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Mechanisms of ROS-mediated interactions between *Bacillus aryabhattai* LAD and maize roots to promote plant growth

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Abstract

Background Plant growth-promoting rhizobacteria (PGPR), as a group of environmentally friendly bacteria growing in the rhizosphere of plants, play an important role in plant growth and development and resistance to environmental stresses. However, their limited understanding has led to the fact that their large-scale use in agriculture is still scarce, and the mechanisms by which beneficial bacteria are selected by plants and how they interact with them are still unclear.

Method In this study, we investigated the interaction between the auxin-producing strain *Bacillus aryabhattai* LAD and maize roots, and performed transcriptomic and metabolomic analyses of *Bacillus aryabhattai* LAD after treatment with maize root secretions(RS).

Results Our results show that there is a feedback effect between the plant immune system and bacterial auxin. Bacteria activate the immune response of plant roots to produce reactive oxygen species(ROS), which in turn stimulates bacteria to synthesize IAA, and the synthesized IAA further promotes plant growth. Under the condition of co-culture with LAD, the main root length, seedling length, root surface area and root volume of maize increased by 197%, 107%, 89% and 75%, respectively. In addition, the results of transcriptome metabolome analysis showed that LAD was significantly enriched in amino acid metabolism, carbohydrate metabolism and lipid metabolism pathways after RS treatment, including 93 differentially expressed genes and 45 differentially accumulated metabolites.

Conclusion Our findings not only provide a relevant model for exploring the effects of plant-soil microbial interactions on plant defense functions and thereby promoting plant growth, but also lay a solid foundation for the future large-scale use of PGPR in agriculture for sustainable agricultural development.

Keywords *Bacillus aryabhattai*, PGPR, Maize, IAA, ROS, Feedback effect

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Background

Plants have been in a symbiotic relationship with soil microorganisms during their growth and development. As a key member of the soil ecosystem, plant growth-promoting rhizobacteria (PGPR) is mainly distributed in the roots and rhizosphere of plants and participates in a variety of biological activities in the soil ecosystem. PGPR can improve crop productivity and resistance to pathogens by inducing complex changes in plant growth and development [1, 2]. In addition, PGPR also competitively settles in plant roots and plays a crucial role in promoting plant growth through different mechanisms such as phosphate solubilization, nitrogen fixation, IAA production, siderophores, biofilm synthesis, VOCs production, and induction of plant systemic resistance [1–3]. To date, many PGPR strains have been isolated for extensive and in-depth research, which is of great significance for promoting sustainable agricultural development [4, 5]. However, so far, the mechanism of plant-rhizosphere microbiome interaction is extremely complex, and little is known about how beneficial bacteria interact with the plant immune system.

In general, the growth and stress effects of plants under the action of PGPR could be determined by changes in hormone levels and related signaling pathways. Each plant has its specific microflora, and the structure of the microflora in the rhizosphere of plants is composed of root secretions produced by plant roots and signal molecules produced by plants and microorganisms [6–8]. With the help of these signaling molecules, a variety of close relationships have been established between plant roots and microorganisms. Acturally, plant roots can interact with a large number of bacteria in the surrounding soil. When bacteria migrate from bulk soil to the root surface and colonize further, the bacterial diversity decreases, which seems to indicate that plants exert selective forces on their colonizing bacteria [9]. An early filter used by plants to identify and respond to bacteria is their immune system [10], which recognizes bacterial flagella, peptidoglycans and bacterial elongation factors through MAMPs [11]. When plant roots recognize these molecules, a series of molecular events will occur. The earliest stage is the outflow of calcium ions and the outbreak of reactive oxygen species (ROS) [12, 13]. Under the condition of pathogen infection, the recognition and activation of plant immune system has been widely described, and MAMP receptors can recognize molecules in all bacteria, and it is found that beneficial bacteria can also induce immune responses similar to pathogenic bacteria [14, 15]. Therefore, the effect of plant immune system on healthy root microbiota and how beneficial microorganisms respond to plant immune system is still an active research field, and there are still many issues that need to be explored in depth [16].

Previously, we found that *B. aryabhattai* LAD, as a good PGPR strain, significantly promotes maize growth [17, 18]. Therefore, in order to understand the interaction between *B. aryabhattai* LAD and maize roots to further understand the interaction between beneficial bacteria and plant immune system, we co-cultured the strain LAD with maize to explore its IAA synthesis level, intracellular ROS level and the relationship between ROS and IAA synthesis. Subsequently, we further analyzed the changes of intracellular transcriptome and metabolome of *B. aryabhattai* LAD under the stimulation of maize root secretions. These results provide a relevant model for exploring the effects of plant-soil microbial interactions on plant defense functions and thus promoting plant growth, and also lay a solid foundation for the future application of PGPR to field production for sustainable agricultural development.

Materials and methods

Strain and culture conditions

The strain *B. aryabhattai* LAD used in this study was isolated from the rhizosphere soil of maize and preserved in the China General Microbiological Culture Collection Center (CGMCC18653), the NCBI accession number is PRJNA716506. *B. aryabhattai* LAD was inoculated in beef extract peptone medium and cultured to logarithmic phase at 37 °C and 180 rpm. Subsequently, the LAD bacterial suspension was transferred to 50 mL beef extract peptone liquid medium at 1% inoculation amount, and cultured at 37 °C and 180 rpm for 24 h as seed solution.

The plant material used in this study was maize and the seeds were sourced from Dalian Baisite Seed Co., Ltd. The maize seeds were surface sterilized by soaking in 70% ethanol for 2 min and then in 1% sodium hypochlorite solution for 20 min. After soaking, the seeds were rinsed three times in sterile desalinated water and prepared for use. Plants were kept in climate cabinets at 21 °C (180 μmol light $m^{-2}{\cdot}s^{-1}$ at plant level; 16 h:8 h, light: dark).

Effects of exogenous IAA and *B. aryabhattai* LAD on the growth of maize seedlings

The *B. aryabhattai* LAD was inoculated into beef extract peptone medium at 1% inoculation amount, and cultured at 37 °C and 180 rpm for 72 h until OD600 nm was 1.4–1.5. The fermentation broth was taken and the IAA content was detected by Angilent ultra-high performance liquid chromatograph 1290 Infinity II (Agilent Technologies Inc., California, USA). The chromatographic column was Agilent Polaris C18-A (250 mm × 4.6 mm), the flow rate was 1 mL / min, the injection volume was 10 μL, the column temperature was 35 °C, the wavelength was 254 nm, the mobile phase A was pure methanol, and the mobile phase B was glacial acetic acid aqueous solution with pH value of 3.2 [V (A) : V (B)=5.5 : 4.5].

In addition, the bacterial suspension cultured for 72 h was diluted in a gradient of 10 times, and the *B. aryabhattai* LAD bacterial suspension diluted to 10^2 CFU mL^{-1} was used to soak the maize seeds for 24 h. The seeds were placed in a hydroponic tank containing *B. aryabhattai* LAD bacterial suspension and IAA for 14 days. The normal culture medium was used as a blank control to measure the roots of maize seedlings. The root development was measured by WinRHIZO Reg STD4800 Root Analysis System (Beijing Ruiding Environmental Technology Co., Ltd., Beijing, China). The growth promotion indexes of maize seedlings and their roots were counted using percentage values calculated as [(treatment group - control group)/control group]*100%.

Collection of maize root secretions

To test the effect of maize root secretions on IAA synthesis in LAD, root secretions were collected by hydroponics. The seeds soaked with LAD were cultured in a hydroponic tank containing *B. aryabhattai* LAD bacterial suspension, and the normal culture medium was used as a blank control. After 14 days of culture, the root secretions were collected and filtered with 0.22 μm filter membrane and stored at 4 °C. At the same time, the IAA content was detected by Angilent ultra-high performance liquid chromatograph 1290 Infinity II (Agilent Technologies Inc., California, USA).

Effects of maize root secretions and H_2O_2 on*B. aryabhattai* LAD

The *B. aryabhattai* LAD was inoculated into beef extract peptone medium supplemented with 0.1%, 0.2% and 0.4% H_2O_2 and 1%, 2% and 4% maize root secretions at a 1% inoculation amount, respectively. The blank beef extract peptone medium inoculated with *B. aryabhattai* LAD bacterial liquid was used as a blank control. One part was placed in a 12-well plate at 37 °C, 180 rpm for 24 h in a microplate reader, and the OD (600 nm) was measured every 1 h. The other part was in a 50 mL conical flask. IAA production was determined by high performance liquid chromatography after 72 h of shaking culture at 180 rpm.

Determination of ROS levels in cells and maize roots

Maize roots were collected at 2,4,6,8,10 and 12 h, respectively. At the same time, bacterial cells were collected by centrifugation at 8000 rpm for 10 min, and then the bacterial cells were fully washed with PBS (pH 7.0) and suspended in 1 mL phosphate buffer.

The relative levels of intracellular reactive oxygen species (ROS) in *B. aryabhattai* LAD were determined by fluorescence assay, mainly using the oxidant-sensitive probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China). The fluorescence intensity was detected using a Bio Tek Cytation5 microplate reader (Beijing Derica Biotechnology Co., Ltd, Beijing, China), and the emission wavelength was set at 525 nm and the excitation wavelength was set at 488 nm. The relative level of ROS in maize roots was measured by Plant Reactive Oxygen Species ELISA Kit instruction at 450 nm to reflect the ROS level. Each sample was performed using three independent biological replicates.

Total RNA extraction and transcriptome analysis

- *B. aryabhattai* LAD treated with maize root secretions was selected as the treatment group (G), and *B. aryabhattai* LAD cultured under normal conditions was used as the control group (CK). 1 mL bacterial suspension was centrifuged at 10,000 r min^{-1} for 2 min at 4 °C in a 1.5 mL enzyme-free tube, and the supernatant was discarded to collect the bacteria and quickly frozen in liquid nitrogen for 15 min. The total RNA was extracted using the Trizol kit (takara, dalian, China) according to the kit instructions.

High-quality RNA samples were collected by NEB Next Ultra RNA Library Preparation Kit (NEB, CA) for the preparation of cDNA library construction. Next-Generation Sequencing (NGS) was used to perform transcriptome sequencing analysis and comparison of samples based on the Illumina HiSeq sequencing platform. GO and KEGG enrichment analysis was performed using Blast2GO (v2.5) and KOBAS (v3.0) software. Differentially expressed genes (DEGs) were performed using the DESeq (v1.32.0) program, and log2 (fold change) ≥ 1 and p (padj) ≤ 0.05 were considered to be DEGs. Pathway analysis of DEGs was performed using the KEGG PATHWAY database and Blast2GO.

Metabolite extraction and metabolome analysis

2 mL of pre-cooled 60% methanol water was placed in a 5 mL centrifuge tube, and then 2 mL of bacterial solution was quickly added to it. The mixture was mixed manually for 5 s. The quenched bacterial solution was centrifuged at 3,000 r min^{-1}, 4 °C for 10 min, and the supernatant was removed and placed in dry ice. The metabolites were detected by Thermo Vanquish (Thermo Fisher Scientific, USA) ultra-high performance liquid chromatography system combined with Thermo Orbitrap Exploris 120 mass spectrometry detector (Thermo Fisher Scientific, USA).

The identification of relevant metabolites in the metabolome was based on the databases of HMDB (http://www.hmdb.ca), Metlin (http://metlin.scripps.edu), MassBank (http://www.massbank.jp), LipidMaps (http://www.

lipidmaps.org) and mzClound (https://www.mzcloud.org) databases were performed. Differently accumulated metabolites (DAMs) were assessed by partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA) models using variable importance in prediction (VIP) scores. Thresholds for the analytical models were set at VIP ≥ 1, fold change ≥ 1.5 or ≤ 0.667, and $p \leq 0.05$. Integration analyses of metabolomics and transcriptomics were performed via Pearson correlation coefficients, as previously described [19, 20].

Real-time quantitative polymerase chain reaction (qPCR) analysis

To further elucidate the ROS-mediated interactions between *B. aryabhattai* LAD and maize rhizosphere to promote growth, we first examined the changes in the expression of genes related to growth hormone synthesis in *B. aryabhattai* LAD after exogenous additions of root secretions and H_2O_2. Subsequently, changes in the expression of growth hormone synthesis and antioxidant-related genes in the maize root system after exogenous addition of *B. aryabhattai* LAD treatment were further examined to determine the relationship between activation of the plant immune system and growth hormone synthesis following *B. aryabhattai* LAD interactions with the maize root system.

The SYBR Green Premix Pro Taq HS qPCR Kit (AG11718, ACCURATE BIOTECHNOLOGY, HUNAN, Co., Ltd, Changsha, China) was used to perform qPCR in a Quant Studio6 Flex system (Thermo Fisher Scientific, USA). 16 S rDNA and Actin were selected as the internal reference genes for *B. aryabhattai* LAD and maize roots, respectively. To analyze the qPCR results, the relative expression of each gene was calculated by the comparative crossover (CP) method and expressed as $2^{-\Delta\Delta CT}$. Each gene expression analysis was performed using three independent biological replicates. Specific primers used for qPCR are listed in Table S1.

Statistical analysis

IBM SPSS 25.0 (SPSS Inc., Chicago, IL, USA) software was used to statistically analyze the experimental data. One-way ANOVA (One-way ANOVA) was used to determine significant differences between the following: different treatments on maize root growth, different treatments on LAD growth and its IAA yield, and different treatments on the level of ROS in LAD and in the maize root system. All trials were conducted in triplicate and data were averaged and expressed as mean ± standard error (SE).

The qPCR data of selected gene expression profiles were analyzed using the software package IBM SPSS 25.0 of Windows (SPSS Inc., Chicago, IL, USA). Prior to analysis, data were normalized by transforming as ln (x+1). All data were expressed as means ± standard error of at least triplicates of independent cultures. The means of different treatments were compared by one-way ANOVA using Turkey's honestly signifcant difference test at the 5% probability level ($p < 0.05$). Metabolomics analysis was evaluated using variable importance in the projection (VIP) in the frst principal component of each OPLS-DA model. Thresholds with $P < 0.05$ and VIP > 1 were considered statistically significant. MetaboAnalyst software (version 3.0) was used for pathway enrichment analysis [21]. Differentially expressed genes were analyzed for differences using DESeq, and the threshold for identifying DEGs was set at |log2 (fold change)| ≥ 1,padj ≤ 0.05.

Results

Effect of exogenous addition of *B. aryabhattai* LAD and IAA on the growth of maize roots

The results of root development of maize seedlings under different treatment conditions measured by root analyzer are shown in Fig. 1 and Table S2. The main root length of maize seedlings increased by 197% and 167% respectively after exogenous addition of *B. aryabhattai* LAD and IAA. The main root thickness increased by 22% and 16% respectively, and the seedling length of maize seedlings increased by 107% and 78% respectively. Exogenous addition of *B. aryabhattai* LAD and IAA increased root surface area by 89% and 61%, root volume by 75% and 34%, and total root length by 97% and 63%, respectively. Exogenous addition of *B. aryabhattai* LAD and direct exogenous addition of IAA with the same content could promote the growth of maize seedlings, but the growth of maize roots in the exogenous addition of *B. aryabhattai* LAD group was significantly higher than that in the IAA group with the same concentration.

Effect of root secretion and H_2O_2 addition on the growth and IAA production of *B. aryabhattai* LAD

The effects of root secretion and H_2O_2 addition on the growth of *B. aryabhattai* LAD and its IAA production are shown in Fig. 2A, B. The growth of *B. aryabhattai* LAD was strongly inhibited by the addition of H_2O_2 treatment, and its level of IAA production was significantly lower than that of the control group, and the levels of IAA synthesized after the addition of 0.1%, 0.2%, and 0.4% of H_2O_2 to the *B. aryabhattai* LAD culture for 72 h were 15.1, 17.5, and 15.8 μg/mL, respectively, while the growth inhibition of *B. aryabhattai* LAD was strongly alleviated after the addition of IAA to its culture. Subsequently, the growth of *B. aryabhattai* LAD was also inhibited by the addition of root secretion, but the level of IAA production was significantly higher than that of the control group and the other treatment groups, and the levels of IAA synthesized after 72 h of incubation of

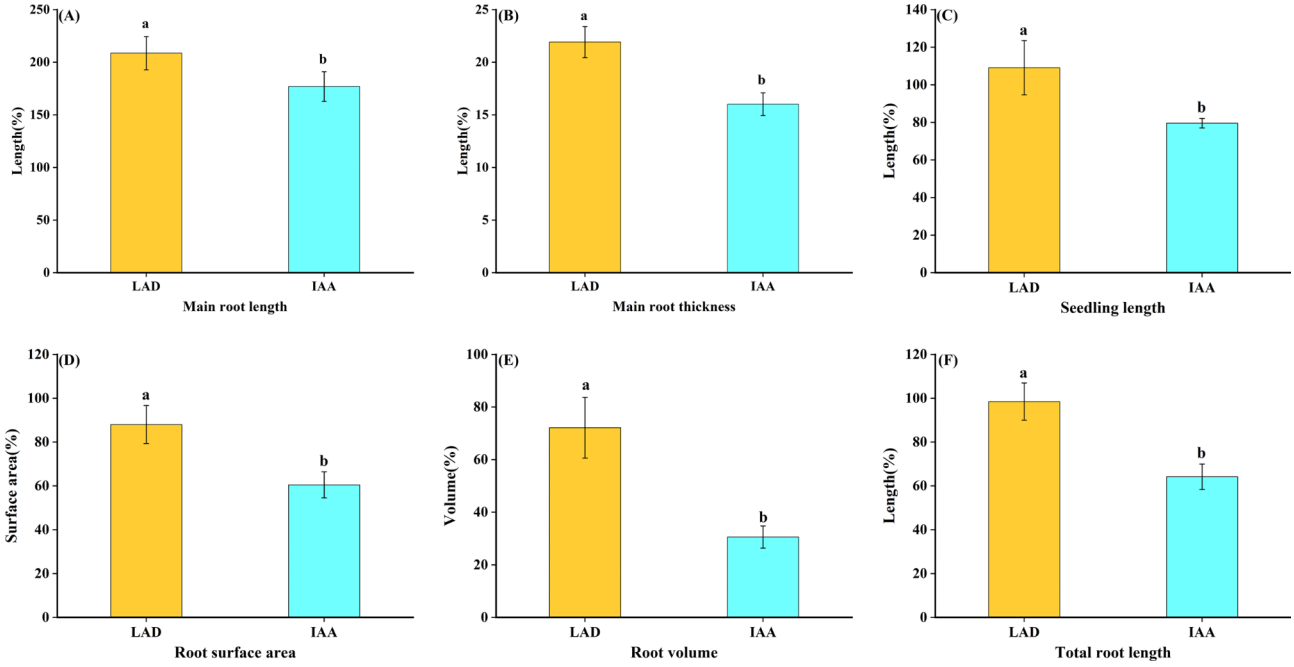

Fig. 1 Effect of exogenous addition of *B. aryabhattai* LAD and IAA on root growth of maize seedlings. All data are from three independent biological replicate means ± SE ($n = 3$). Different letters (a-b) indicate the degree of significant difference between treatments. Values with the same letter above the bar graph indicate no significant difference according to the Tukey test

B. aryabhattai LAD with 1%, 2%, and 4% of root secretion were 26.7, 24.2, and 23.4 μg/mL, respectively.

Effect of different treatment conditions on ROS levels in *B. aryabhattai* LAD and maize roots

The ROS levels in the roots of maize at different times are shown in Fig. 2C, and the ROS levels were determined by taking the maize roots on the third day from the start of the submerged seed culture, and both the control group and the treatment group showed a trend of increasing and then decreasing, and the ROS levels of the maize roots were significantly higher than those of the control group after the addition of the *B. aryabhattai* LAD treatment.

The ROS levels of *B. aryabhattai* LAD strains at different culture stages were shown in Fig. 2D, which combined with the *B. aryabhattai* LAD growth curve showed that the intracellular ROS in the culture to 2 h, the concentration of the bacteria was small, and the difference of its intracellular ROS levels was not significant, after 2 h, the bacteria multiplied a lot and entered into the logarithmic growth period, and the ROS levels in the control group began to be significantly higher than that of the treatment group, and by 8 h, the growth of the strains began to slow down, and the ROS levels in the treatment group began to decrease and the control group was still at a high level.

Transcriptome analysis

To identify DEGs in *B. aryabhattai* LAD under maize root secretion treatment conditions, we constructed 6 cDNA libraries and filtered the sequencing data of transcriptome samples to obtain a total of 17.71 Gb of data. Gene expression was differentially analyzed using DESeq, and the threshold for identifying DEGs was set at $|\log_2(\text{fold change})| \geq 1$, padj ≤ 0.05. Compared with the control group, there were 93 DEGs in *B. aryabhattai* LAD under root secretion treatment conditions, of which 57 genes were up-regulated and 36 genes were down-regulated .

Subsequently, we performed GO and KEGG enrichment analysis to further understand the biological functions of these DEGs and the related biological processes involved. As shown in Fig. 3A, in terms of GO enrichment analysis, the DEGs of *B. aryabhattai* LAD under root secretions treatment were mainly enriched in molecular functions (MF) and biological processes (BP). The top three GO terms significantly enriched in the MF category were phosphoribosylformylglycinamidine synthase activity (GO:0004642), carbon-nitrogen ligase activity, with glutamine as amido-N-donor (GO:0016884), ligase activity, forming carbon-nitrogen bonds (GO:0016879). In the BP category, the top three GOs significantly enriched were phospholipid catabolic process (GO:0009395), urea cycle (GO:000050), and urea metabolic process (GO:0019627). KEGG enrichment analysis showed (Fig. 3B) that the top 20 significantly enriched pathways of DEGs in *B. aryabhattai* LAD under

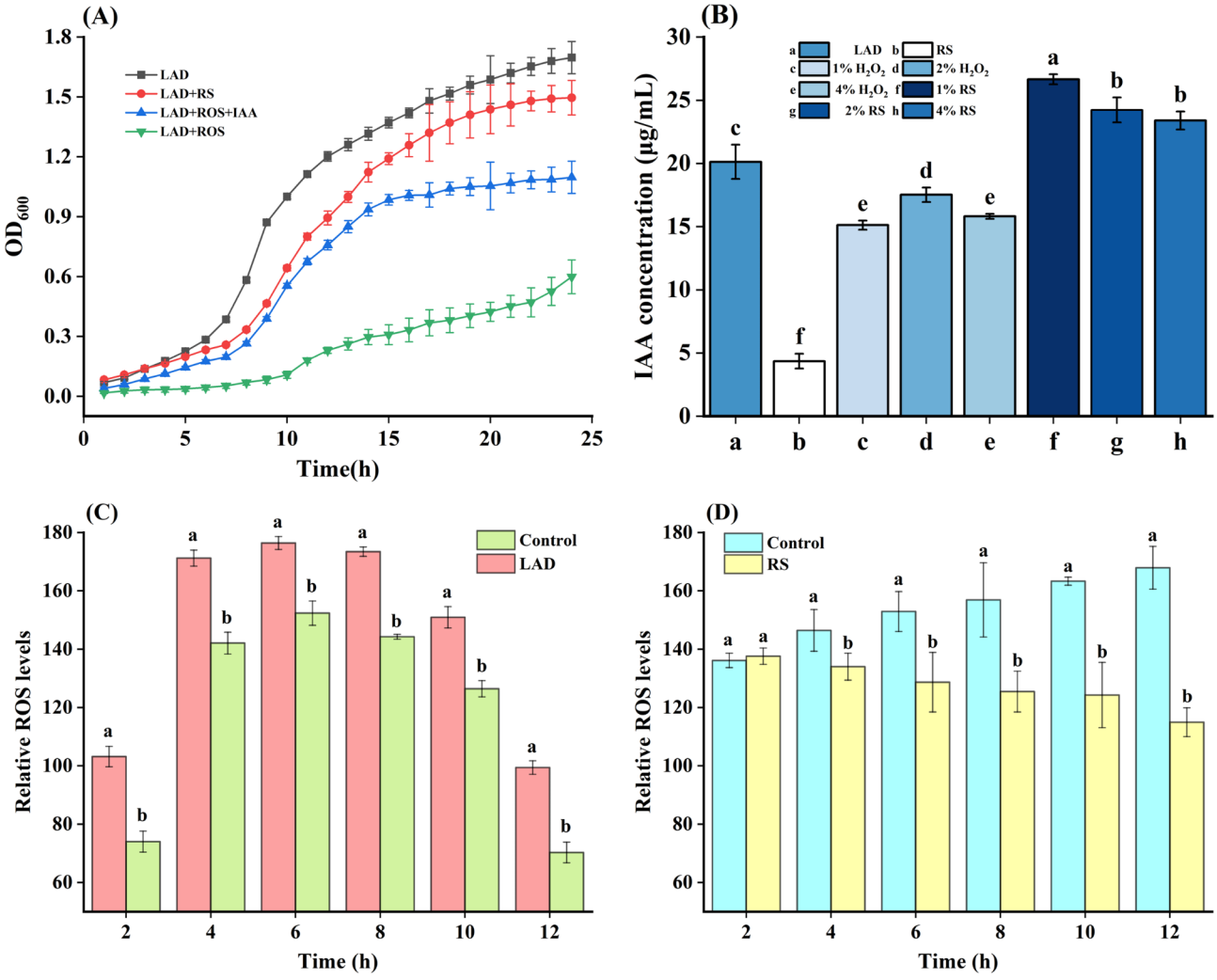

Fig. 2 Effects of root secretions and H_2O_2 addition on the growth (**A**) and IAA production (**B**) of *B. aryabhattai* LAD and changes in ROS levels in cells after interaction between *B. aryabhattai* LAD (**C**) and maize roots (**D**). All data are from three independent biological replicate means ± SE ($n = 3$). Different letters (a-b) indicate the degree of significant difference between treatments. Values with the same letter above the bar graph indicate no significant difference according to the Tukey test

root secretion treatment conditions were mainly associated with Metabolism, Genetic Information Processing, Environmental Information Processing in KEGG level 1 and Purine metabolism (ko00230), Inositol phosphate metabolism (ko00562), Alanine, aspartate and glutamate metabolism (ko00250), Glycerophospholipid metabolism (ko00564), ABC transporters (ko02010), Tyrosine metabolism (ko00350), Lysine degradation (ko00310), Oxidative phosphorylation (ko00190) Glycolysis / Gluconeogenesis (ko00010), Pyruvate metabolism (ko00620) in KEGG level 2. These results may be importantly related to the promotion of maize growth and resistance to environmental stresses by *B. aryabhattai* LAD.

Metabolome analysis

In order to evaluate the changes of intracellular metabolism of *B. aryabhattai* LAD after root secretions treatment, LC-MS / MS was used to evaluate the metabolic profile. The results showed that a total of 1,572 metabolites were identified based on the MS2 spectral database. The identified metabolites were classified according to chemical classification statistics, including 82 classifications, of which Carboxylic acids and derivatives were up to 361. Secondly, there are 113 Steroids and steroid derivatives, 105 Benzene and substituted derivatives, 105 Fatty Acyls, 105 Prenol lipids, 94 Organooxygen compounds, 46 Indoles and derivatives, 45 Glycerophospholipids and 30 Organonitrogens (Fig. 4A). At a threshold setting of VIP ≥ 1, fold change ≥ 1.5 or ≤ 0.667, and $p \leq 0.05$, a total of 1,574 metabolites were detected in the treatment and control groups, of which 967 were detected in the positive ion mode and 607 were detected in the negative ion mode. Differential analysis of the detected metabolites showed a total of 45 differential

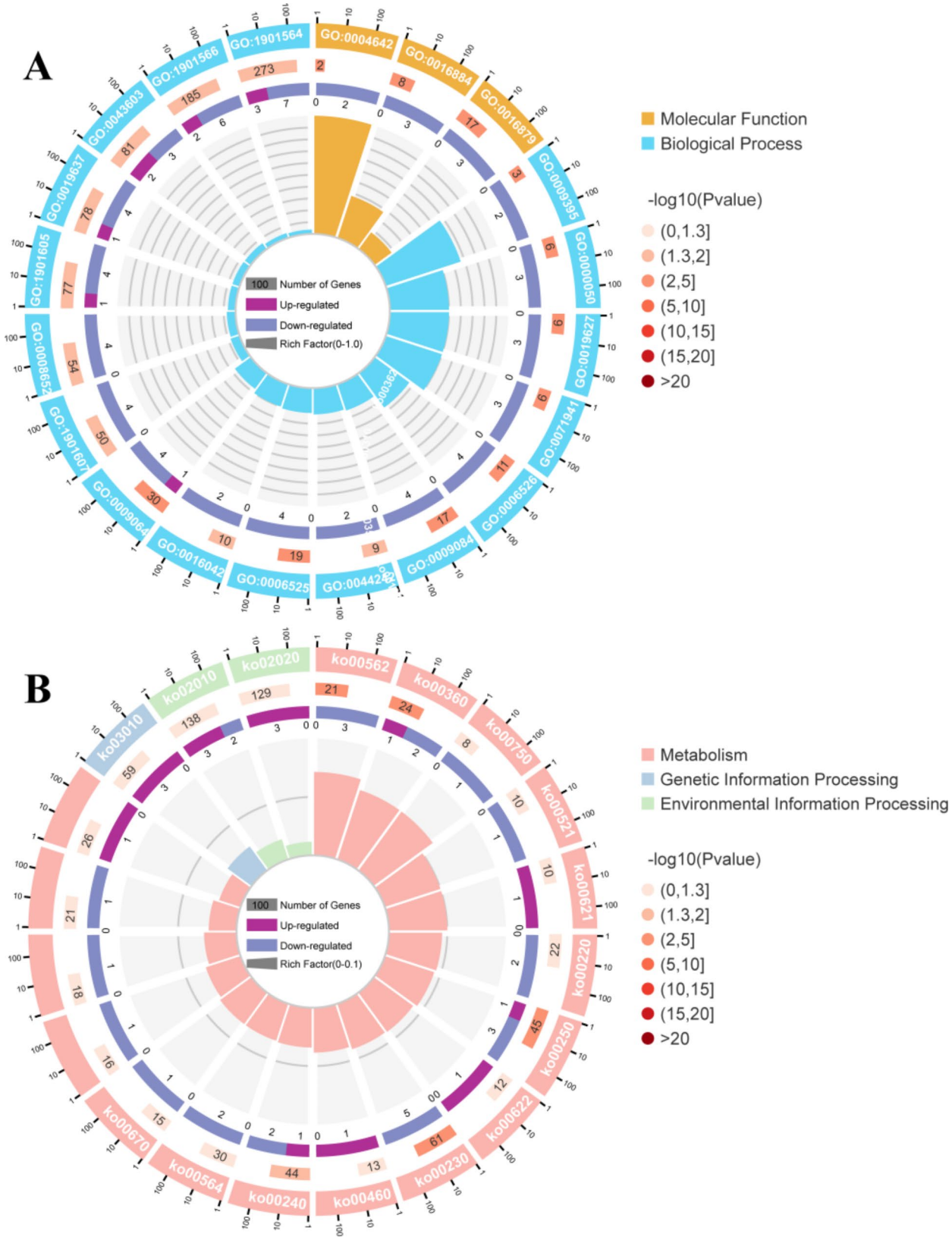

Fig. 3 Transcriptome analysis of G and CK in *B. aryabhattai* LAD. (**A**) Circos plots depicting GO enrichment analysis of DEGs. (**B**) Circos plots illustrating KEGG enrichment analysis of DEGs. Starting from the outer circle and moving inward the representation includes the top 20 enriched GO terms or KEGG pathways (ring 1), the number of background genes in the genome (ring 2), the number of upregulated and downregulated genes (ring 3), and the rich factor of DEGs in corresponding GO terms or KEGG pathways (ring 4) are represented

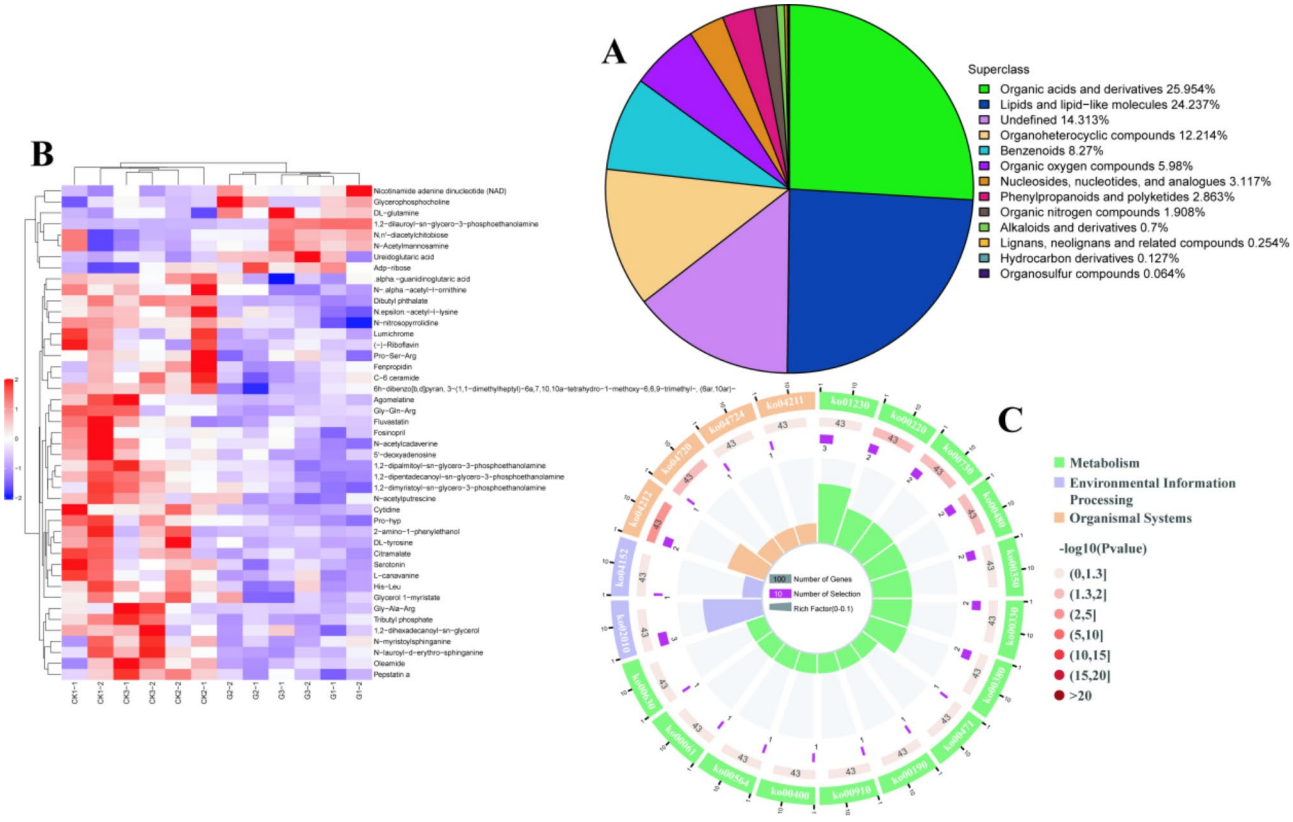

Fig. 4 Metabolome analysis of G and CK in *B. aryabhattai* LAD. (**A**) Quantitative weight of the identified metabolites in each chemical classification. (**B**) Heat map of hierarchical clustering of significantly different metabolites. (**C**) Circos plots illustrating KEGG enrichment analysis of DAMs. Starting from the outer circle and moving inward the representation includes the top 20 enriched GO terms or KEGG pathways (ring 1), the number of background genes in the genome (ring 2), the number of upregulated and downregulated genes (ring 3), and the rich factor of DEGs in corresponding GO terms or KEGG pathways (ring 4) are represented

metabolites, of which 8 differential metabolites were up-regulated and 37 differential metabolites were down-regulated (Fig. 4B). Subsequently, we performed KEGG enrichment analysis to better understand the biological functions of these differential metabolites and their involvement in relevant biological processes. The results showed that a total of 87 KEGG pathways were enriched by differential metabolites under root secretion treatment conditions. As shown in the figure, the first 20 significantly enriched pathways were mainly distributed in Metabolism, Environmental Information Processing and Organismal Systems at KEGG level 1, and Amino acid metabolism, Carbohydrate metabolism and Lipid metabolism at KEGG level 2 (Fig. 4C). Therefore, these metabolic pathways are important for *B. aryabhattai* LAD to respond to root secretions to resist environmental stresses and promote plant growth.

Furthermore, the differentially expressed metabolites and all transcripts were simultaneously mapped to the KEGG pathway database, and there were 69 relevant metabolic pathways, including 213 genes. The main metabolic pathways included Tryptophan biosynthesis, Tryptophan metabolism, Zeatin synthesis, ABC transport system and Two-component system. Among them, 9 differential genes and 14 differential metabolites were annotated in the Tryptophan Metabolism and Tryptophan Biosynthesis pathways, which are closely related to IAA synthesis. Among them, K03781 (catalase: katE, CAT, catB, srpA), KO00453 (tryptophan 2,3 dioxygenase: TOD2, kynA), K00817 (histidyl-phosphate aminotransferase: hisC), K00891 (manganic acid kinase: aroK, aroL), K03786 (3-dehydroquinate dehydratase II: aroQ, qutE), KO01426 (amidase: amiE), and KO00128 (aldehyde dehydrogenase: ALDH) genes were significantly up-regulated after root secretion treatment; K16901 (monooxygenase), K01696 (tryptophan synthase β-chain: trpB), and K01817 (phosphoribosyl o-aminobenzoate isoform: trpF) were significantly down-regulated after root secretion treatment, and the results of integration of differential genes and differential metabolites related to IAA synthesis are shown in Fig. 5.

Changes in the expression of genes related to IAA synthesis in *B. aryabhattai* LAD and maize roots

The expression changes of IAA synthesis related genes *YUC*, *GH3* and *PIN* genes in maize roots under the

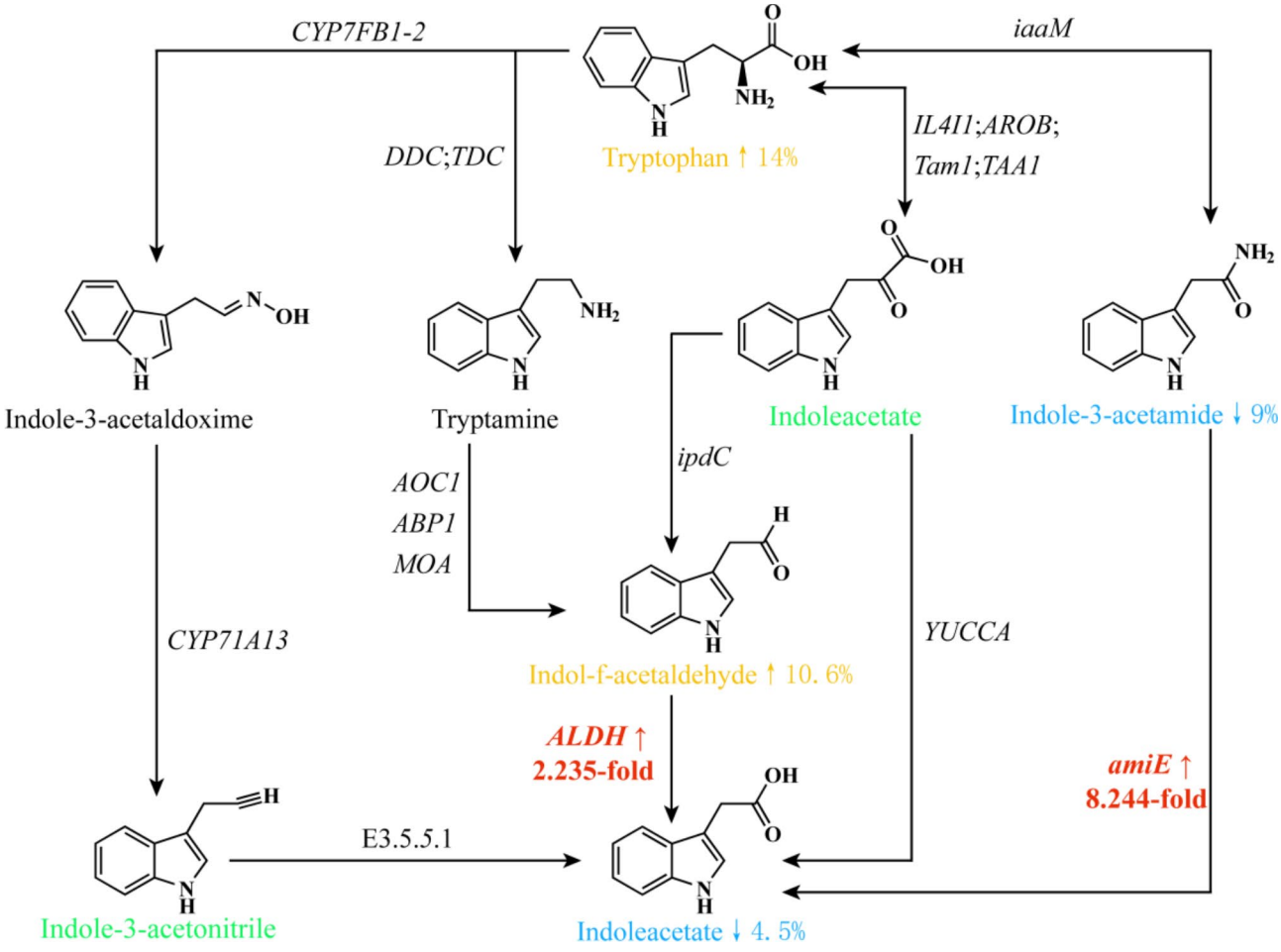

Fig. 5 Analysis of IAA synthesis pathway in *B. aryabhattai* LAD after root secretions treatment

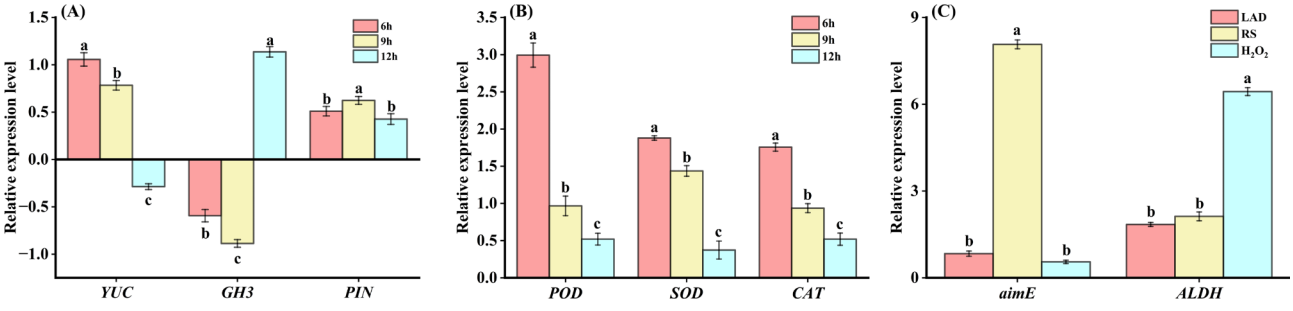

Fig. 6 Changes in the expression of IAA (**A**) and antioxidant genes (**B**) in maize roots and genes related to IAA synthesis (**C**) in *B. aryabhattai* LAD

condition of interaction with *B. aryabhattai* LAD are shown in the Fig. 6A. Among them, *YUC* gene was up-regulated on the 6d and 9d, and down-regulated on the 12d. The *GH3* gene was down-regulated on 6d and day 9d, and the *GH3* gene began to be up-regulated on 12d. *PIN* gene showed an upward trend at 6,9 and 12 d. The changes in the expression of antioxidant genes POD, SDO and CAT genes in maize roots showed that the expression of antioxidant-related genes decreased gradually on the 6d, 9d and 12d after adding *B. aryabhattai* LAD to maize roots (Fig. 6B).

In the *B. aryabhattai* LAD strain, both root secretion and hydrogen peroxide treatments up-regulated the *amiE* and *ALDH* genes (Fig. 6C). The *amiE* gene was significantly up-regulated by root secretion, and the strain was induced by hydrogen peroxide to synthesize more growth hormone by up-regulating the transcript level of *ALDH* gene.

Discussion

Our results indicate that there is a feedback loop between the plant immune system and bacterial auxin synthesis (Fig. 7). When bacteria colonize the roots of plants, they will trigger the immune response of plants and the production of ROS, while ROS in turn will induce bacteria to synthesize auxin to reduce the toxicity of ROS to cells. At the same time, it promotes the spread of bacteria on roots and the formation of colonies, induces the expression of immune receptors in plants, and further accelerates the feedback loop [9]. Moreover, auxin, as an important plant hormone, plays a vital role in plant development [22]. Many bacterial species, such as *Agrobacterium tumefaciens* and *Pseudomonas syringae*, can control plant growth by synthesizing and secreting auxin [23–25]. However, despite decades of research on the production of bacterial auxin and its effects on plants, little is known about the role of bacterial auxin in bacterial physiology and its interaction with plants.

In this study, we found that when *B. aryabhattai* LAD responded to maize root secretions, the root secretion produced a stress response to *B. aryabhattai* LAD resulting in a significant increase in intracellular ROS levels, which acted as a feedback to regulate *B. aryabhattai* LAD intracellularly to respond to this stress effect, such as the increase in the synthesis of IAA production, while amino acid metabolism, carbohydrate metabolism and lipid metabolism pathways were significantly enriched. Free amino acids, carbohydrates and lipids are important regulators of cellular responses to environmental stresses and promote plant growth [21, 26–28]. The joint analysis showed that tryptophan synthesis, tryptophan metabolism, glutamate metabolism, cysteine metabolism, glycine metabolism and glutathione metabolism were significantly enriched in amino acid metabolic pathways and metabolites such as glutathione were significantly accumulated. Tryptophan synthesis and tryptophan metabolism are the main metabolic pathways for IAA synthesis. When *B. aryabhattai* LAD feels environmental stress, cells act as a feedback effect to activate IAA synthesis-related pathways to synthesize IAA to help cells resist environmental stress and promote plant growth. In the *B. aryabhattai* LAD tryptophan metabolic pathway, the tryptophan content was significantly elevated and the amiE gene transcript level was significantly upregulated. Under the action of amiE, indoleacetamide was converted to indoleacetic acid, which in turn was secreted extracellularly through the ABC transport system and was absorbed and utilized by plants. In addition, the enrichment of glutamate metabolism, cysteine

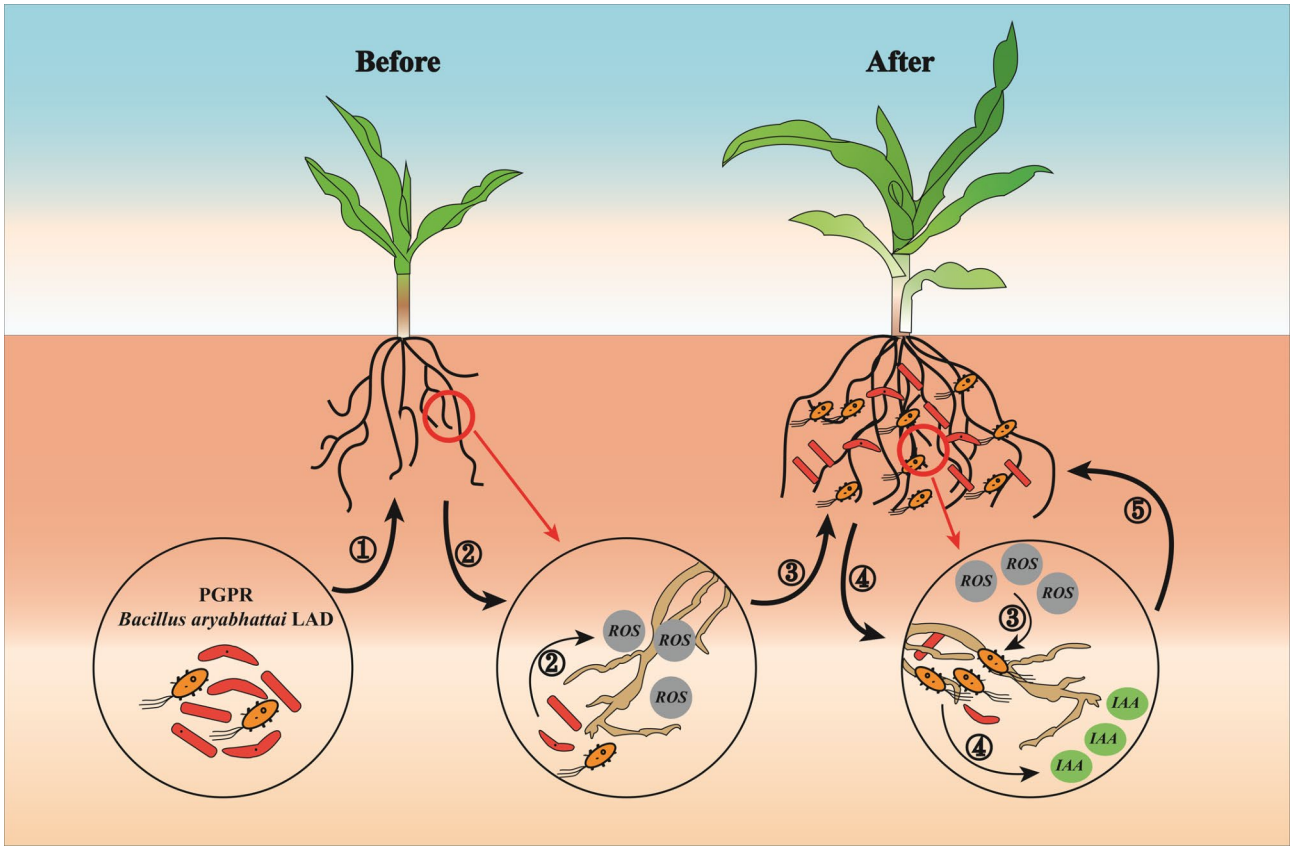

Fig. 7 Regulatory mechanisms of ROS and IAA in the promotion of maize root development by *B. aryabhattai* LAD

metabolism, and glycine metabolism may be mainly used for the synthesis of glutathione, because glutathione, as a substance possessing an extremely strong antioxidant capacity to scavenge ROS, inhibit the formation of free radicals, and lipid peroxidation, helps the *B. aryabhattai* LAD to play an important role in regulating the intracellular ROS in the event of environmental stress [29, 30].

In carbohydrate metabolism, glycolysis and gluconeogenesis, fructose and mannose metabolism, galactose metabolism, starch and sucrose metabolism, glyoxylate and dicarboxylic acid metabolism, oxidative phosphorylation and other metabolic pathways were significantly enriched, among which glucose, fructose, UDP-D-G, NAD and so on were significantly accumulated. As energy substances of cells, on the one hand, they provide energy sources for their own growth to accelerate cell metabolism, on the other hand, they can help cells improve their ability to resist osmotic stress and help cells resist environmental stress [31–33]. Moreover, it is worth noting that glyoxalate and dicarboxylic acid metabolism were also significantly enriched in *B. aryabhattai* LAD, and the antioxidant enzyme CAT was significantly up-regulated. Previous studies have shown that the glyoxylate cycle plays an important role in stress defense and resistance to environmental stress, especially when some bacteria activate the glyoxylate cycle to help cells resist environmental stress and activate related antioxidant enzyme systems to scavenge the toxic effects of ROS on the cells when they are subjected to environmental stress [34, 35]. Furthermore, inositol is also significantly accumulated in *B. aryabhattai* LAD, which is involved in a variety of physiological and biochemical processes as a precursor of many important metabolites. Cells resist the stress response produced by plant roots to maintain their own growth by up-regulating inositol [36, 37]. In the lipid metabolism pathway, the metabolic pathways related to glycerol synthesis were also significantly enriched, including glycerolipid metabolism and glycerol phospholipid metabolism, and the accumulation of lipids such as glycerol was significantly increased. It can not only help *B. aryabhattai* LAD resist the stress effect of plant roots, but also provide substrates and energy for other metabolic pathways, and improve the antioxidant capacity of cells in an indirect way.

The cross-talk between auxin and ROS has been increasingly emphasized. On the one hand, stress treatments cause an increase in ROS in the plant, which in turn regulates the activity of the root zone by controlling the accumulation of auxin; on the other hand, auxin can also reduce the redox state in the cell by modulating the activity of scavenging enzymes in vivo, which can alter the growth and development of the plant. In addition, ROS produced by plant roots also activates the immune response of rhizosphere bacteria to synthesize more IAA to further promote plant growth [38, 39]. In this study, it was shown that exogenous addition of *B. aryabhattai* LAD could significantly promote the growth of maize seedlings. The verification of the expression changes of IAA synthesis-related genes in maize showed that the IAA synthesis-related genes *YUC*, *GH3* and *PIN* in maize roots were significantly up-regulated. They are closely related to the synthesis of IAA in plants and play an important role in the growth and development of plants [40–42], in which the *YUC* gene was up-regulated on the 6th and 9th days, indicating that IAA was synthesized in large quantities in maize roots at this time, while on the 12 th day, the *YUC* gene was down-regulated. It may be that the accumulation of IAA in the plant itself reaches a certain level, and the synthesis rate begins to decline. The *GH3* gene was down-regulated on the 6th and 9th days, indicating that the IAA metabolism was relatively slow at the accumulation stage. At 12 days, the *GH3* gene began to be up-regulated, and the IAA effect of plant roots may be relatively obvious. After exogenous addition of *B. aryabhattai* LAD, the *PIN* gene in maize roots mainly controlled IAA transport, and most of its genes were up-regulated in each period to promote maize root development.

Together, plants promote their own growth through this ROS-mediated interaction with *B. aryabhattai* LAD, and plants interact with a wide variety of bacteria in nature. The composition of these microbial populations is affected by microbial diversity, immune system activation, bacteria and other bacteria, fungi and phages. The interaction of other organisms and other factors, understanding each of these components can reasonably manipulate the plant microbiome to benefit plants. Therefore, these results may provide some ideas for understanding microbial-plant interactions and provide a theoretical basis for the development of biofertilizers and sustainable agriculture.

Conclusion

Here, we explored the interaction between *B. aryabhattai* LAD and maize roots. The results showed that the interaction between auxin-secreting bacteria *B. aryabhattai* LAD and maize roots promoted their connection with plants to further help plant growth, and found that there was a feedback effect between plant immune system and bacterial auxin. Bacteria activate the immune response of plant roots to produce ROS, which in turn stimulates bacteria to synthesize IAA, and the synthesized IAA further promotes plant growth. This result provides a theoretical basis for exploring the endogenous mechanism of maize development, and also provides a relevant model for exploring the effect of plant-soil microbial interaction on plant defense function and promoting plant growth.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03479-y.

Supplementary Material 1

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Author contributions

Chao Deng: Conceptualization, Investigation, Data curation, Writing - Original draft. Nan Zeng: Conceptualization, Software, Data curation, Writing - Original draft, Writing—review and editing. Chunji Li: Writing—review and editing. Jiahe Pang: Data curation. Ning Zhang: Investigation, Supervision. Bingxue Li: Supervision, Resources, Project administration, Visualization, Writing—review and editing.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. The raw RNA-seq data has been formally deposited with the National Center for Biotechnology Information under project PRJNA1091454.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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