
| **Quorum sensing** |                                         |                       |                |
| PPSC2_02180 | peptide ABC transporter substrate-binding protein | oppF1                 | 2.61           |
| PPSC2_02185 | peptide ABC transporter permease             | oppB1                 | 4.2            |
| PPSC2_02190 | peptide ABC transporter permease             | oppC1                 | 3.94           |
| PPSC2_02195 | ABC transporter substrate-binding protein   | oppA1                 | 4.92           |
| PPSC2_05715 | peptide ABC transporter substrate-binding protein | oppD                  | 2.65           |
| PPSC2_05720 | transports peptides consisting of two or three amino acids | oppB                  | 2.71           |
| PPSC2_05725 | peptide ABC transporter permease             | oppC3                 | 2.31           |
| PPSC2_10965 | ABC transporter substrate-binding protein   | oppA5                 | 2.11           |
| PPSC2_10970 | diguanylate cyclase                          | opp8                  | 2.2            |
| PPSC2_10975 | peptide ABC transporter permease             | oppC5                 | 2.85           |
| PPSC2_10980 | peptide ABC transporter ATPase               | oppD3                 | 2.88           |
| PPSC2_10985 | peptide ABC transporter substrate-binding protein | oppF3            | 2.58           |
| PPSC2_13025 | nickel transporter permease NikC             | oppC3                 | 2.23           |
| PPSC2_24700 | ABC transporter ATPase                       | oppD7                 | 2.07           |
| PPSC2_25400 | peptide ABC transporter substrate-binding protein | oppF9            | 2.94           |
| PPSC2_25405 | peptide ABC transporter ATPase               | oppD9                 | 3.6            |
| PPSC2_25410 | peptide ABC transporter permease             | oppC9                 | 3.35           |
| PPSC2_25415 | diguanylate cyclase                          | oppB9                 | 3.26           |
| PPSC2_25420 | ABC transporter substrate-binding protein   | dbp                   | 2.91           |
| **Chemotaxis** |                                         |                       |                |
| PPSC2_08335 | chemotaxis protein AER                      | aer                   | 3.25           |
| PPSC2_18805 | chemotaxis protein                          |                       | 4.65           |
| PPSC2_09975 | histidine kinase CheA                       | cheA                  | 2.11           |
| PPSC2_09985 | cheC                                         | cheC                  | 1.71           |
| PPSC2_09990 | chemotaxis protein CheD                     | cheD                  | 1.49           |
| PPSC2_14595 | chemotaxis protein CheR                     | cheR                  | 2.73           |
| PPSC2_21235 | chemotaxis protein CheR                     | cheR                  | 2.53           |
| PPSC2_09980 | coupling protein CheW                       | cheW                  | 2.04           |
| PPSC2_09970 | effector protein CheY                       | cheY                  | 2.26           |
| PPSC2_09915 | flagellar motor switch protein FilM         | filM                  | 2.16           |
| PPSC2_09920 | flagellar motor switch protein FilN         | filN                  | 2.16           |
| **Biofilm formation** |                                         |                       |                |
| PPSC2_00125 | AbrB family transcriptional regulator       | abrB                  | 3.06           |
| PPSC2_23095 | Transcriptional regulatory protein DegU      | degU                  | 2.02           |
| PPSC2_05800 | tyrosine protein kinase                      | epsB                  | 6.69           |
| PPSC2_05810 | multidrug MFS transporter                    | epsE                  | 6.25           |
| PPSC2_25275 | transcriptional regulator                   | sinR                  | 1.2            |
| PPSC2_03845 | histidine kinase                            | kinA                  | 2.34           |
| PPSC2_03710 | Regulator Abh                               | abh                   | 2.74           |
growth of pathogenic bacteria and fungi, respectively. Under the stimulation of pepper, expression of genes related to polymyxin and fusaricidin biosynthesis in P. polymyx SC2 was significantly up-regulated by 2.93- to 6.13-fold and 2.77- to 7.88-fold, respectively (Table 4). The ectB gene (PPSC2_11845) encoding aminotransferase for polymyxin production was up-regulated by 2-fold. Up-regulation of genes related to polymyxin biosynthesis may increase the production of polymyxin, which may strengthen the resistance of pepper to bacterial pathogens in nature. Genes related to fatty acid synthesis (accB, fabG5, fabH3, fabD3, and fabG13) were all up-regulated (3.74-, 2.43-, 2.93-, 3.31-, and 2.93-fold, respectively). The changes in these genes may be beneficial for the synthesis of fusaricidin because this process requires fatty acid side chains.

The antagonistic results of P. polymyx SC2 against the pathogenic bacterium Xanthomonas citri were shown in Fig. 6. Fermentation broth of P. polymyx SC2 supplemented with MS medium (which has been used for culturing pepper) had the best antagonistic effect on X. citri. The effects of P. polymyx SC2 were also tested on the growth of Fusarium moniliforme, but the antagonistic circle in the treated group was smaller than that in the control group (data not shown).

| Table 3 Genes related to metabolism in P. polymyx SC2 |
|---------------------------------|-----------------|------------------|
| **Type**                       | **gene**        | **product**      | **log_2FC(SH/S)** |
| Fructose and mannose metabolism | PPSC2_05870     | glycosyl hydrolase | 2.69             |
|                                 | PPSC2_05910     | GDP-mannose 4,6-dehydratase | 7.84             |
|                                 | PPSC2_05865     | mannose-1-phosphate guanylyltransferase | 3.29             |
|                                 | PPSC2_02440     | PTS fructose transporter subunit IIB | 3.46             |
|                                 | PPSC2_24735     | rhamnose isomerase | 2.09             |
|                                 | PPSC2_02450     | PTS mannose transporter subunit IID | 2.2              |
|                                 | PPSC2_05905     | GDP-L-fucose synthase | 6.89             |
|                                 | PPSC2_02445     | PTS mannose transporter subunit IIC | 2.51             |
|                                 | PPSC2_24740     | rhamnulose-1-phosphate aldolase | -2.43            |
|                                 | PPSC2_02435     | PTS mannose transporter subunit IID | 3.96             |
| Starch and sucrose metabolism   | PPSC2_05860     | UDP-glucose 6-dehydrogenase | 4.22             |
|                                 | PPSC2_05940     | UDP-glucose 6-dehydrogenase | 6.53             |
|                                 | PPSC2_16055     | pectin methylesterase | 3.4              |
|                                 | PPSC2_03770     | levansucrase | 3.25             |
|                                 | PPSC2_05805     | UTP-glucose-1-phosphate uridylyltransferase | 5.83             |
|                                 | PPSC2_03430     | PTS beta-glucoside transporter subunit IIA/BC | -4.99            |
|                                 | PPSC2_03425     | trehalose-6-phosphate hydrolase | -4.69            |
|                                 | PPSC2_13470     | glucose-1-phosphate adenylyltransferase | -3.71            |
| Glycerolipid metabolism         | PPSC2_09660     | phosphate acyltransferase | 2.53             |
|                                 | PPSC2_00395     | aldehyde dehydrogenase | 6.65             |
|                                 | PPSC2_15995     | glycerol dehydrogenase | -2.61            |
| Alanine, aspartate and glutamate metabolism | PPSC2_01185 | asparagine synthetase | 4.66             |
|                                 | PPSC2_25355     | adenylosuccinate synthetase | -2.86            |
|                                 | PPSC2_14255     | aspartate ammonia-lyase | 3.69             |
|                                 | PPSC2_12155     | acetylornithine aminotransferase | 6.28             |
|                                 | PPSC2_06000     | asparagine synthase | 5.58             |
|                                 | PPSC2_22115     | glutamine–fructose-6-phosphate aminotransferase | -2.9             |
|                                 | PPSC2_16495     | aspartate carboxamidotransferase | -2.11            |
| Valine, leucine and isoleucine biosynthesis | PPSC2_09040 | transferase | 2.34             |
|                                 | PPSC2_07085     | 2-isopropylmalate synthase | 2.76             |
|                                 | PPSC2_07090     | 3-isopropylmalate dehydrogenase | -2.59            |
|                                 | PPSC2_00400     | transferase | 7.88             |
While pepper influenced the expression of genes of \textit{P. polymyxa SC2}, the bacterium also played an important role in the expression of pepper genes. \textit{P. polymyxa SC2} stimulated an up-regulation in expression of transcription factors (TFs) (Table 5), including WRKY2 \textit{c33870_g1}, WRKY3 \textit{c39207_g1}, WRKY40 \textit{c50361_g2}, and WRKY33 \textit{c66538_g3}, which were up-regulated by 1.17- to 4.2-fold, respectively. This finding suggests that \textit{P. polymyxa SC2} induces systemic resistance in pepper. Other pepper genes were also up-regulated after inoculation with \textit{P. polymyxa SC2}. These included ethylene response factor 1 (ERF1) gene, up-regulated by 4.86-fold; the gene \textit{c78851_g1} encoding Pti5, which can activate defense responses of plants to aphid and bacteria, up-regulated by 6.14-fold; genes directly involved in plant defense (such as CML and PIK1); genes involved in the jasmonic acid signaling pathway (JAZ), isoquinoline alkaloid biosynthesis (TAT), and phenylpropanoid biosynthesis (MYB); and genes DNAJC7, PPID, HSPA1, and RNF5, belonging to chaperone, heat shock protein, and ubiquitin, up-regulated by 4.12- to 5.86-fold. These results indicated that \textit{P. polymyxa SC2} could improve the defense ability of pepper.

**Table 4** DEGs related to secondary metabolic clusters in \textit{P. polymyxa SC2}

| Gene product Name | log\textsubscript{2}FC(SH/S) |
|-------------------|-----------------------------|
| **polymyxin biosynthesis** |                         |
| PPSC2_22060 polymyxin synthetase E | \textit{pmxE} | 4.93 |
| PPSC2_22065 multidrug ABC transporter permease | \textit{pmxD} | 6.13 |
| PPSC2_22070 multidrug ABC transporter permease | \textit{pmxC} | 5.13 |
| PPSC2_22075 ATP-dependent asparagine adenylase | \textit{pmxB} | 4.95 |
| PPSC2_22080 synthetase 2 Gramicidin S synthetase II | \textit{pmxA} | 2.93 |
| **fusaricidin biosynthesis** |                         |
| PPSC2_00380 membrane protein | \textit{ymcC} | 4.92 |
| PPSC2_00385 enoyl-ACP reductase | \textit{fusG} | 4.73 |
| PPSC2_00390 peptide synthetase | \textit{fusF} | 2.77 |
| PPSC2_00395 aldehyde dehydrogenase | \textit{fusE} | 6.65 |
| PPSC2_00400 acetalactate synthase large subunit | \textit{fusD} | 7.88 |
| PPSC2_00405 3-oxoacyl-ACP synthase | \textit{fusc} | 7.74 |
| PPSC2_00410 3-hydroxymyristoyldehydrotase | \textit{fusB} | 7.81 |
| PPSC2_00415 bacitracin synthetase | \textit{fusA} | 7.5 |

**Fig. 6** Antagonistic assay of \textit{P. polymyxa SC2} against \textit{Xanthomonas citri}. The antagonistic assay comprised three treatment groups. Control: fermentation medium was inoculated \textit{P. polymyxa SC2}. MS group: fermentation medium was supplemented with 1 mL MS medium and then inoculated with \textit{P. polymyxa SC2}. PM group: fermentation medium was supplemented with 1 mL MS medium (which has been used for culturing peppers) and then inoculated with \textit{P. polymyxa SC2}. Values indicate means ± SD (\(n = 3\); * \(P < 0.05\), Student’s t-test).
The PGPR minor (duckweed) and the dicot *Lemna* increased the chlorophyll content of monocot *p23As*, as a member of PGPR, significantly increasing the chlorophyll content of plants. *Actinetobacter* *thesis* [31]. PGPR can promote plant growth by increasing the chlorophyll content of wheat [33]. Chlorophyll participates in energy transfer also significantly increased the chlorophyll content of pepper leaves. Chlorophyll participates in energy transfer by absorbing light energy in the process of photosynthesis [31]. PGPR can promote plant growth by increasing the chlorophyll content of pepper, a pot experiment and transcriptome analysis of *P. polymyxa* and pepper in co-cultured conditions were conducted.

In the pot experiment, *P. polymyxa* SC2 not only increased the stem diameter and leaf size of pepper, but also significantly increased the chlorophyll content of pepper leaves. Chlorophyll participates in energy transfer by absorbing light energy in the process of photosynthesis [31]. PGPR can promote plant growth by increasing the chlorophyll content of plants. *Actinetobacter calcoaceticus* p23As, as a member of PGPR, significantly increased the chlorophyll content of monocot *Lemma minor* (duckweed) and the dicot *Lactuca sativa* (lettuce) [32]. The PGPR *Bacillus megaterium* M3 and *B. subtilis* OSU142, which were previously reported as plant-growth-promoting and potential biocontrol agents, could increase the chlorophyll content of wheat [33]. Chlorophyll contents of chickpea were significantly increased following inoculation with *P. polymyxa* [34]. Increasing the chlorophyll content may also increase the biomass of plants. However, in the current study, differences in biomass of pepper were not obvious in the group inoculated with *P. polymyxa* SC2 (data not shown). This may be because the growth of pepper in the pot is limited by space.

Interaction between *P. polymyxa* SC2 and pepper resulted in numerous differences in gene expression in the transcriptome of *P. polymyxa* SC2. Chemotaxis is advantageous for bacterial colonization in the rhizosphere of plants [35, 36], and can mediate beneficial bacteria-plant interactions [37]. After interacting with pepper, expression of chemotactic genes of *P. polymyxa* SC2 changed in varying degrees. Expression of genes encoding histidine kinase CheA and the coupling protein CheW was up-regulated by 2.11- and 2.04-fold, respectively. These genes help *P. polymyxa* SC2 respond to chemical stimuli. Up-regulation of effector protein CheY and the flagellar motor switch proteins FlIM and FlIN could promote motility of *P. polymyxa* SC2. These results indicated that pepper roots may secrete signaling molecules that can attract *P. polymyxa* SC2 to move towards pepper. Previous studies have reported that root exudate components can attract PGPR motility. Phazna et al. showed that six organic acids in the root exudates of *Capsicum chinense* were chemotactic for *Pseudomonas, Burkholderia*, and *Bacillus* [38]. Biofilm formation also plays an important role in the colonization of PGPR [39]. The proteins SinR and AbrB are negative regulators in biofilm formation [40]. After interacting with pepper, expression of the genes *sinR* and *abrB* was down-regulated in *P. polymyxa* SC2. This suggested that the root exudates may induce TasA operon expression [41]. Meanwhile, expression of genes related to biofilm formation (*kinA, epsB, epsE, degU*, and *abh*) in *P. polymyxa* SC2 was up-regulated by 2.02- to 6.69-fold, respectively. This may facilitate *P. polymyxa* SC2 colonization in the rhizosphere of peppers.

Phytase plays an important role in phosphate solubilization [42]. After interacting with pepper, genes encoding phytase were up-regulated in *P. polymyxa* SC2. This indicated that *P. polymyxa* SC2 may enhance the ability of pepper to absorb phosphorus. Improving phosphorus uptake may promote pepper growth to some extent. Previous studies also found that phytase-secreting bacteria can enhance the phosphorous content in plants [43]. Pepper also stimulated the expression of metabolic genes in *P. polymyxa* SC2. In the RNA-seq of *P. polymyxa* SC2, various genes related to metabolism were up-regulated, including genes involved in fructose and mannose metabolism, which were up-regulated by 2.20- to

### Table 5 DEGs related to defense mechanisms in pepper

| Gene     | Name     | log_{2}FC (PH/P) | Gene     | Name     | log_{2}FC (PH/P) |
|----------|----------|------------------|----------|----------|------------------|
| c119678_g1 | CML      | 6.52             | c118659_g1 | TAT      | 4.68             |
| c68413_g9 | CYP710A  | 5.43             | c33870_g1 | WRKY 2   | 1.17             |
| c48212_g2 | DNAJC7   | 5.31             | c73380_g1 | WRKY 27  | 0.7              |
| c56938_g1 | ERF1     | 4.86             | c39207_g1 | WRKY 3   | 1.45             |
| c55034_g2 | HSFF     | 4.52             | c50361_g2 | WRKY 40  | 3.5              |
| c48321_g3 | HSPA1    | 4.29             | c66538_g3 | WRKY33   | 4.2              |
| c69002_g1 | JAZ      | 5.17             | c119537_g1 |         | 4.67             |
| c89163_g1 | MYBP     | 5.28             | c48656_g1 |         | 5.52             |
| c68645_g1 | PKI1     | 4.53             | c68046_g1 |         | 6.74             |
| c50209_g1 | PPID     | 5.86             | c98990_g1 |         | −5.21            |
| c78851_g1 | Ph5      | 6.14             | c94743_g1 |         | 4.02             |
| c61710_g1 | RNF5     | 4.12             |          |          |                  |

**Discussion**

*P. polymyxa* SC2 promotes the growth of pepper and inhibits several phytopathogens [30]. To understand the interactional relationship of *P. polymyxa* SC2 and pepper, a pot experiment and transcriptome analysis of *P. polymyxa* SC2 and pepper in co-cultured conditions were conducted.

In the pot experiment, *P. polymyxa* SC2 not only increased the stem diameter and leaf size of pepper, but also significantly increased the chlorophyll content of pepper leaves. Chlorophyll participates in energy transfer by absorbing light energy in the process of photosynthesis [31]. PGPR can promote plant growth by increasing the chlorophyll content of plants. *Actinetobacter calcoaceticus* p23As, as a member of PGPR, significantly increased the chlorophyll content of monocot *Lemma minor* (duckweed) and the dicot *Lactuca sativa* (lettuce) [32]. The PGPR *Bacillus megaterium* M3 and *B. subtilis* OSU142, which were previously reported as plant-growth-promoting and potential biocontrol agents, could increase the chlorophyll content of wheat [33]. Chlorophyll contents of chickpea were significantly increased following inoculation with *P. polymyxa* [34]. Increasing the chlorophyll content may also increase the biomass of plants. However, in the current study, differences in biomass of pepper were not obvious in the group inoculated with *P. polymyxa* SC2 (data not shown). This may be because the growth of pepper in the pot is limited by space.

Interaction between *P. polymyxa* SC2 and pepper resulted in numerous differences in gene expression in the transcriptome of *P. polymyxa* SC2. Chemotaxis is advantageous for bacterial colonization in the rhizosphere of plants [35, 36], and can mediate beneficial bacteria-plant interactions [37]. After interacting with pepper, expression of chemotactic genes of *P. polymyxa* SC2 changed in varying degrees. Expression of genes encoding histidine kinase CheA and the coupling protein CheW was up-regulated by 2.11- and 2.04-fold, respectively. These genes help *P. polymyxa* SC2 respond to chemical stimuli. Up-regulation of effector protein CheY and the flagellar motor switch proteins FlIM and FlIN could promote motility of *P. polymyxa* SC2. These results indicated that pepper roots may secrete signaling molecules that can attract *P. polymyxa* SC2 to move towards pepper. Previous studies have reported that root exudate components can attract PGPR motility. Phazna et al. showed that six organic acids in the root exudates of *Capsicum chinense* were chemotactic for *Pseudomonas, Burkholderia*, and *Bacillus* [38]. Biofilm formation also plays an important role in the colonization of PGPR [39]. The proteins SinR and AbrB are negative regulators in biofilm formation [40]. After interacting with pepper, expression of the genes *sinR* and *abrB* was down-regulated in *P. polymyxa* SC2. This suggested that the root exudates may induce TasA operon expression [41]. Meanwhile, expression of genes related to biofilm formation (*kinA, epsB, epsE, degU*, and *abh*) in *P. polymyxa* SC2 was up-regulated by 2.02- to 6.69-fold, respectively. This may facilitate *P. polymyxa* SC2 colonization in the rhizosphere of peppers.

Phytase plays an important role in phosphate solubilization [42]. After interacting with pepper, genes encoding phytase were up-regulated in *P. polymyxa* SC2. This indicated that *P. polymyxa* SC2 may enhance the ability of pepper to absorb phosphorus. Improving phosphorus uptake may promote pepper growth to some extent. Previous studies also found that phytase-secreting bacteria can enhance the phosphorous content in plants [43]. Pepper also stimulated the expression of metabolic genes in *P. polymyxa* SC2. In the RNA-seq of *P. polymyxa* SC2, various genes related to metabolism were up-regulated, including genes involved in fructose and mannose metabolism, which were up-regulated by 2.20- to
Vančura and Hovádik reported that sucrose and fructose were components of red pepper root exudates [44], and this would explain the results of our study. Pepper also stimulated the expression of genes related to alanine, aspartate, and glutamate metabolism. We found that alanine could be used as a nitrogen source for growth of P. polymyxa SC2 (unpublished data). Together, these results suggest that root exudates of pepper may provide nutrients for the growth of P. polymyxa SC2.

In the presence of pepper, the expression of genes related to polymyxin and fusaricidin in P. polymyxa SC2 was up-regulated. Expression of the aminotransferase gene ectB was also up-regulated. L-2,4-diaminobutyric acid (L-Dab), the precursor of polymyxin synthesis, is synthesized by EctB [45]. In the initial stage of polymyxin synthesis, increasing Dab can increase the yield of polymyxin E [46]. However, after fermentation for 35 h, addition of L-Dab inhibited the production of polymyxin and suppressed ectB expression [45]. In this study, P. polymyxa SC2 interacted with pepper for 20 h before transcriptome sequencing. This short culture time may account for the increase in endogenous precursor Dab increasing the yield of polymyxin. In the antagonistic test, the antagonistic ability of P. polymyxa SC2 was confirmed to be stronger after adding the medium that had been used to culture pepper. This may be related to the up-regulated expression of polymyxin biosynthesis gene cluster in P. polymyxa SC2 under the effects of pepper root exudates. Although the expression of genes related to fusaricidin biosynthesis cluster was up-regulated, the ability of P. polymyxa SC2 to inhibit fungi (F. moniliforme) did not change significantly after adding the medium that had been used to culture pepper. This may be because polymyxin and fusaricidin compete for the same transporter genes to be secreted [47]. It is also possible that the MS medium contains substances that inhibit the synthesis of fusaricidin. Thus, genes related to fusaricidin biosynthesis in P. polymyxa SC2 were only up-regulated at the level of gene transcription.

For plants, WRKY is a superfamily of transcription factors that play important roles in many biological processes [48–53]. In the current study, the genes encoding WRKY2 and WRKY40 (c33870_g1 and c103783_g1, respectively) were up-regulated in pepper. For the pepper, C. annuum, the gene encoding WRKY2 (CaWRKY2) was regarded as an early component of defense signaling and it was rapidly induced following inoculation with host or non-host pathogens [54]. The gene CaWRKY40 was reported to be regulated by salicylic acid, jasmonic acid, and ethylene signaling in response to Ralstonia solanacearum infection and heat stress in pepper [55]. Combining our data with the above research leads us to speculate that P. polymyxa SC2 could induce the systemic resistance of pepper and enhance the resistance of pepper to some pathogens.

Conclusion
In this study, P. polymyxa SC2 effectively improved the agronomic characteristics of peppers. The root exudates of pepper enhanced the antagonistic ability of P. polymyxa SC2 against pathogenic bacteria. Meanwhile, based on the transcriptomics data, pepper can induce the expression of genes related to polymyxin biosynthesis. Pepper could stimulate the expression of genes related to quorum sensing, chemotaxis, and biofilm formation in P. polymyxa SC2. Concurrently, P. polymyxa SC2 may also induce the systemic resistance of pepper by stimulating the expression of some TFs. This interplay relationship between pepper and P. polymyxa SC2 is the result of multiple pathways and coordinated regulation of various reactions. This study described the growth-promoting effects of P. polymyxa SC2 on pepper and contributes to elucidating the growth-promoting mechanisms of P. polymyxa.

Methods
Strains and plants
P. polymyxa SC2 was isolated from the rhizosphere soil of pepper in Guizhou, China and stored at 4 °C in the dark. Strain SC2 was activated on Luria-Bertani (LB) agar plates and then cultured at 37 °C for 24–48 h. A single colony of the strain was inoculated in 5 mL LB liquid medium, shaken at 37 °C overnight, then 5 mL was subcultured into 50 mL fresh LB and shaken at 37 °C for a further 12 h. Cells suspensions, diluted to ~10⁸ cells/mL, were used for pot experiments. Cells were collected by centrifugation and were resuspended in 1× PBS buffer to an OD₆₀₀ of 1.0. It was used for cocultivation with pepper in a sterile environment. Xanthomonas citri, a pathogen causing citrus canker [56, 57], was used in the antagonistic test. Xanthomonas citri was inoculated on LB agar and cultured at 30 °C for 24–48 h. A single colony of Xanthomonas citri was then inoculated into 5 mL LB liquid medium and shaken at 30 °C overnight for the test. Pepper seeds (Capsicum annuum L. (Shengfeng)) were purchased from Nongda Seed Company, Tai’an, China.

Promotion effects of P. polymyxa SC2 on pepper in pot conditions
Pot experiments were conducted in the greenhouse of Shandong Agricultural University. Approximately four pepper seeds were planted in each hole of aperture disks containing vermiculites, and were cultured at 25 °C. After germination, a seedling remained in each hole. Seedlings with 4–5 euphylia were ready for pot experiments. Approximately 3 kg healthy soil (or continuous
cropping soil) was placed in a pot (25 cm diameter and 15 cm depth). Pepper seedlings in a similar growth trend were selected and one seedling was transplanted into each pot and irrigated with 500 mL Hoagland nutrient solution [58]. Experimental treatments commenced after 3 days. Treated group: each pot of peppers was irrigated with 5 mL *P. polymyxa* SC2 fermentation broth (1 × 10⁸ CFU/mL) and diluted with water to 200 mL. Control group: 5 mL sterilized LB medium was poured into the rhizosphere soil of peppers in the same way as the treated group. Fifteen biological replicates were set per treatment. Agronomic traits including stem diameter (diameter at the ground base), plant height (vertical height from the soil surface to the highest point of the main stem), leaf width, and leaf length were investigated at 30, 40, and 50 dpi. Fresh weights of above ground and underground parts were determined after pot experiments. At 40 dpi, the leaves of five pepper plants were randomly selected for chlorophyll determination by the ethanol method [59].

**Interaction treatments of *P. polymyxa* SC2 and pepper in sterile conditions**

To better analyze the interaction mechanisms between *P. polymyxa* SC2 and peppers, a co-culture experiment was carried out under sterile conditions. To achieve these conditions, the peppers needed to be sterile. The surfaces of pepper seeds were sterilized by dipping in 75% (vol/vol) ethanol for 5 min, then the seeds were immersed in 1%o (vol/vol) mercury dichloride for 20 min before rinsing with sterile distilled water for 5–7 times. Sterilized seeds were placed on wire mesh in tissue culture vessels containing Murashige and Skoog (MS) liquid medium prepared according to Guan’s method [60]. Culture vessels were then placed in a plant growth chamber at 25 °C with 16 h light (day) period (13,200 lx) and 8 h dark (night) period. Twenty days post-germination, seedlings were used for the interaction experiment, and at this time, roots of the seedlings grow in the liquid MS medium. *P. polymyxa* SC2 was inoculated into liquid medium to study the possible interactions between pepper and *P. polymyxa* SC2. The bacteria negative control comprised 1 mL *P. polymyxa* SC2 suspension applied to sterilized MS medium; *P. polymyxa* SC2 cells were collected after 20 h and named as [S]. The plant negative control comprised pepper seedlings treated with 1 mL of 1× PBS buffer; roots of the pepper seedlings were collected after 20 h and marked as [P]. The *P. polymyxa* SC2-pepper co-cultured group comprised peppers treated with 1 mL *P. polymyxa* SC2; bacterial cells and pepper roots were collected after 20 h and named as [SH] and [PH], respectively. Each treatment was designed with three biological replicates. The collected bacteria and pepper roots were frozen in liquid nitrogen and stored at −80 °C until further processing.

**RNA extraction and RNA-seq**

Total RNA of *P. polymyxa* SC2 and peppers was extracted and purified using the TRIzol (Invitrogen) method. Total RNA quantity and quality were assessed using a NanoDrop 2000 spectrophotometer. RNA integrity number (RIN) was investigated using an Agilent 2100 Bioanalyzer. RNA quality is an essential factor in RNA-seq, therefore only RNA samples with RIN > 6, 230/260 and 260/280 ratios > 2 were used. The mRNA of pepper was isolated from the crude RNA via Oligo (dT) according to the manufacturer’s (NEBNext™ Ultra™ RNA Library Prep Kit for Illumina®) instructions. The mRNA of *P. polymyxa* SC2 was isolated by removing rRNA. The mRNA was randomly fractured into small fragments of approximately 200 bp, and was reversed to a single strand of cDNA by random primers. Two-strand cDNA was further synthesized to form a stable double-strand structure. End Repair Mix was used to complement double-stranded cDNA into flat ends, and then an A base was added at the 3’ end. The library was enriched and sequencing of cDNA fragments was conducted using an Illumina Hiseq4000 platform.

**Bioinformatics analyses**

Raw RNA-seq data were stored in a fastq file format. To ensure the accuracy of subsequent bioinformatics analysis, raw reads were filtered by SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle). In this stage, the reads without inserting fragments due to the self-connection of connectors, and the reads with N ratio over 10%, and the reads less than 20 bp were removed. Then the high-quality clean data were assembled and aligned. Clean reads of *P. polymyxa* SC2 were mapped to the reference genome of *P. polymyxa* SC2 (https://www.ncbi.nlm.nih.gov/nuccore/NC_014622.2). Bowtie2 was used to align the clean reads and reference genome [61]. Since there was no reference genome for pepper, Trinity software (http://trinityrnaseq.sourceforge.net/, vision:trinityrnaseq-r2013-02-25) was used to assemble the short fragment sequences of pepper after obtaining high-quality sequencing data from RNA-seq [62], and predict the ORFs. The ORFs were searched through HMMER3, and the annotated proteins were aligned with NR, String, SwissProt, and KEGG databases to obtain corresponding annotation information through Blastx (Version 2.2.25, E value<1e⁻⁵). The software edgeR was used to analyze differentially expressed genes [63]. GO enrichment analysis of differentially expressed genes was performed
using GOATOOLS software (https://github.com/tanghaibao/GOatools) [64]. KEGG pathway enrichment was conducted with KOBASE [65]. Fisher’s exact test was used to analysis of GO/KEGG enrichment. The p-value (p_fdr) ≤ 0.05 indicated that the GO/KEGG function was enriched significantly.

Expression profiling by RT-qPCR

One microgram of purified total RNA was used as a template for first-strand cDNA synthesis using an Evo M-MLV RT Kit with gDNA Clean for qPCR (Accurate Biotechnology (Hunan) Co., Ltd). Selected genes for each treatment were amplified to validate the RNA-seq results. These genes were selected from the DEG lists obtained for each condition. Primer sequences were designed using Beacon Designer 7 and are listed in Additional File 7: Table S5. The gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of pepper was used as the reference gene and GAPDH of P. polymyxa SC2 was used as an endogenous control. Relative expression levels were calculated using the ΔΔCt method [66]. Three biological replicates were used for real-time quantitative PCR.

Antibacterial activity assay

An experiment was designed to test the effects of pepper root exudates on antibiotic production by P. polymyxa SC2. There were three treatment groups. Control: fermentation medium (sucrose 43.6 g/L, (NH4)2SO4 6.66 g/L, CaCO3 6.26 g/L, KH2PO4 0.2 g/L, NaCl 0.2 g/L, MgSO4 0.2 g/L) inoculated with P. polymyxa SC2. Treated group 1 (MS): fermentation medium supplemented with 1 mL MS medium and then inoculated with P. polymyxa SC2. Treated group 2 (PM): fermentation medium supplemented with 1 mL MS medium (which has been used for culturing peppers) and then inoculated with P. polymyxa SC2.

P. polymyxa SC2 was inoculated into 5 mL LB liquid medium and cultured at 37 °C, 180 rpm for 8–12 h, before being subcultured into the appropriate fermentation medium at 2% inoculation volume and incubated at 37 °C, 180 rpm for 72 h. For the antagonistic test, the P. polymyxa SC2 cultures were centrifuged at 12000 rpm for 10 min to remove cells, and the supernatants were used. Sterile water with 2% agar was added into Petri dishes and allowed to coagulate, then Oxford cups were placed in the Petri dishes. Next, 2 mL Xanthomonas citri cells were mixed with 200 mL LB medium containing 1% agar and cooled below 55 °C to prepare the plates. A total of 100 µL P. polymyxa SC2 culture supernatant was loaded into the well of the Oxford cup and incubated at 37 °C for 24 h to observe the growth inhibition effect on X. citri. All treatments had three replicates.

Statistical analysis

Statistical analyses were performed using the Student’s t-test in SPSS 19.0. Columns were drawn using GraphPad Prism 7. P < 0.05 (*) in columns means there was a significant difference, and P < 0.01 (**) means there was an extremely significant difference.

Abbreviations

PGPR: Plant growth-promoting rhizobacteria; ACC: 1-aminocyclopropane-1-carboxylate; IAA: Indole-3-acetic acid; IAN: Indole-3-acetonitrile; dpi: Days post-inoculation; DEGs: Differential expression genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; TFs: Transcription factors; LB: Luria-Bertani; MS: Murashige and Skoog; OD: Optical density; RNA: RNA Integrity Number

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-021-02132-2.

Additional file 1. Detailed statistics of reads mapping: Table S1
Mapping proportion statistics in RNA-seq of strain SC2, Table S2: Mapping proportion statistics in RNA-seq of peppers.

Additional file 2: Fig. S1: PCA analysis of samples based on the gene expression level (FPKM) in RNA-seq.

Additional file 3: Table S3: Annotation of differentially expressed genes in P. polymyxa SC2.

Additional file 4: Table S4: Annotation of differentially expressed genes in pepper.

Additional file 5: Fig. S2: Heatmap of DEGs in P. polymyxa SC2.

Additional file 6: Fig. S3: Heatmap of DEGs in pepper.

Additional file 7: Table S5: Primers for RT-qPCR.

Acknowledgements

Not applicable.

Authors’ contributions

BD, YD, and CW designed and supported the study. HL and YL performed the laboratory work and analyzed the data. HL and CW wrote and revised the manuscript. KG and KL advised on the manuscript. The authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (31700094 and 31770115), the National Key Research and Development Program of China (2017YFD0200804), the funds of Shandong “Double Tops” Program (SYL2017XTTD03), and the Key Field Research and Development Program of Guangdong Province (2018B020218009).

Availability of data and materials

The raw data of the transcriptome has been uploaded to the Sequence Read Archive (SRA) database in National Center for Biotechnology Information (NCBI). The accession numbers are SRP242237 (https://trace.ncbi.nlm.nih.gov/Traces/sra/?study=SRP242237) and SRP242239 (https://trace.ncbi.nlm.nih.gov/Traces/sra/?study=SRP242239).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.
References
1. Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Abdulaziz Univ Sci. 2014;26(1):20–1.
2. Castagnino LN, Estrella MJ, Sannazzaro AI, Grassano AE, Ruiz OA. Phosphate-solubilization mechanism and in vitro plant growth promotion activity mediated by Pantoea eucalypti isolated from Lotus tenuis rhizosphere in the Salado River basin (Argentina). J Appl Microbiol. 2011;110:1151–65.
3. Luduena LM, Anzuy MG, Angelini JG, McIntosh M, Becker A, Rupp O, Goemann A, Blom J, Fabra A, Taurian T. Strain Seriato1 sp. S119: a potential biofertilizer for peanut and maize and a model bacterium to study phosphate solubilization mechanisms. Appl Soil Ecol. 2018;126:107–12.
4. Gómez-Sagasti MT, Marino D. PGPRs and nitrogen-fixing legumes: a perfect team for efficient cd phytoimmunization. Front Plant Sci. 2015;6:81.
5. Erturk Y, Erçili S, Hanzedar A, Cakmakci R. Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (Actinidia delicosa) stem cuttings. Birol. 2010;43:95–9.
6. Himadri Bhusan B, Subhasis D, Dangar TK, Adhya TK. ACC deaminase and its response to the root exudates. Int Microbiol. 2020;23:241–5.
7. Tank N, Saraf M. Enhancement of plant growth and decontamination of nickel-spiked soil using PGPR. J Basic Microbiol. 2010;50:85–93.
8. Liu H, Wang J, Sun HM, Han XB, Peng YL, Liu J, Liu K, Ding YQ, Wang CQ. Du BH. Transcriptome Profiles Reveal the Growth-Promoting Mechanisms of Paenibacillus polymyxa YCO136 on Tobacco (Nicotiana tabacum L.). Front. Microbiol. 2020;11:584174.
9. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M, Kegg. Koyo encyclopedia of genes and genomes. Nucleic Acids Res. 1999;27:29–34.
10. Hou X, Yu X, Du B, Liu K, Yao L, Zhang S, Selin C, Fernando WG, Wang C, Ding Y. A single amino acid mutation in Spo0A results in sporulation deficiency of Paenibacillus polymyxa SC2. Res Microbiol. 2016;167:42–7.
11. Reinbothe S. Chlorophyll biosynthesis: spotlight on protochlorophyllide reduction. Trends Plant Sci. 2010;15:64–74.
12. Suzuki W, Sugawara M, Miwa K, Morikava M. Plant growth-promoting bacteria Azotobacter calcoaceticus P23 increases the chlorophyll content of the monocot Lemma minor (duckweed) and the dicot Lactuca sativa (lettuce). J Biosci Bioeng. 2014;118:841–9.
13. Turan M, Gulluce M, Sahin F. Effects of plant-growth-promoting Rhizobacteria on yield, growth, and some physiological characteristics of wheat and barley plants. Commun Soil Sci Plant Anal. 2012;43:1658–73.
14. Akhtar MS, Siddiqui ZA. Bioclorination of a chickpea root rot disease complex with Glomus intraradices, Pseudomonas putida and Paenibacillus polymyxa. Austral Plant Pathol. 2007;36:175–80.
15. Wu K, Yuan S, Xun G, Shi W, Pan B, Guan H, Shen B, Shen Q. Root exudates from two tobacco cultivars affect colonization of Rhizobium sonorense and the disease index. Eur J Plant Pathol. 2015;141:667–77.
16. Johnson KS, Ottermann KM. Colonization, localization, and inflammation: the role of H. pylori chemotaxis in vivo. Curr Opin Microbiol. 2017;41:51–7.
17. Scharf BE, Hynes MF, Alexandre G. Chemotaxis signaling systems in model beneficial plant-bacteria associations. Plant Mol Biol. 2016;90:549–59.
18. PD TA, Sahoo D, Atta SA, Sharma C, Kalita MC, JS DS. Bacterial rhizosphere community profile at different growth stages of Lomorok (Capsicum chinense) and its response to the root exudates. Int Microbiol. 2020;23:241–51.
19. Yuan J, Zhang N, Huang Q, Raza W, Li R, Vivanco JM, Shen Q. Organic acids from root exudates of banana help root colonization of PGPR strain Bacillus amyloliquefaciens NIN-6. Sci Rep. 2015;5:13438.
20. Vlamakis H, Chai Y, Beaujeaurg P, Logue R, Kolter R. Sticking together: building a biofilm the Bacillus subtilis way. Nat Rev Microbiol. 2013;11:157–68.
21. Murray EJ, Strauch MA, Stanley-Wall NR. cdp is involved in controlling Bacillus subtilis biofilm architecture through the AbrB homologue Abd J. Bacteriol. 2009;191:6822–32.
22. Behera BC, Singdevsachan SK, Mishra RR, Dutta SK, Thakot HI. Diversity, mechanism and biotechnology of phosphate solubilising microorganism in mangrove-a review. Biotaalysts Agricultural Biotechnol. 2014;39:7–110.
43. Ramirez CA, Kloepper JW. Plant growth promotion by Bacillus amyloliquefaciens FZB45 depends on inoculum rate and P-related soil properties. Biol Fertil Soils. 2010;46:835–44.

44. Vančura V, Hovadík A. Root exudates of plants. Plant Soil. 1965;22:21–32.

45. Yu Z, Guo C, Qiu J. Precursor amino acids inhibit Polymyxin E biosynthesis in Paenibacillus polymyxa. Probably by Affecting the Expression of Polymyxin E Biosynthesis-Associated Genes Biomed Res Int. 2015;2015:11.

46. Kuratsu Y, Arai Y, Inuzuka K, Suzuki T. Stimulatory effect of aspartic acid on Colistin production by Bacillus polymyxa. Agric Biocl Chem. 1983;47:2607–12.

47. Shaheen M, Li J, Ross AC, Vederas JC, Jensen SE. Paenibacillus polymyxa PKB1 produces variants of Polymyxin B-type antibiotics. Chem Biol. 2011;18:1640–8.

48. Li J, Brader G, Kariola T, Palva ET. WRKY70 modulates the selection of signaling pathways in plant defense. Plant J. 2006;46:477–91.

49. Aditya B, Aradhya R. WRKY Proteins. Signaling and Regulation of Expression during Abiotic Stress Responses. Scientific World J. 2015;2015:807560.

50. Phukan UJ, et al. WRKY transcription factors: molecular regulation and stress responses in plants. Front Plant Sci. 2016;7:760.

51. Jiang J, Ma S, Ye N, Jiang M, Cao J, Zhang J. WRKY transcription factors in plant responses to stresses. J Integr Plant Biol. 2017;59:96–101.

52. Yang X, Li H, Yang Y, Wang Y, Mo Y, Zhang R, Zhang Y, Ma J, Wei C, Zhang X. Identification and expression analyses of WRKY genes reveal their involvement in growth and abiotic stress response in watermelon (Citrullus lanatus). PLoS One. 2018;13:e0191308.

53. Han D, Hou Y, Wang Y, Ni B, Li Z, Yang G. Overexpression of a Malus baccata WRKY transcription factor gene (MbWRKY5) increases drought and salt tolerance in transgenic tobacco. Can J Plant Sci. 2019;99:173–83.

54. Oh SK, Yi SY, Yu SH, Moon JS, Choi D. CaWRKY2, a chili pepper transcription factor, is rapidly induced by incompatible plant pathogens. Mol Cells. 2006;22:58–64.

55. Dang FF, Wang YN, Yu L, Eulgem T, Lai Y, Liu QZ, Wang X, Qiu AL, Zhang TX, Lin J. CaWRKY40, a WRKY protein of pepper, plays an important role in the regulation of tolerance to heat stress and resistance to Ralstonia solanacearum infection. Plant Cell Environ. 2013;36:757–74.

56. Bull CT, De Boer SH, Denny TP, Firmao G, Saux MF, Saddler GS, Scottichini M, Stead DE, Takikawa Y. Comprehensive list of names of plant pathogenic bacteria, 1980-2007. J Plant Pathol. 2010;92:551–92.

57. Hasse CH. Pseudomonas citri, the cause of citrus canker. J Agric Res. 1915;4:97–100.

58. Chaney RL, Chen KY, Li YM, Angle JS, Baker AJ. Effects of calcium on nickel tolerance and accumulation in Alyssum species and cabbage grown in nutrient solution. Plant Soil. 2008;311:151–60.

59. Fritschi FB, Ray JD. Soybean leaf nitrogen, chlorophyll content, and chlorophyll a/b ratio. Photosynthetica. 2007;45:92–8.

60. Guan P, Wang J, Xie C, Wu C, Yang G, Yan K, Zhang S, Zheng C, Huang J. SES1 positively regulates heat stress resistance in Arabidopsis. Biochem Biophys Res Commun. 2019;513:582–8.

61. Langmead B, Salzberg SL. Fast gapped-read alignment with bowtie 2. Nat Methods. 2012;9:357–9.

62. Grabherr M, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol. 2011;29:554–64.

63. Robinson MD, McCarthy D, Smyth GK. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Biogeoosciences. 2010;26:139–40.

64. Klopsteinen DN, Zhang L, Pedersen BS, Ramirez F, Verszoczy AW, Naldi A, Mungall CJ, Yusnes JM, Botvinnik O, Weigel M. GOATOOLS A Python library for Gene Ontology analyses. Sci Rep. 2018;8:10872.

65. Xia C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li C, Wei L. KOBAS 2.0, a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res. 2011;39:316–22.

66. Livak K, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^-△△Ct_ method. Methods. 2001;25:402–8.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
• fast, convenient online submission
• thorough peer review by experienced researchers in your field
• rapid publication on acceptance
• support for research data, including large and complex data types
• gold Open Access which fosters wider collaboration and increased citations
• maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions