

# Endophytic microbes *Bacillus* sp. LZR216-regulated root development is dependent on polar auxin transport in *Arabidopsis* seedlings

Jianfeng Wang · Yongqiang Zhang · Ying Li · Xiaomin Wang ·  
Wenbin Nan · Yanfeng Hu · Hong Zhang · Chengzhou Zhao ·  
Feng Wang · Ping Li · Hongyong Shi · Yurong Bi

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## Abstract

**Key message** Endophytic microbes *Bacillus* sp. LZR216 isolated from *Arabidopsis* root promoted *Arabidopsis* seedlings growth. It may be achieved by promoting the lateral root growth and inhibiting the primary root elongation.

**Abstract** Plant roots are colonized by an immense number of microbes, including epiphytic and endophytic microbes. It was found that they have the ability to promote plant growth and protect roots from biotic and abiotic stresses. But little is known about the mechanism of the endophytic microbes-regulated root development. We isolated and identified a *Bacillus* sp., named as LZR216, of

endophytic bacteria from *Arabidopsis* root. By employing a sterile experimental system, we found that LZR216 promoted the *Arabidopsis* seedlings growth, which may be achieved by promoting the lateral root growth and inhibiting the primary root elongation. By testing the cell type-specific developmental markers, we demonstrated that *Bacillus* sp. LZR216 increases the *DR5::GUS* and *DR5::GFP* expression but decreases the *CYCB1;1::GUS* expression in *Arabidopsis* root tips. Further studies indicated that LZR216 is able to inhibit the meristematic length and decrease the cell division capability but has little effect on the quiescent center function of the root meristem. Subsequently, it was also shown that LZR216 has no significant effects on the primary root length of the *pin2* and *aux1-7* mutants. Furthermore, LZR216 down-regulates the levels of PIN1-GFP, PIN2-GFP, PIN3-GFP, and AUX1-YFP. In addition, the wild-type *Arabidopsis* seedlings in the present of 1 or 5  $\mu\text{M}$  NPA (an auxin transport inhibitor) were insensitive to LZR216-inhibited primary root elongation. Collectively, LZR216 regulates the development of root system architecture depending on polar auxin transport. This study shows a new insight on the ability of beneficial endophytic bacteria in regulating postembryonic root development.

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J. Wang and Y. Zhang contributed equally to this work.

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J. Wang · Y. Zhang · Y. Li · X. Wang · H. Zhang · C. Zhao ·  
F. Wang · P. Li · H. Shi · Y. Bi (✉)  
Ministry of Education Key Laboratory of Cell Activities and  
Stress Adaptations, School of Life Sciences, Lanzhou  
University, Lanzhou, Gansu 730000, People's Republic of China  
e-mail: yrbi@lzu.edu.cn

W. Nan  
Chongqing Key Laboratory of Molecular Biology of Plant  
Environmental Adaptations, College of Life Sciences,  
Chongqing Normal University, Chongqing 401331,  
People's Republic of China

Y. Hu  
Key Laboratory of Mollisols Agroecology, Northeast Institute  
of Geography and Agroecology, Chinese Academy of Sciences,  
Nangang District, Harbin, China

**Keywords** *Arabidopsis* · *Bacillus* sp. LZR216 ·  
Endophytic microbe · Polar auxin transport · Root  
architecture

## Abbreviations

CFU Colony-forming units  
GFP Green fluorescent protein  
GUS  $\beta$ -Glucuronidase  
KB King's B medium  
NPA 1-*N*-Naphthylphthalamic acid

PAT	Polar auxin transport
PGPB	Plant growth-promoting bacteria
PGPR	Plant growth-promoting rhizobacteria
QC	Quiescent center
YFP	Yellow fluorescent protein

## Introduction

Like animals, plants have their own microbiome that protects them from various adverse environmental conditions (Berendsen et al. 2012). The plant rhizosphere is a noticeable ecological environment as myriad microorganisms colonize in and out of the roots of growing plants. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere (Berg and Smalla 2012). Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil microorganisms that colonize in/on roots and stimulate shoot and root growth. Such bacteria have been able to enhance crop yields (Kloepper et al. 1980, 1989). Within the rhizobacteria, some microbes can promote plant growth and provide a better environment for plant growth through indirect or direct methods (Coullero et al. 2009; Richardson et al. 2009). Some PGPR can help plants withstand environment stresses including heavy metal contaminations or other pollutants (Jing et al. 2007; Tak et al. 2013). Some PGPR can promote plants in nutrition uptake via associative nitrogen fixation and phosphate solubilization (Richardson et al. 2009). They can suppress a broad spectrum of bacterial, fungal, and nematode diseases. Similarly, beneficial rhizobacteria from rice rhizosphere confer high protection against biotic and abiotic stresses inducing systemic resistance in rice seedlings (Lucas et al. 2014). Plant growth-promoting bacteria confer resistance to salt stress in tomato plants (Mayak et al. 2004). Therefore, utilizing PGPR is a new and promising approach for improving plant growth and development.

Some microbial species can alter root system architecture by producing phytohormones including auxin, ethylene, and cytokinins (Bowen and Rovira 1999). The effect of hormones from bacterial suspensions on root morphogenesis includes enhancing root hairs and lateral roots (Persello-Cartieaux et al. 2003). Understanding the function of PGPR on root development is important. One strategy is to inoculate roots with PGPR in vitro and monitor the resultant effects on plants. This method showed that many PGPR reduce the growth rate of the primary root (Dobbelaere et al. 1999) and increase the number of lateral roots (Combes-Meynet et al. 2011). Root surface areas were increased by inoculation with PGPR. So plants uptake minerals and water more favorably, and the fresh weight of the whole plant can be increased (Walker et al.

2012). Studies have shown that the molecular mechanisms of those observations are dissected at the genetic and molecular levels (López-Bucio et al. 2007). Root growth depends on two basal developmental processes: cell division in the root apical meristem and elongation of cells that leave the root meristem. The root system architecture is defined by the primary root formation, lateral root (LR), and root hair (RH) formation. Auxin is known to alter the root system architecture, including primary root elongation (Alonso et al. 2003) and root hair formation and elongation (Pitts et al. 1998; Rahman et al. 2002, 2007).

*Arabidopsis* has been chosen as a model system to study plant–microbe interactions because of its short life cycle and the availability of many mutants impaired in hormone response pathways, including auxin and ethylene (Persello-Cartieaux et al. 2003; Ryu et al. 2005). Several PGPR can produce auxin and influence the root system architecture (Barazani and Friedman 1999; Patten and Glick 2002). Inoculation with *Phyllobacterium brassicacearum* strain STM196 stimulated the expression of several auxin biosynthesis pathway enzymes in *Arabidopsis* shoots with a potential redistribution of auxin into roots (Contesto et al. 2010). Auxin transport inhibitor treatment blocked auxin accumulation at sites of synthesis in shoots of *Arabidopsis*, inoculated with *Bacillus subtilis*, suppressed the levels of auxin in roots and reduced the growth-promoting effects (Zhang et al. 2007). Application of the auxin transport inhibitor, 1-naphthylphthalamic acid (NPA), restricted auxin accumulation to sites of synthesis (Keller et al. 2004). Inoculation of *Arabidopsis* auxin transport mutants *aux1-7* and *pin2* with *Trichoderma virans* reduced growth compared with wild-type plants (Contreras-Cornejo et al. 2009). The auxin-responsive promoter *DR5::GUS* lines as the object of study provide insights indirectly into changes of auxin levels, signaling, or responses during plant–microbe interaction. Besides the above important information, very little is known about the cellular, developmental, and physiological responses of plants to endophytic bacteria. We identified a new endophytic bacteria strain of *Bacillus subtilis* LZR216 from *Arabidopsis thaliana*, which is able to promote the growth of *Arabidopsis* plants. Our results showed that *Bacillus subtilis*. LZR216 can dramatically change the root system architecture, including increased growth of lateral roots and inhibited primary root elongation involving polar auxin transport.

## Materials and methods

### Isolation and identification of endophytic microbes

*Arabidopsis* (Col-0) seeds were surface-sterilized with 70 % ethanol for 30 s and subsequent 2 % bleach for 15 min, washed three times with autoclaved water, and

then kept at 4 °C for 2 days for stratification. After that, the seeds were transferred to 1/2 MS solid medium for growth. 5-day-old seedlings were transferred to new tissue culture bottles containing 1/2 MS medium for further growth. The endophytic bacteria were isolated from roots of 4-week-old *Arabidopsis* seedlings. Each sample was triturated with autoclaved mortar and pestle and with autoclaved water, transferred to 5 mL of King's B (KB) liquid medium, and then cultured at 28 °C with a constant shaking at 150 rpm. After 48 h, several concentrations of bacterial suspensions were plated on KB agar media and then incubated at 28 °C for another 48 h until colonies formed. To confirm the formation of endophytic bacteria, PCR reactions were performed with the universal 16S primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3'). The PCR products were sequenced and the resultant 16s rDNA sequences were compared to GenBank databases using BLAST.

#### Plant materials and methods

Wild-type Col-0, mutants *tir1-1*, *pin2*, *aux1-7*, and *axr1-12*, and several transgenic plants *DR5::GUS*, *CycB1;1::GUS*, *PIN1-GFP*, *PIN2-GFP*, *PIN3-GFP*, and *AUX1-YFP* were used in this study. Seeds were surface-sterilized and stratified at 4 °C for 2 days on 1/2 MS agar-solidified medium. To test the effect of LZR216 on plant growth, 5-day-old seedlings were transferred and positioned vertically in a growth chamber under a long photoperiod (16 h light/8 h dark) and 65–70 % relative humidity at 22 °C. For inhibition of polar auxin transport, indicated concentrations of NPA were added in the media.

*Bacillus* sp. LZR216 was cultured on KB liquid medium with constant shaking at 150 rpm for 24 h at 28 °C. After 24 h of growth, cells were collected in autoclaved water, washed twice, and resuspended in autoclaved water after centrifugation at 7500 rpm for 10 min. The bacterial titer was adjusted to an optical density at 600 nm of 0.5 ( $5 \times 10^8$  colony-forming units mL<sup>-1</sup>). For treatment, 20 µL of bacterial suspension (autoclaved water as a control) was spotted at 1/2 MS petri plates. In order to test the effect of LZR216 on promoting *Arabidopsis* growth, seedlings were grown on horizontal plates for 12 days, and then the shoot fresh weight and total leaves surface area were measured. To determine the effect of LZR216 on the root system architecture, seedlings were grown on vertical plates for 7 days, and then the primary root length and lateral root number per plant were measured.

#### GUS histochemical staining

Histochemical assay of the GUS activity was performed as described by Ulmasov et al. (1997) with minor

modifications. Five-day-old seedlings were treated with LZR216 for another 7 days, and then incubated in GUS staining buffer containing 1 mM X-Gluc, 100 mM sodium phosphate (pH 7.5), 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 10 mM EDTA, and 0.1 % Triton X-100. After incubation at 37 °C for 12 h, the stained roots were cleared in a mixture of chloral hydrate:glycerol:water (8:1:2, g/v/v) and observed with Nomarski optics.

#### Quantitative real-time PCR (qRT-PCR) analysis

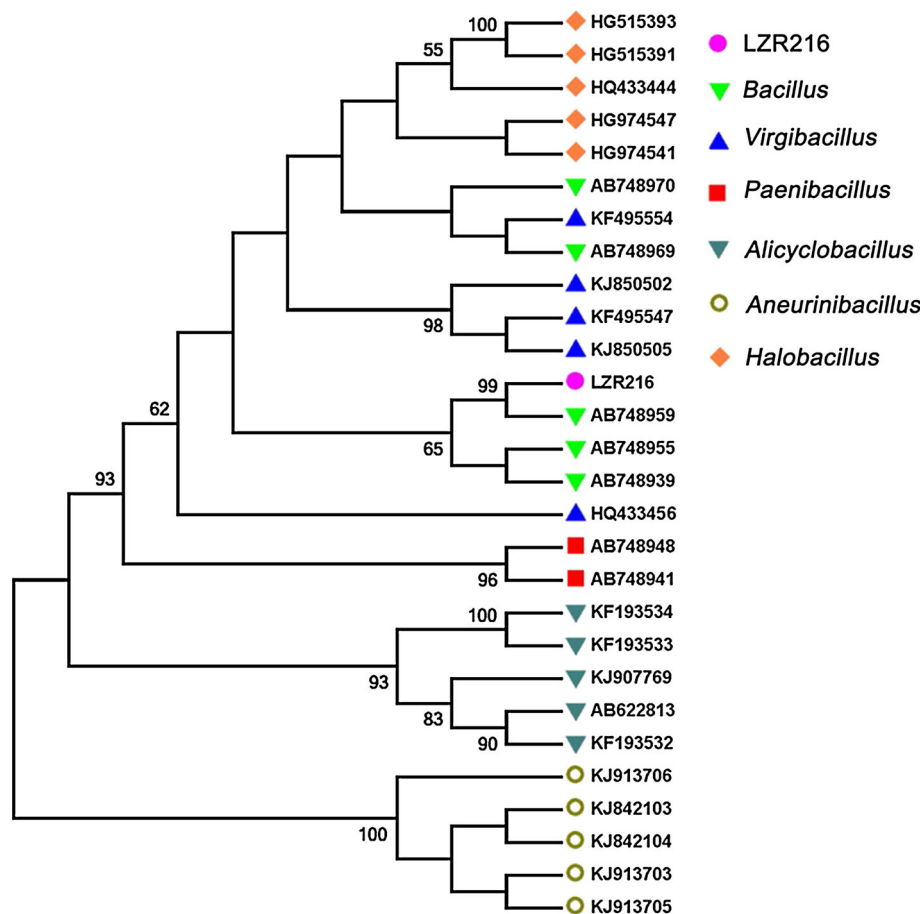
Total RNA was extracted with Trizol (TaKaRa) from shoots and roots, and then treated with RNase-free DNase (Promega, Madison, WI, USA). First-strand cDNA was synthesized with the PrimeScript II 1st Strand cDNA Synthesis Kit (TaKaRa, Mountain View, CA, USA). Quantitative real-time PCR (qRT-PCR) was performed using the SYBR PrimeScript RT-PCR Kit (Perfect Real Time; TaKaRa). PCR was performed using a CFX 96 Real-Time system (Bio-Rad, Hercules, CA, USA) with the following standard cycling conditions: 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. The cycle threshold  $2^{(-\Delta\Delta C(T))}$ -based method was used for relative quantitation of gene expression. Expression levels of genes were normalized to *ACTIN2* levels. The primer sequences used in the study are shown in supplementary table S1.

#### Fluorescence microscopy

Confocal images were captured with a confocal laser scanning microscope (Olympus, FV1000) equipped with a 20× (water immersion) objective lens, a HeNe laser (543 nm), and an argon laser (488, 514 nm). Emission filters for GFP, YFP, and FM4-64 were set at 505–530, 490–510, and 560 nm, respectively. All images within a single experiment were captured with the same gain and exposure settings. For FM4-64 staining, seedlings were incubated for 10 min with 4 µM FM4-64 (Invitrogen) from a 10 mM stock solution in water.

#### Statistical analysis

Each experiment was repeated at least three times. Values were expressed as mean ± SE. Statistical analyses were done with one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for independent samples or with Student's *t* test when two means were compared (SPSS version 17.0). In all cases, the confidence coefficient was set at  $P < 0.05$ .



**Fig. 1** LZR216 phylogenetic tree of the partial 16S rDNA sequences. The sequence data for several closely related type cultures were recovered from GenBank and included in the tree. The accession numbers for the sequences are as follows: *Paenibacillus apiarius* strain AECSP01, AB748948; *Paenibacillus xylanilyticus* strain AECSP02, AB748941; *Virgibacillus halodenitrificans* strain GUFB-Sama06-18, KF495554; *Virgibacillus* sp. GUFBSama06-11, KF495547; *Virgibacillus* sp. enrichment culture clone DSFB408, KJ850502; *Virgibacillus* sp. enrichment culture clone ZSTA205, KJ850502; *Virgibacillus* sp. B6B(2010), HQ433456; *Bacillus* sp. AECSB16, AB748970; *Bacillus* sp. AECSB15, AB748969; *Bacillus* sp. AECSB02, AB748939; *Bacillus atrophaeus* strain AECSB18, AB748959; *Bacillus* sp. AECSB10, AB748955; *Halobacillus* sp.

S4B(2010), HQ433444; *Halobacillus naozhouensis* isolate 0612R1-8.2, HG515393; *Halobacillus naozhouensis* isolate 0612P19-10, HG515391; *Halobacillus* sp. JC256, HG974547; *Halobacillus* sp. JC250, HG974541; Uncultured *Alicyclobacillus* sp. clone METASED16, KJ907769; *Alicyclobacillus* sp. PS1, KF193534; *Alicyclobacillus* sp. PSS3, KF193533; *Alicyclobacillus* sp. CPP55, KF193532; *Alicyclobacillus cellulosilyticus*, AB622813; *Aneurinibacillus thermoaerophilus* strain Billoris-VO22, KJ913706; *Aneurinibacillus thermoaerophilus* strain Billoris-VO18, KJ913705; *Aneurinibacillus thermoaerophilus* strain Billoris-VO16, KJ913703; *Aneurinibacillus thermoaerophilus* strain VO26, KJ842104; *Aneurinibacillus thermoaerophilus* strain VO25, KJ842103

## Results

### Isolation and identification of endophytic bacterium *Bacillus* sp. LZR216

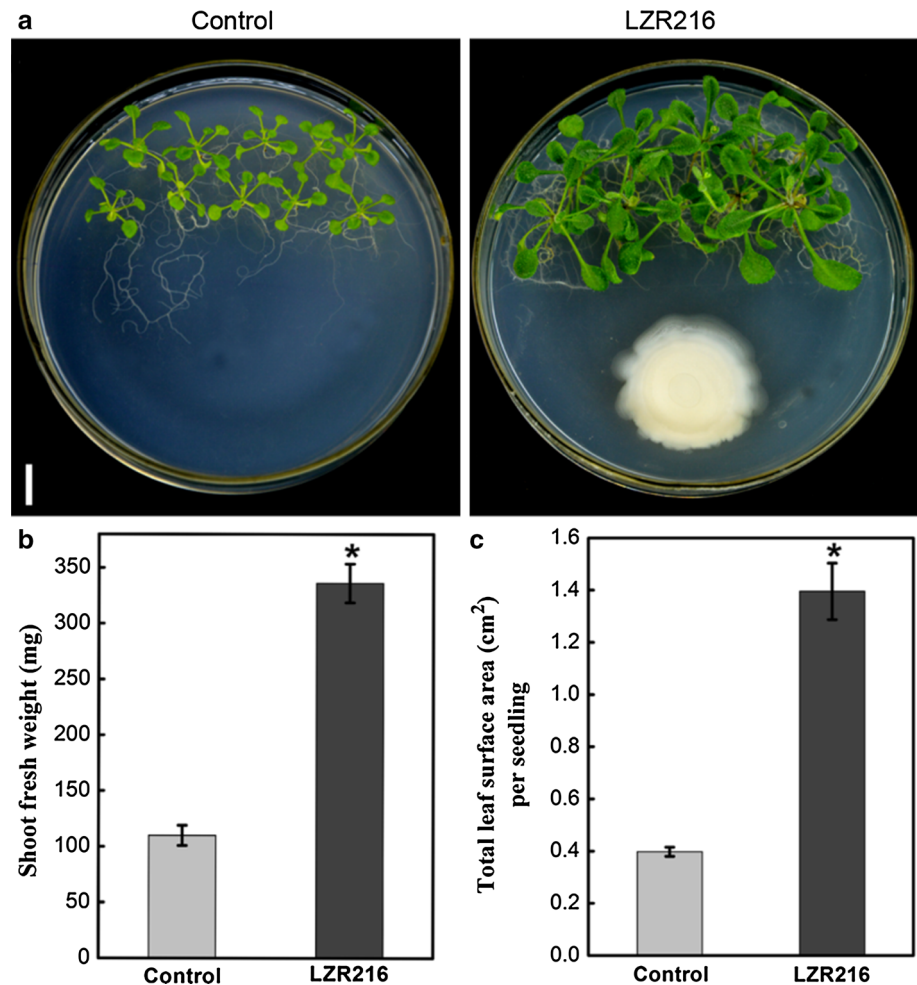
Endophytic bacterium was isolated from roots of 4-week-old *Arabidopsis* tissue culture seedlings, one of which was named as LZR216. For comparison, other six *Bacillus* in Bacillaceae, including *Virgibacillus* sp., *Bacillus* sp., *Halobacillus* sp., *Alicyclobacillus* sp., and *Aneurinibacillus thermoaerophilus* were selected from the GenBank for sequence alignment. Based on the phylogenetic tree

constructed with MEGA6, this endophytic bacterium LZR216 belong to *Bacillus* sp. (Fig. 1).

### *Bacillus* sp. LZR216 affects *Arabidopsis* seedling growth

Because *Bacillus* sp. LZR216 is an endophytic bacterium, we proposed that LZR216 may promote *Arabidopsis* growth. To verify this hypothesis, *Arabidopsis* seeds were germinated and vertically grown for 5 days on petri dishes containing agar-solidified 1/2 MS medium. After that, the seedlings were transferred to a new 1/2 MS medium

**Fig. 2** Effects of *Bacillus* sp. LZR216 inoculation on the growth of *Arabidopsis* seedlings. **a** Representative images of 17-day-old seedlings after 12 days of growth under control and LZR216-inoculated conditions. **b** Effects of *Bacillus* sp. LZR216 inoculation on shoot fresh weight. **c** Effects of *Bacillus* sp. LZR216 inoculation on total leaf surface area. Data are mean  $\pm$  SE of three independent experiments ( $n = 10$ ). Asterisks indicate statistically significant differences compared with the control (Student's *t* test;  $P < 0.05$ ). The experiments were repeated three times with similar results



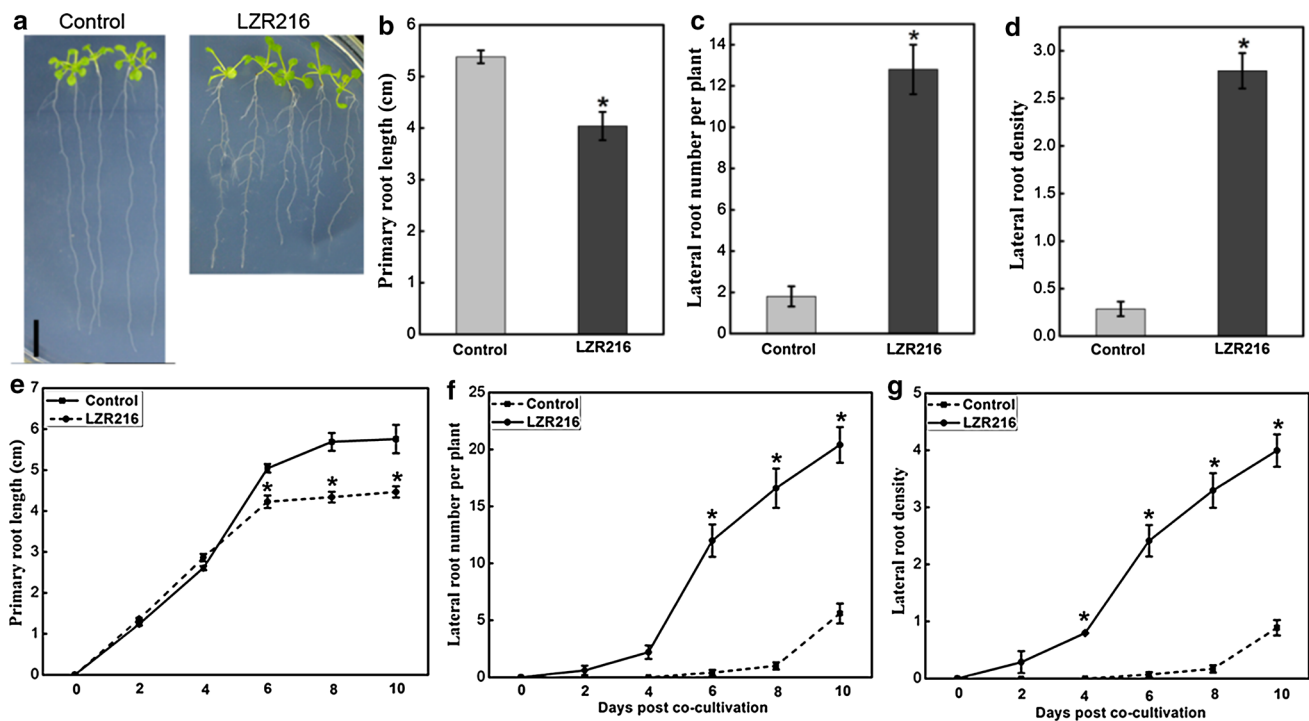
spotted with 20  $\mu$ L distilled sterilized water (as the control) or 20  $\mu$ L  $5 \times 10^8$  CFU mL<sup>-1</sup> LZR216 ( $OD_{600} = 0.5$ ) suspension. Twelve days later, a significant increase in shoot biomass and total leaf surface area was observed in LZR216-treated seedlings compared to the control (Fig. 2), indicating that *Bacillus* sp. LZR216 can promote *Arabidopsis* shoot growth.

It has been shown that many PGPB strains could cause alterations in the root system architecture of host plants by promoting the formation of secondary roots and thus improving the root exploratory capacity (Zamioudis et al. 2013). The effect of LZR216 on root system architecture of *Arabidopsis* was tested. As shown in Fig. 3a, b, the primary root length of seedlings exposed to LZR216 was reduced by approximately 37 % after inoculation with LZR216 for 1 week compared to the control, suggesting a suppressive effect of LZR216 on the primary root elongation. Furthermore, in the time course experiment, we found that the inhibition of LZR216 on the primary root elongation was significantly exhibited after 6-day treatment (Fig. 3e). By the contrary, LZR216 observably enhanced both the number and the density of lateral roots, with more than six- and ninefold

increase, respectively, after 7-day treatment (Fig. 3c, d). With the growth time elongation, the lateral root number as well as the density increased rapidly in LZR216-treated seedlings, but only slightly in control plants (Fig. 3f, g).

LZR216 negatively affects cell division in *Arabidopsis* roots

To clarify the mechanism by which LZR216 restrains the primary root growth, the effect of LZR216 on quiescent center (QC) was first analyzed. It was found that LZR216 has little effect on the expression of QC-localized marker *WOX5-GFP* in the meristem (Fig. 4a). Next, the effect of LZR216 on the cortical cell length was measured in the elongation zone (EZ) of primary roots. The results showed that LZR216 can markedly induce the elongation of cortical cells (Fig. 4b, c), suggesting a positive role of such endophytic microbe on cortical cell elongation. Meanwhile, meristematic zone length was also measured. Interestingly, the meristematic zone in LZR216-treated roots is significantly shorter than that in the control (Fig. 4d, e), suggesting that LZR216 inhibits the cell



**Fig. 3** Effects of LZR216 on root system architecture in *Arabidopsis* seedlings. **a** Representative images of 12-day-old seedlings after 7 days of growth under control and LZR216-inoculated conditions. **b** Effects of LZR216 on primary root length. **c** Effects of LZR216 on lateral root numbers. **d** Effects of LZR216 on lateral root density. **e** Time course of primary root length in response to LZR216. **f** Time course of lateral root numbers in response to LZR216. **g** Time course

of lateral root density in response to LZR216. In **e**, **f**, and **g**, at time point 0, LZR216 bacteria were spotted on the plates. Data are mean  $\pm$  SE of three independent experiments ( $n = 30$ ). Asterisks indicate statistically significant differences compared with control treated (Student's *t* test;  $P < 0.05$ ). The experiments were repeated three times with similar results

division. Consistently, in transgenic plant *CYCB1;1::GUS*, one cell cycle marker, LZR216 obviously represses the GUS activity compared to the control (Fig. 4f). Taken together, these data indicate that LZR216 inhibition of primary root may mainly result from its negative effect on cell division.

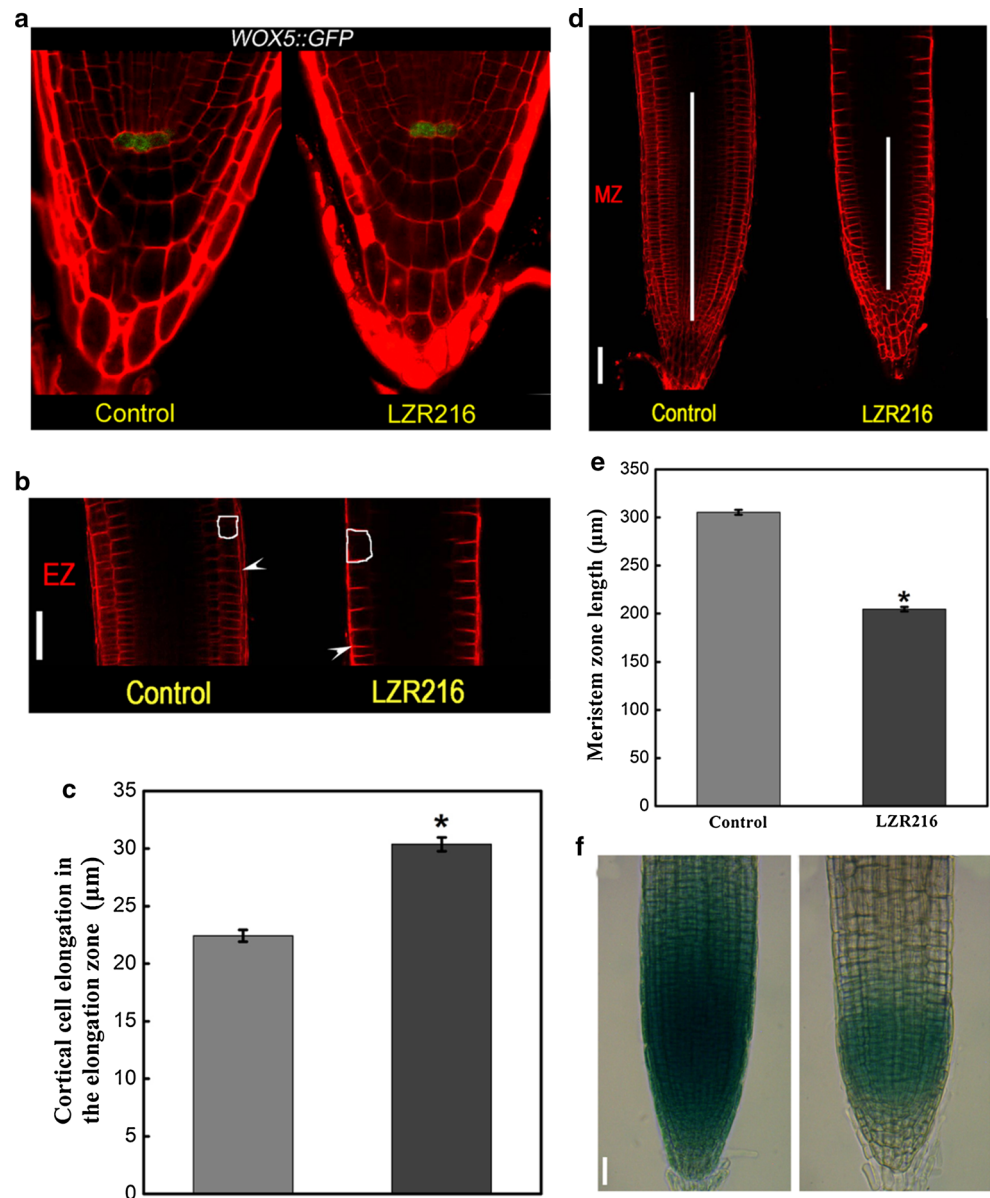
Auxin is involved in LZR216 regulation of root system architecture

Phytohormone auxin plays an important role in the regulation of root system architecture, including the increase of lateral root and root hair development (Casimiro et al. 2001; Reed et al. 1998). The above results indicate that LZR216 inhibits the primary root elongation but enhances the lateral root formation. So it is reasonable to investigate whether auxin plays any roles in the LZR216 regulation of root growth. Firstly, using transgenic plants, expressing the synthetic auxin reporters *DR5::GUS* and *DR5::GFP*, we found that a strong auxin response was detected toward the root tips in the root meristem of primary roots (Fig. 5a), suggesting an induction role of LZR216 on auxin response. To test whether LZR216 affects auxin biosynthesis at the

transcriptional level, the transcripts of *NIT1*, *TAA1*, and *YUCCA1*, key genes in auxin biosynthesis, were tested using qRT-PCR. The results indicated that LZR216 treatment notably induces the transcriptional levels of *NIT1*, *TAA1*, and *YUCCA1* compared to the control (Fig. 5b). Hence, these data indicated that the LZR216 enhancement of auxin responses may be achieved by, at least partially, enhancing the auxin biosynthesis at the transcriptional level.

To determine how LZR216 affects auxin responses in the regulation of *Arabidopsis* root system, effect of LZR216 on the growth of four auxin signaling mutants was tested. Through measuring the primary root length, lateral root number per plant, lateral root density, shoot fresh weight, and total leaf surface area per seedling, we found that the responses of these mutants to LZR216 were more or less impaired compared to wild type. For example, after LZR216 treatment, the lateral root number per plant was increased with about threefold in wild type, but only with 1.7-, 1.6-, 1.1-, and 2.5-fold in mutants *tir1-1*, *pin2*, *aux1-7*, and *axr1-12*, respectively (Fig. 6). These data suggest that auxin signaling and polar transport are necessary for the LZR216 effect on plant growth.

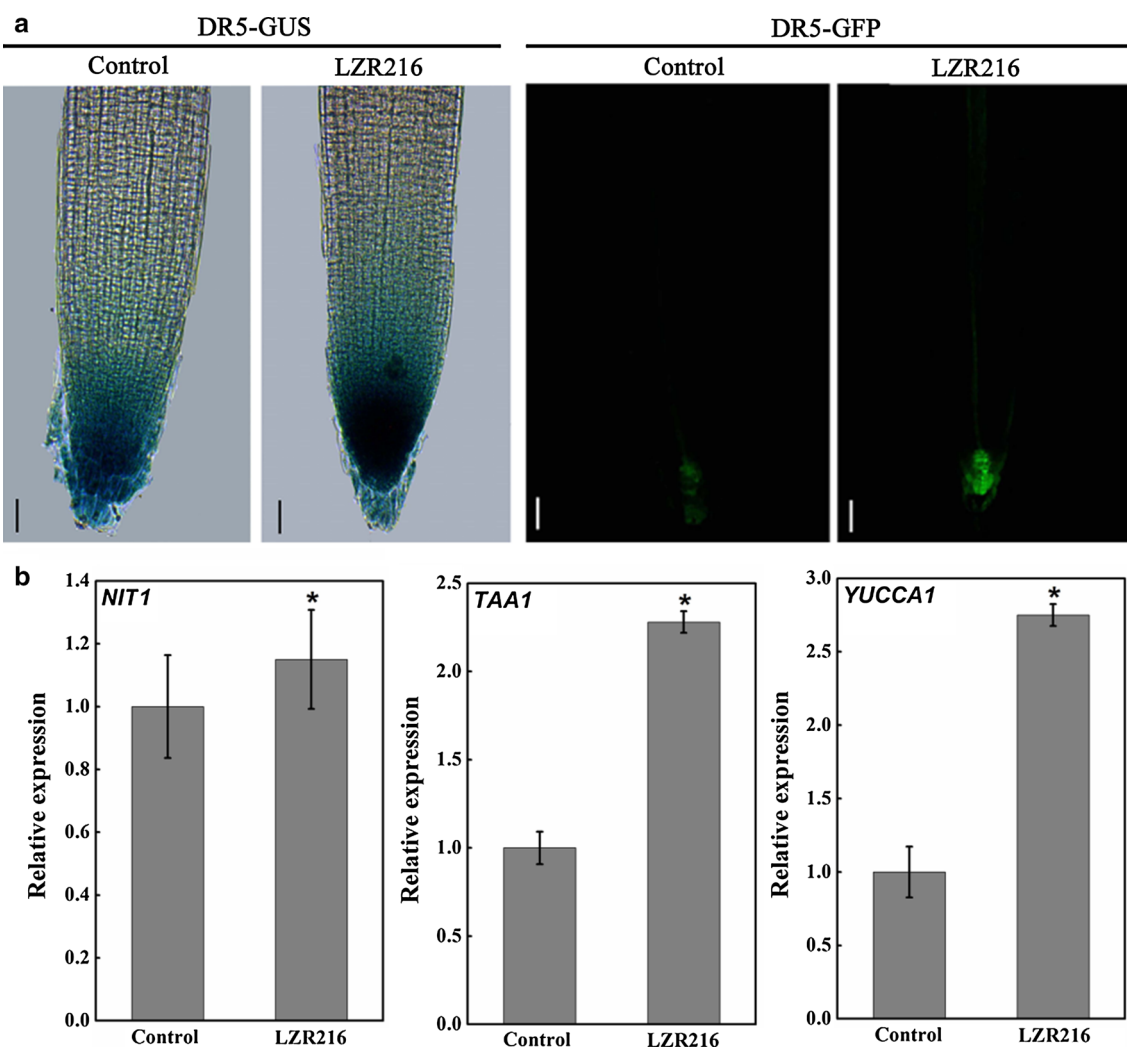
**Fig. 4** Effects of LZR216 on *WOX5*-GFP, *CYCB1;1::GUS* and meristem size of *Arabidopsis* roots. **a** Confocal images showing the expression patterns of the QC-localized marker *WOX5*-GFP under control and LZR216-inoculated conditions. **b** Cortical cell elongation in the elongation zone (EZ) of roots grown in the presence or absence of LZR216. **c** Quantitative graphs of **b**. **d** Meristem size of control- and LZR216-treated roots. **e** Quantitative graphs of **d**. **f** Expression of cell division marker *CYCB1;1::GUS* in the primary root meristem of control plants and LZR216-inoculated plants. Data are mean  $\pm$  SE of three independent experiments ( $n = 30$ ). Asterisks indicate statistically significant differences compared with the control (Student's *t* test;  $P < 0.05$ ). The experiments were repeated three times with similar results



LZR216 regulation of root system architecture is likely attributed by affecting polar auxin transport

Given the necessity of auxin polar transport for LZR216-mediated plant growth, the expression levels of *AUX1* and *PIN2* together with other two efflux carriers *PIN1* and *PIN3* were tested using transgenic plants *AUX1*-YFP, *PIN2*-GFP, *PIN1*-GFP, and *PIN3*-GFP, respectively, in the presence or absence of LZR216 treatment. LZR216 significantly reduces the expressions of these four proteins compared to the control (Fig. 7), suggesting LZR216-induced alteration of the root system architecture involves polar auxin transport. To further confirm this notion, we used the specific inhibitor of polar auxin transport, NPA. As shown in Fig. 8a, b, the LZR216-

induced increase of total leaf surface area per seedling was significantly impaired in the presence of 1 or 10  $\mu$ M NPA, suggesting that LZR216-mediated growth promotion is interrupted if the auxin transport is blocked. Similarly, in the absence of NPA, LZR216 represses the growth of primary roots; but in the presence of NPA, with concentrations from 0.5 to 5  $\mu$ M, the primary root length of LZR216-treated seedlings becomes comparable with the control plants (Fig. 8c). Furthermore, the ability of LZR216-stimulated LR formation is severely attenuated by 0.5 or 1  $\mu$ M NPA. The LR formation in response to LZR216 is almost completely abolished after 5  $\mu$ M NPA treatment (Fig. 8d, e). Together, we concluded that polar auxin transport is necessary for LZR216 in the regulation of plant growth.



**Fig. 5** Effects of LZR216 on auxin level in *Arabidopsis* roots. **a** Representative images of *DR5::GUS* (bar 30  $\mu\text{m}$ ) and *DR5::GFP* (bar 50  $\mu\text{m}$ ) with or without LZR216 treatment. **b** Quantitative real-time PCR analysis of the relative transcript abundance of *NIT1*, *TAA1* and *YUCCA1* genes in WT seedlings. 5-day-old seedlings were treated with 20  $\mu\text{L}$   $5 \times 10^8$  CFU  $\text{mL}^{-1}$  suspension in water (20  $\mu\text{L}$

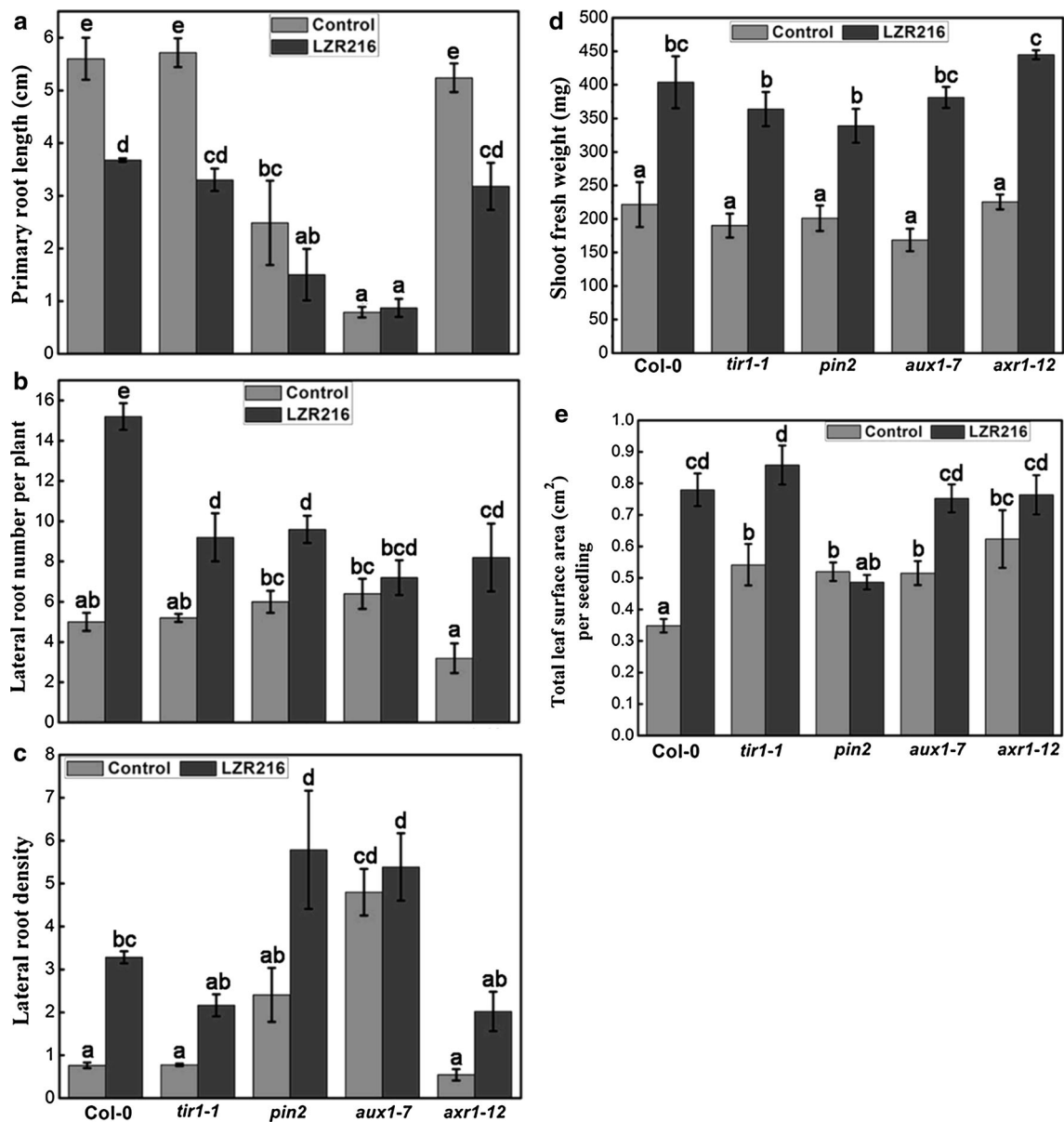
sterilized water as control) for 7 days. All gene transcripts were normalized to *ACTIN2*. Data are mean  $\pm$  SE of three independent experiments. Asterisks indicate statistically significant differences compared with control treated (Student's *t* test;  $P < 0.05$ ). The experiment was repeated three with similar results

## Discussion

In natural ecosystems, the environment influences plant growth and development. Some beneficial microbes can enhance stress resistance against various adverse environment conditions (Berendsen et al. 2012). Rhizosphere microorganisms have significant effects on plant performance in agricultural and marginal soils by influencing growth and development of roots and improving nutrient availability in the rhizosphere (Tinker 1984; Rodríguez and Fraga 1999; Chaiarn et al. 2009). Most studies on mechanisms for plant growth promotion by PGPR are focused on bacterial traits without examining the detailed phenotypic, cellular, and

physiological responses of host plants. This is because root architecture is difficult to observe, quantify, and/or interpret. Roots grow in soil, an opaque medium from which they cannot be extricated or readily observed without introducing artifacts and destroying the native root architecture. In recent years, significant progress has been made in our understanding of how the root system architecture alters in response to soil microbes. But studies on how endophytic microbes affect plant growth and development are still limited. In this work, we used *Arabidopsis thaliana* as a model system to characterize the phenotypic and physiological basis by which endophytic microbe *Bacillus* sp. LZR216 regulates plant growth and root system architecture.



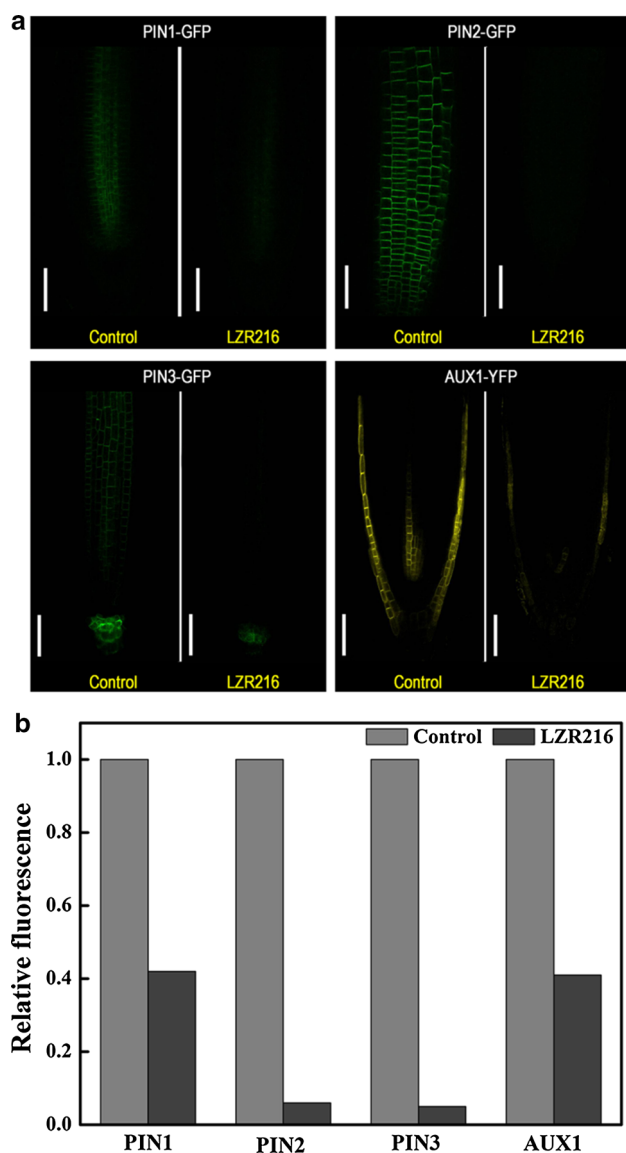


**Fig. 6** Influence of auxin signaling and transport on LZR216-mediated primary root length and LR formation in *Arabidopsis* roots. **a** Primary root length under control and LZR216-inoculated conditions. **b** Lateral root length under control and LZR216-inoculated conditions. **c** Lateral root density under control and LZR216-inoculated conditions. **d** Shoot fresh weight under control and

LZR216-inoculated conditions. **e** Total leaf surface area under control and LZR216-inoculated conditions. Data are mean  $\pm$  SE of three independent experiments ( $n = 30$ ), bars with different letters are significantly different at the level of  $P < 0.05$ . The experiments were repeated three times with similar results

PGPR have been extensively studied in respect of their capability to induce biomass production and systemic resistance in plants (Lugtenberg and Kamilova 2009; Pozo and Azcón-Aguilar 2007). Our results indicated that endophytic microbe *Bacillus* sp. LZR216 stimulates the growth of *Arabidopsis* (Fig. 2), suggesting that this bacterial strain likely acts as a novel plant growth-promoting bacterium. Strains of *B. subtilis* and *Enterobacter cloacae* promote plant growth by releasing several volatiles, of

which acetoin shows the highest level of growth promotion (Ryu et al. 2003). Previously, it was shown that *Pseudomonas fluorescens* WCS417 enhances the shoot fresh weight and alters the root system architecture (Zamioudis et al. 2013). Similarly, our results showed that LZR216 inhibits the primary root elongation and promotes the lateral root formation (Fig. 3). There was a report that the expression of *CYCBI;I::GUS* is clearly reduced in plants inoculated with *Bacillus megaterium* (López-Bucio et al.



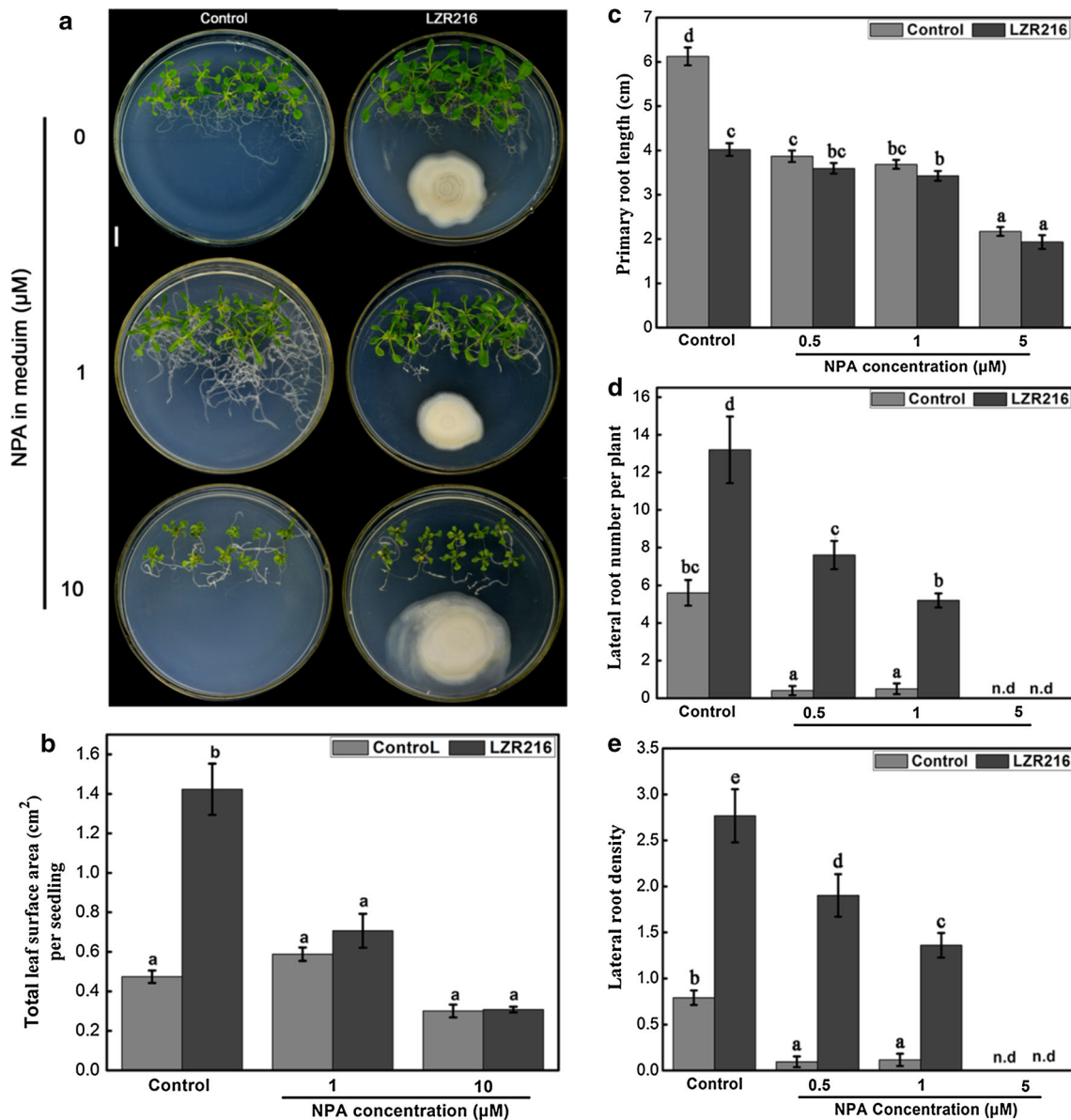
**Fig. 7** Effect of LZR216 on the protein level of *PIN1/PIN2/PIN3/AUX1*. **a** Five-day-old seedlings containing indicated markers were transferred to medium with or without LZR216 for 7 days and GFP expression was monitored. **b** Quantification of GFP/YFP fluorescence by image analysis of confocal sections as described in **a**. Images shown are representatives of at least three independent experiments. Bars 50  $\mu$ m

2007). Our microscopic survey showed that LZR216 decreases the expression of the *CYCBI;1::GUS* reporter in the meristematic zone of root tips and reduces the meristem zone length, and enhances the length of cells entering the elongation zone (Fig. 4). These results imply that LZR216 inhibits cell division and the meristem zone length.

Auxin is an essential plant hormone in the maintenance of stem cell identity and function. Both primary root elongation and lateral root formation require auxin transport. Transcripts of *GH3* genes which are involved in IAA

conjugation, *PtaGH3-1*, *PtaGH3-2*, *PtaGH3-7*, and *PtaGH3-8*, were induced in populus with *Laccaria bicolor* co-culture (Felten et al. 2009). Similarly, *Phyllobacterium brassicacearum* strain STM196 induces the expression of *DR5::GUS* in *Arabidopsis* primary as well as lateral root tips (Contesto et al. 2010). Our results showed that LZR216 enhances *DR5::GUS* and *DR5::GFP* levels in primary root tips (Fig. 5a), suggesting that auxin responses can be strengthened by the inoculation of this endophytic microbe. Furthermore, our results indicated that LZR216 treatment notably induces the transcriptional levels of *NITI*, *TAA1*, and *YUCCA1* (Fig. 5b), suggesting that LZR216 also regulates auxin biosynthesis at the transcriptional level. On the other hand, the effect of LZR216 was impaired in several mutants defective in auxin signaling or polar transport (Fig. 6). Ryu and co-workers (2005) also reported that the *Arabidopsis* growth promotion by several PGPR involves complex hormonal signaling. More recently, growth responses to *Trichoderma virens* or *Phyllobacterium brassicacearum* inoculation are reduced in *axr1-3* compared to wild type (Contesto et al. 2010; Contreras-Cornejo et al. 2009). So affecting plant growth through auxin modulation may be a universal pathway for some endophytic microbes, including LZR216.

Auxin influx and efflux transporter genes in populus are transiently induced by *Laccaria bicolor* co-culture (Felten et al. 2009). In *Arabidopsis*, when co-cultured with *Laccaria bicolor*, *aux1*, and *pin3* mutants exhibited similar induction of lateral roots compared to wild type, but less stimulation was observed in the *pin2* and *pin2,3,4,7* mutant lines (Felten et al. 2009). These aforementioned results provide evidence that auxin transport machinery is involved in plant–microbe interactions. This study on LZR216-mediated root development provides important information on the mechanism by which candidate molecules with auxin activity are perceived and transported within the roots. Our results indicated that LZR216 can still inhibit the primary root elongation in *tir1-1* and *axr1-12* mutants, with similar extent exhibited in wild type (Fig. 6a), which may be because TIR1 is just one of auxin receptors, whereas AXR1 is not important for LZR216 inhibition of the primary root elongation. LZR216 has little effect on the primary root elongation of *pin2* and *aux1-7* mutants (Fig. 6a), indicating that polar auxin transport might be the key factor for LZR216 in affecting the root development of *Arabidopsis* seedlings. LZR216 inhibition of PIN1, PIN2, PIN3, and AUX1 accumulation in root tips strengthens this viewpoint. Moreover, NPA eliminated the roles of LZR216 on promoting plant growth and affecting root system architecture (Fig. 8), further suggesting that the LZR216 function in plants is very likely through the interference of the polar auxin transport.



**Fig. 8** Effects of the polar auxin transport inhibitor NPA on LZR216-promoted *Arabidopsis* growth and LZR216-altered root system architecture. **a** Photograph of 17-day-old *Arabidopsis* (Col-0) seedlings grown on the surface of 1/2 MS medium containing different concentrations of NPA. 5-day-old seedlings were treated with 20 μL autoclaved water or with 20 μL LZR216 bacterial suspension for 12 days and photographed. Bar 1 cm. **b** Total leaf surface area under control, and LZR216-induced conditions in different concentrations of NPA after 12 days of co-cultivation.

**c** Primary root length under control and LZR216-induced conditions in different concentrations of NPA after 7 days of co-cultivation. **d** Lateral root formation under control and LZR216-induced conditions in different concentrations of NPA after 7 days of co-cultivation. **e** Lateral root density under control and LZR216-induced conditions in different concentrations of NPA after 7 days of co-cultivation. Data are mean ± SE of three independent experiments ( $n = 30$ ). Within each set of experiments, bars with different letters are significantly different at the level of  $P < 0.05$

Microorganisms interact with plants either beneficially or as pathogens and may influence plant growth and development. Perception of microorganisms by plants is highly coordinated through cellular processes that determine the final outcome of the relationship, ranging from parasitism to mutualism (Bais et al. 2004; Pozo and Azcón-

Aguilar 2007). Important biological processes mediated by root exudates, such as communication with symbiotic microorganisms and alterations in chemical and physical properties of the soil justify such an energy investment (Bais et al. 2004, 2006). In this work, we used an endophytic microbe to study its effects on plant growth and

development. We can understand how plant growth and development is affected when endophytic microbes overgrow. It is expected that endophytic microbe-induced alterations in the root system architecture would greatly facilitate plant nutrition, thereby conferring significant ecological benefits to host plants.

**Author contribution statement** The experiments were conceived and designed by Yurong Bi, Jianfeng Wang, Yongqiang Zhang, and Ying Li. The experiments were performed by Jianfeng Wang, Yongqiang Zhang, Ying Li, Yanfeng Hu, Wenbin Nan, and Hong Zhang. The data were analyzed by Jianfeng Wang, Feng Wang, Ping Li, Chengzhou Zhao, and Hongyong Shi. Yurong Bi, Jianfeng Wang, Yongqiang Zhang, and Xiaomin Wang wrote the paper.

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**Conflict of interest** The content in this paper is new. It is not being submitted to any other journal. All the authors listed have approved the manuscript for publication.

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