DOI: 10.1007/s10535-016-0585-7 BIOLOGIA PLANTARUM 60 (2): 299-310, 2016

Involvement of polar auxin transport in the inhibition of *Arabidopsis* seedling growth induced by *Stenotrophomonas maltophilia*

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Abstract

A wide range of microorganisms found in the rhizhosphere are able to regulate plant growth and development, but little is known about the mechanism by which epiphytic microbes inhibit plant growth. Here, an epiphytic bacteria *Stenotrophomonas maltophilia*, named as LZMBW216, were isolated and identified from the potato (*Solanum tuberosum* L. cv. Da Xi Yang) leaf surface. They could decrease primary root elongation and lateral root numbers in *Arabidopsis* seedlings. The inhibitory effects of LZMBW216 on plant growth were not due to a reduced indole-3-acetic acid (IAA) content, as exogenously applied IAA did not recover the inhibition. Furthermore, LZMBW216 did not affect the expression of *DR5*::*GUS* and *CycB1*;*1*::*GUS*. However, we found that LZMBW216 exhibited little effect on the primary root elongation in the *pin2* mutant and on the lateral root numbers in the *aux1-7* mutant. Moreover, LZMBW216 decreased expressions of AUX1 and PIN2 proteins. Together, these results suggest that root system architecture alterations caused by LZMBW216 may involve polar auxin transport.

Additional key words: IAA, LZMBW216, mutants, potato, root elongation.

Introduction

Roots as underground organs are in constant interplay with many soil microorganisms (Cesarz *et al*. 2013). Root morphogenesis and growth are under the control of both environmental stimuli and endogenous hormones. In both cases, plant hormones coordinate adaptive changes in cell division and differentiation that lead to changes in root system architecture (López-Bucio *et al*. 2003).

It has been found that inoculation with beneficial microorganisms has positive effects on plant growth, leading to increased yields (Misaghi and Donndelinger 1990, Sturz *et al*. 2000). There are a lot of microbes around roots in soil, including species of the genera *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Serratia* which have beneficial effects on various plants (Gray and Smith 2005). The composition of soil microbiota, chemicals in root exudates, and plant factors, such as the age of plants and microbial host specificity, are driving forces in determining the net plant-microbial relationships (Slankis 1974, Morgan *et al*. 2005, Badri and Vivanco 2009, Johnson *et al*. 2012). Host plants and microorganisms communicate through secreting signalling factors, proteins, metabolites, and/or volatile organic compounds. For example, blends of volatile chemicals emitted from specific strains of plant growth promoting bacteria trigger growth in *Arabidopsis* (Ryu *et al*. 2005). In general, some microbial species can alter root development and plant growth through three major mechanisms: *1*) by

Submitted 14 February 2015, *last revision* 2 July 2015, *accepted* 3 August 2015.
Abbreviations: ACC - 1-aminocyclopropane-1-carboxylic acid; ET - ethylene; GFP - green fluorescent protein; IAA - indole-3-acetic acid; GUS - β-glucuronidase; KB - King's B medium; MS - Murashige and Skoog; PCR - polymerase chain reaction; YFP - yellow fluorescent protein.
Acknowledgements: This work was supported by the Major State Basic Research Development Program of China (2012CB026105), the National High Technology Research and Development Program (2007AA021401), the National Natural Science Foundation of China (31170225; 31201145), the Foundation of Science and Technology Program of Gansu Province (1107RJYA005), the Scientific research project of Qinghai-Tibetan DC Interconnection Project in State Grid Corporation of China, and the Foundation of Science and Technology Program of Gansu Province (1208RJZA224). The first two authors contributed equally to this work.
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production of plant growth-regulating substances including auxins, gibberellins, and cytokinins (Karadeniz *et al*. 2006); *2*) by production of volatile compounds such as acetoin (Ping and Boland 2004, Ryu *et al*. 2005); *3*) by modulation of plant ethylene (ET) content by the bacterial enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Glick 2005).

Auxins are versatile plant hormones that play important roles in plant growth and development (Ljung 2013). Indole-3-acetic acid (IAA) is perceived by a co-receptor complex consisting of an F-box protein from the TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX PROTEINS (TIR1/AFBs) family and a member of the Auxin/INDOLE ACETIC ACID (Aux/IAA) family of transcriptional repressors (Dharmasiri *et al*. 2005a,b, Dreher *et al*. 2006, Calderon Villalobos *et al*. 2012). Transcription is regulated by the transcription factors AUXIN RESPONSE FACTOR (ARFs) (Tiwari *et al*. 2003, Weijers *et al*. 2005, Guilfoyle and Hagen 2007). The IAA regulates various growth processes by modulating gene transcription through a SCF-TIR1/AFB-Aux/IAA-ARF nuclear signalling module.

Ethylene affects many aspects of plant growth and development. Ethylene is perceived by ETR1⁄ETR2, ET-response sensors 1 and 2, and EIN4 in *Arabidopsis* (Chang *et al*. 1993, Hua *et al*. 1995a, 1998b, Sakai *et al*. 1998). Ethylene binds to its receptors *via* a copper cofactor which is probably delivered by the copper transporter RAN1, which results in the inactivation of receptor function (Hua and Meyerowitz 1998) indicating that, in the absence of ET, the receptors are active. In the absence of ET, the receptors activate a Raf-like serine⁄threonine kinase CTR1 which is negative regulator of the pathway (Kieber *et al*. 1993). The CTR1 de-represses EIN2 leading to the activation of EIN3 and EIN3-like transcription factors. In the nucleus, ERF1 belongs to a large family of APETALA2 domain-containing transcription factors that bind to a GCC-box present in the promoters of many ET-inducible defense genes (Hao *et al*. 1998, Brown *et al*. 2003). The ERF1, 2, and 5 activate and ERF3, 4, 7, 10, 11, and 12 repress GCC-box-containing genes (Fujimoto *et al*. 2000, Ohta *et al*. 2000).

Interactions between auxin and ET regulate root system architecture (Růžička *et al*. 2007, Ivanchenko *et al*. 2008). Numerous observations suggested a tight correlation between auxin and lateral root formation (Casimiro *et al*. 2001, Swarup *et al*. 2008). Elevated content of auxin, either by auxin production or by auxin transport, affect lateral root formation (Boerjan *et al*. 1995, Celenza *et al*. 1995, Casimiro *et al*. 2001, Wilmoth *et al*. 2005, Shkolnik-Inbar and Bar-Zvi 2010, Arase *et al*. 2012). A few reports in the literature documented the role of ET in lateral root formation (D'Haeze *et al*. 2003, Ivanchenko *et al*. 2008, Negi *et al*. 2008, Lewis *et al*. 2011). The *Neverripe* mutant of tomato has a reduced ET response caused by a dominant negative mutation in one of the ET receptors (O'Malley *et al*. 2005). *Neverripe* has an increased underground root biomass resulting from the inhibition of ET signalling (Clark *et al*. 1999). As noted previously, beneficial plant-microbe interactions can directly or indirectly impact underground and aboveground structures of host plants (Zamioudis *et al*. 2013). Specifically, changes in root structures including overall root growth, primary and lateral root length, and lateral root numbers (Hirsch *et al*. 1997, Ditengou *et al*. 2000, Oldroyd and Downie 2008, Felten *et al*. 2009, Contesto *et al*. 2010).

Arabidopsis has been chosen as model system to study plant-microbe interactions because of its short life cycle and the availability of many mutants impaired in hormone responses including auxins, ET, and cytokinins (Persello-Cartieaux *et al*. 2001, Ryu *et al*. 2003, 2005). Another study showed that *Serratia odorifera* emits high amounts of ammonia, causes alkalization of the neighbouring plant medium, and subsequently reduces the growth of *Arabidopsis* during co-cultivation in compartmented Petri dishes (Weise *et al*. 2013). There are reports in the literature that cyanide production by rhizobacteria is possible mechanism of plant growth inhibition (Alström and Burns 1989). However, little is known about the roles of plant hormones involved in inhibiting root system development by microbes. In this study, we paid attention to LZMBW216's roles in altering root system architecture using several mutants with defects in polar auxin transport or ethylene signalling. Our results show that LZMBW216 inhibited growth of primary roots as well as lateral roots in wild-type plants, but LZMBW216 exhibited little effect on the primary root length and lateral root numbers in the mutants *pin2* and *aux1-7*, respectively.

Materials and methods

Potato (*Solanum tuberosum* L. cv. Da Xi Yang) microplants were obtained from the School of Life Science and Engineering, the Lanzhou University of Technology, Lanzhou, China. The epiphytic bacterium was isolated from leaves of three-week-old potato microplants. Briefly, leaves were rinsed once with autoclaved water and then a 0.2 cm^3 of liquid was taken from the final rinse liquid and transferred to 5 cm^3 King's B (KB) medium (proteose peptone 20 g, K_2HPO_4 1.5 g, $MgSO_4 . 7 H_2O$ 1.5 g, glycerol 20 cm^3, water 1000 cm^3, agar 15 g, pH 7.2), and then subsequently 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 appeared. To confirm endophytic bacteria, polymerase chain reactions (PCRs) were performed with the universal 16S primers 27 F (5′-AGAGTTTGATCC TGGCTCAG-3′) and 1492 R (5′-GGTTACCTTGTTA CGACTT-3′). The PCR products were sequenced and the resultant 16S rDNA sequences were compared to the GenBank databases using *BLAST*, and then a phylo-genetic tree of LZMBW216 was constructed (Fig. 1 Suppl.).

Wild-type *Arabidopsis thaliana* L. cv. Col-0, two transgenic promoter::reporter lines *DR5::GUS* and *CycB1;1::GUS*, two transgenic lines *PIN2-GFP* and *AUX1-YFP*, and mutants *tir1-1*, *pin2*, *aux1-7*, *axr1-12*, *etr1-3*, and *ein3-1* were used in our experiments. All seeds were surface-sterilized in 15 % (v/v) bleach solution for 15 min, extensively rinsed with autoclaved water for three times, and then grown on a half-strength Murashige and Skoog (1/2 MS) medium with 3 % (m/v) sucrose and 1 % (m/v) agar under a 16-h photoperiod, an irradiance of 100 μmol m^{-2} s^{-1}, a temperature of 22 °C, and a relative humidity of 65 - 70 %.

Stenotrophomonas maltophilia, named LZMBW216, was cultured on a KB liquid medium with constant shaking at 150 rpm and 28 °C for 24 h. After 24 h growth, the cells were collected, washed twice with autoclaved water, and resuspended in autoclaved water. For *Arabidopsis* seeds treatment, different bacterial suspensions (10^7, 10^8, or 10^9 CFU cm^{-3}) were used to soak them at room temperature for 12 or 24 h. After treatment, Col-0 seeds were transferred onto a 1/2 MS medium. After 11 and 16 d, plant growth, primary root length, and lateral root numbers of the *Arabidopsis* seedlings were measured. Similarly, for treatment of five-d-old seedling of various plants including Col-0, *tir1-1*, *pin2*, *aux1-7*, *axr1-12*, *etr1-3*, and *ein3-1*, and different bacterial suspensions (10^7or 10^8 CFU cm^{-3}) were used to soak them at room temperature for 12 or 24 h. After the treatments, the seedlings were transferred onto 1/2 MS plates. After 4 d, growth on the 1/2 MS medium, primary root length, and lateral root numbers were measured. The seedlings of lines *DR5::GUS* and *CycB1;1::GUS* were used for β-glucuronidase (GUS) staining after transferring to a new 1/2 MS for 2 d. Four plates were used for one independent experiment, and there were about 15 seeds per plate. Three independent experiments were carried out for all inoculation assays.

Histochemical assay of GUS activity was performed as described by Ulmasov *et al.* (1997) with minor modifications. A GUS staining buffer composed of 100 mM Na_3PO_4 (pH 7.0), 1 mM EDTA, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 0.1 % *Triton X-100*, and 1 mM *X-Gluc*. Samples were put into Eppendorf tubes containing the staining buffer and incubated at 37 °C overnight. The stained roots were cleared in a mixture of chloral hydrate : glycerol : water (8:1:2, m/v/v) and observed with an optical microscope (*35tv*, *Phoenix*, China).

Confocal images were captured with a confocal laser-scanning microscope (*FV1000*, *Olympus*, Tokyo, Japan) equipped with a 20× (water immersion) objective lens, an argon laser excitation was set at 488 nm. Emission filters for the green fluorescent protein (GFP) and yelow fluorescent protein (YFP) were set at 505 - 530 nm and 490 - 510 nm, respectively. The images were processed in *Adobe Photoshop*. The GFP fluorescent proteins were quantified using the *ImageJ* program on confocal sections acquired with the same microscope settings (Růžička *et al*. 2007). Approximately 10 seedlings per image were examined, and at least three independent experiments were repeated.

After indicated times of growth and treatments, the seedlings were put on an agar plate and digital pictures were taken. Primary root length was measured using a standard 1 cm scaled ruler with the *ImageJ* software (Alabadí *et al*. 2004). Values are 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 (*SPSS v. 17.0*). In all cases, the confidence coefficient was set at $\alpha = 0.05$.

Results

The epiphytic bacterium was isolated from leaves of 3-week-old potato seedlings and named LZMBW216. We found that LZMBW216 had a very high similarity with *Stenotrophomonas* by sequence alignment. Furthermore, six species of genus *Stenotrophomonas* were selected for phylogenetic analysis, including *S. acidaminiphila*, *S. humi*, *S. koreensis*, *S. maltophilia*, *S. nitritireducens*, and *S. rhizophila*. The phylogenetic tree of LZMBW216 was constructed by the *MEGA6* software (Fig. 1 Suppl.). From the phylogenetic tree, we found that LZMBW216 belongs to *S. maltophilia*.

To test possible effects of LZMBW216 on plant growth, *Arabidopsis* seeds were inoculated with an LZMBW216 suspension (10^7, 10^8, or 10^9 CFU cm^{-3}) for 24 h and then sown on the 1/2 MS medium for germination and growth. After 11 and 16 d, shoot and root growth significantly decreased in the LZMBW216 pretreated seedlings compared to the control (Fig. 1*A*). Especially at day 16, shoot and root fresh masses decreased considerably in the seedlings pretreated with 10^7 CFU cm^{-3} LZMBW216 compared to the control (Fig. 1). Primary root length and the number of emerged lateral roots were also determined. The seedlings pretreated with the LZMBW216 suspension for 24 h had a significantly decreased primary root length and lateral root number compared to the control. Similarly, when the

seeds were inoculated for 12 h, LZMBW216 still inhibited primary root elongation, decreased lateral root number, and reduced shoot and root fresh masses (Fig. 1*G*,*I*). Taken together, these data demonstrate that growth inhibition of the *Arabidopsis* seedlings by LZMBW216 was mostly not dependent on the concentration of LZMBW216, at least within the tested concentration range (10^7, 10^8, or 10^9 CFU cm^{-3}).

Given that LZMBW216 could inhibit seedling growth after inoculation of the seeds, we asked whether LZMBW216 exhibited similar effects after inoculating the seedlings. For this purpose, five-d-old *Arabidopsis* seedlings were inoculated with LZMBW216 for 12 and 24 h, and primary root length, lateral root number, and shoot and root fresh masses were measured after 4 d of further growth (Fig. 2). Primary root elongation and the number of emerged lateral roots decreased in the seedlings treated with LZMBW216 compared to the control (Fig. 2*E*-*H*). Shoot and root fresh masses also declined when the seedlings were inoculated with LZMBW216 for 12 and 24 h (Fig. 2*A*-*D*). So, regardless of inoculation of the *Arabidopsis* seeds or seedlings,

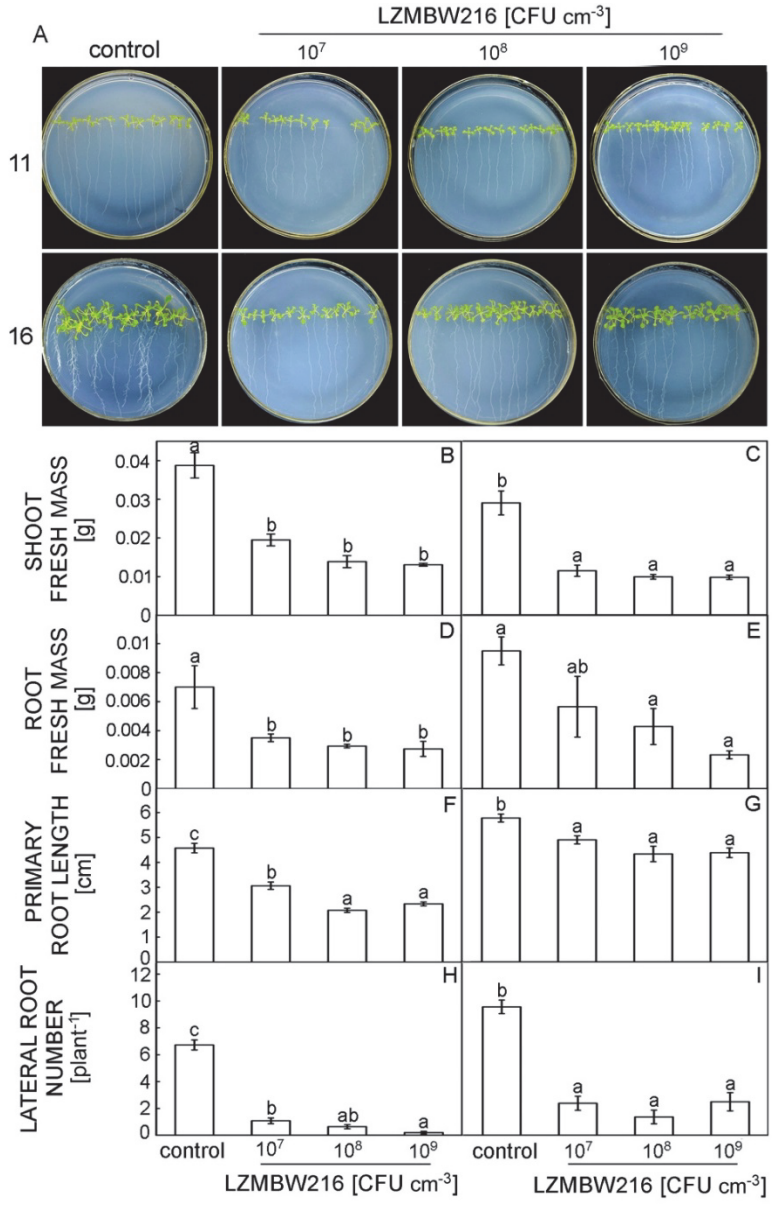

Fig. 1. Effects of LZMBW216 inoculation of seeds (for 24 h) on the subsequent growth of 11-d-old or 16-d-old *Arabidopsis* seedlings (*A*). Growth parameters of seedlings grown from seeds innoculated with LZMBW216 for 24 h (*B*, *D*, *F*, *H*) or 12 h (*C*, *E*, *G*, *I*). Means ± SEs of three independent experiments (n = 10). Within each set of experiments, bars with different letters are significantly different at $P < 0.05$.

LZMBW216 inhibited root growth and development.

To test whether the LZMBW216 inhibition of root growth was caused by affecting seed germination, we inoculated *Arabidopsis* seeds with LZMBW216 for 12 and 24 h and examined seed germination rate (Fig. 3). Although LZMBW216 slightly inhibited seed germination at 36 h after sowing, overall germination rates of the seeds pretreated with LZMBW216 were comparable with the control at 60 h after sowing. So, the LZMBW216 inhibition of primary roots was not caused by an effect on seed germination.

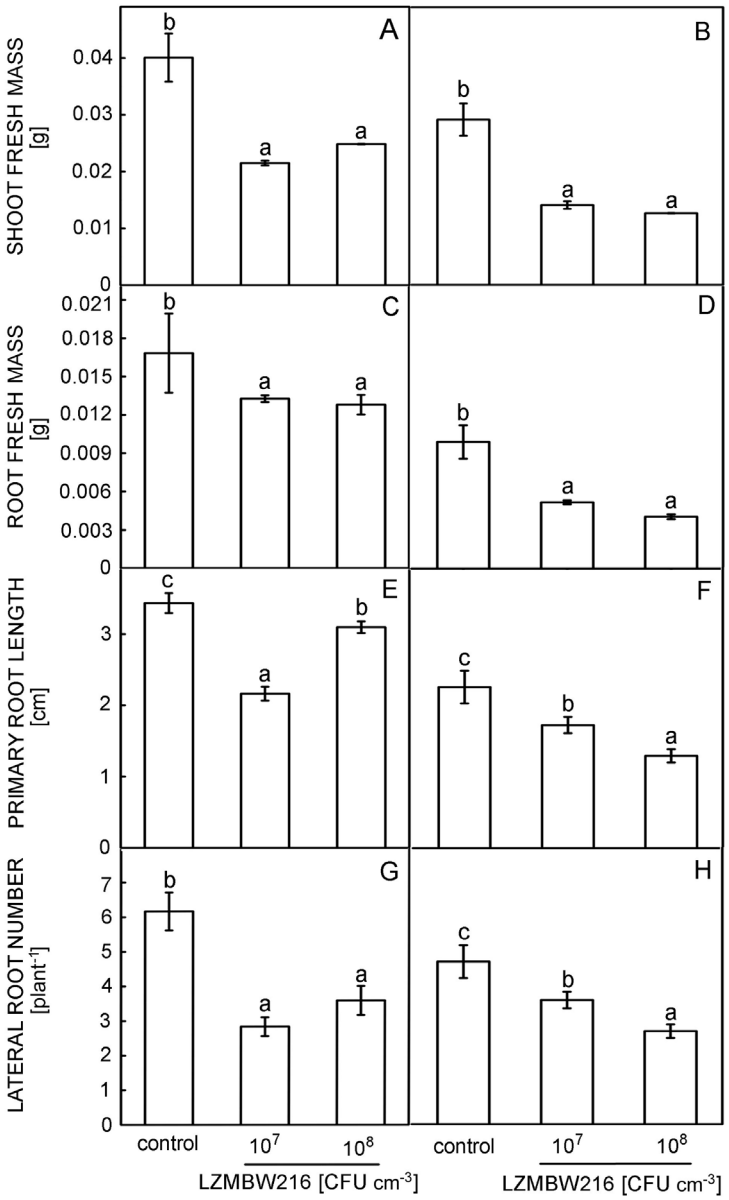

Fig. 2. *Arabidopsis* seedlings (5-d-old) were inoculated with LZMBW216 for 12 h (*A*, *C*, *E*, *G*) or 24 h (*B*, *D*, *F*, *H*) and growth parameters were measured after further 4 d on 1/2 MS medium. Means ± SEs of three independent experiments ($n = 10$). Within each set of experiments, bars with different letters are significantly different at $P < 0.05$.

It is known that IAA can affect root system development. To determine whether IAA played a role in LZMBW216 inhibition of root growth, we transferred the LZMBW216-inoculated seedlings onto the 1/2 MS agar medium containing different concentrations of IAA (0.1, 1.0, and 10 nM) and after 4 d of growth, several physiological parameters were measured. The results show that IAA was not able to rescue or relieve the inhibitory effects of LZMBW216 on the primary and lateral roots (Fig. 4*A*,*D*).

Application of IAA has been found to repress primary root growth in *Arabidopsis* (López-Bucio *et al*. 2002,

Perrine-Walker *et al*. 2014). So, it was reasonable to investigate whether endogenous IAA played a role during LZMBW216 regulation of root growth. With the help of transgenic plants expressing the synthetic auxin reporter *DR5::GUS*, it was found that LZMBW216 did not induce the expression of *DR5::GUS* (Fig. 5*E*,*F*).

To test whether the LZMBW216 inoculation altered cell division or elongation or both, we analyzed the expression of the cell-cycle marker *CycB1;1::GUS* (Colón-Carmona *et al*. 1999) in the *Arabidopsis* transgenic plants. The *CycB1;1::GUS* seedlings were stained for GUS activity (Fig. 4*G*,*H*). The results show that the bacterial inoculation did not affect cell division or elongation.

To further define possible roles of auxin and ET in *Arabidopsis* responses to LZMBW216 inoculation, we examined root architecture in several *Arabidopsis* mutants including *tir1-1*, *pin2*, *aux1-7*, *axr1-12*, *etr1-3,* and *ein3-1*. The loss-of-function mutant *tir1-1* showed a reduced sensitivity to IAA (Ruegger *et al*. 1998). Mutation in *PIN2*, which encodes an efflux carrier of auxin in plant cells, resulted in an increased IAA content in roots due to lack of redistribution of IAA *via* basipetal transport from the root tip to the elongation zone (Friml *et al*. 2004, Perrine-Walker *et al*. 2014). Mutation in *AUX1*, which encodes an influx carrier, was shown to be resistant to auxin transport inhibitors (Fujita and Syono 1996). Mutation in *AUXIN RESISTANT 1* (*AXR1*) also exhibits a severe reduction in auxin responses (Lincoln *et al*. 1990). The loss-of-function mutant *etr1* shows a reduced sensitivity to ET (Hall *et al*. 1999). The mutant *ein3* shows a loss of ET-mediated effects on gene expression, triple responses, and cell growth inhibition (Chao *et al.* 1997). We found that LZMBW216 inhibited growth of primary roots and decreased lateral root number in the control and most mutants (Fig. 5). However, it was interesting to find that LZMBW216 did not inhibit primary root elongation in the *pin2* mutant and lateral root number in the *aux1-7* mutant (Fig. 5*C*,*D*) implying that polar auxin transport might be important for LZMBW216-altered root system architecture. In addition, all mutants analyzed showed decreased shoot and root fresh masses after the inoculation with LZMBW216 (Fig. 5). Given the necessity of auxin polar transport for LZMBW216-mediated plant growth, the expressions of AUX1 and PIN2 were tested using transgenic plants AUX1-YFP and PIN2-GFP, respectively. The results show that LZMBW216 reduced the expressions of these two proteins (Fig. 6), suggesting an important role of polar auxin transport in LZMBW216 alteration of root system architecture.

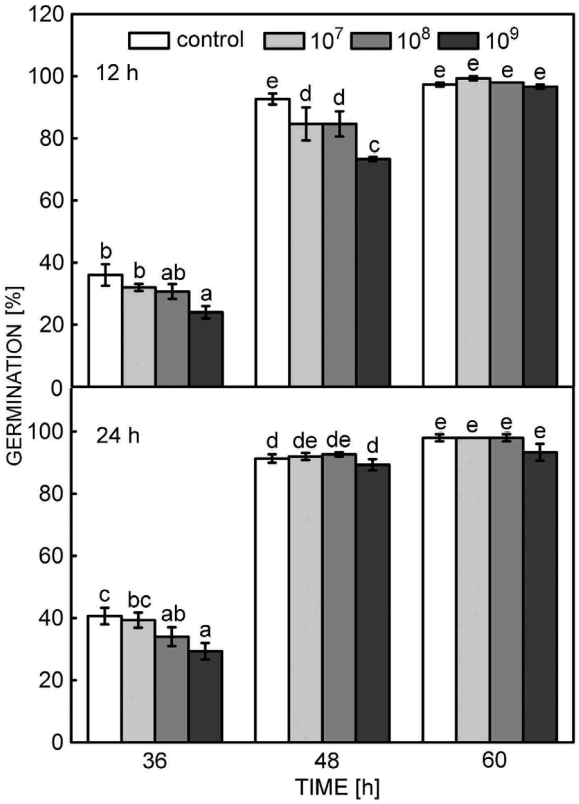

Fig. 3 Effects of LZMBW216 inoculation on *Arabidopsis* seed germination. *Arabidopsis* seeds were treated with bacterial suspensions (10^7, 10^8, or 10^9 CFU cm^{-3}) for 12 h and 24 h. Controls were treated with sterile water. Means ± SEs of three independent experiments (n = 50). Within each set of experiments, bars with different letters are significantly different at $P < 0.05$.

Discussion

Plant growth is affected by a plethora of environmental factors including irradiance, temperature, nutrients, and microorganisms. The region around a root, the rhizosphere, is relatively rich in nutrients because as much as 40 % of plant photosynthesis products is lost from roots (Bais *et al*. 2006). Consequently, the rhizosphere supports large microbial populations capable of exerting beneficial, neutral, or detrimental effects on plant growth. Like other biological surfaces, the phyllosphere harbours a large number and diversity of microorganisms including bacteria, yeasts, oomycetes, fungi, and algae (Lindow and Brandl 2003). Research in the field of plant-microbe interactions is primarily driven by plant pathogens because a number of microorganisms cause diseases, therefore affecting the crop yield. A number of bacterial isolates are known to exhibit beneficial effects. For example, Abanda-Nkpwatt *et al*. (2006) reported that a *Methylobacterium extorquens* strain promotes growth of various plant seedlings. Bacteria directly influence root system architecture and

shoot development by secretion of auxins and cytokinins (Persello-Cartieaux *et al*. 2001, Arkhipova *et al*. 2005). But, the roles of plant hormones on detrimental microbes affecting root growth are still unclear.

Serratia odorifera and six other rhizobacteria emit high amounts of ammonia which causes alkalization of the neighbouring medium and subsequently reduces growth of *Arabidopsis thaliana* (Weise *et al.* 2013). Similarly, it was found that *S. maltophilia* LZMBW216 inhibited the growth of the *Arabidopsis* seedlings (Fig. 1) suggesting that it likely acted as novel plant growth-inhibiting bacterium. We used the LZMBW216 strain to gain a better understanding of the mechanisms of plant growth inhibition.

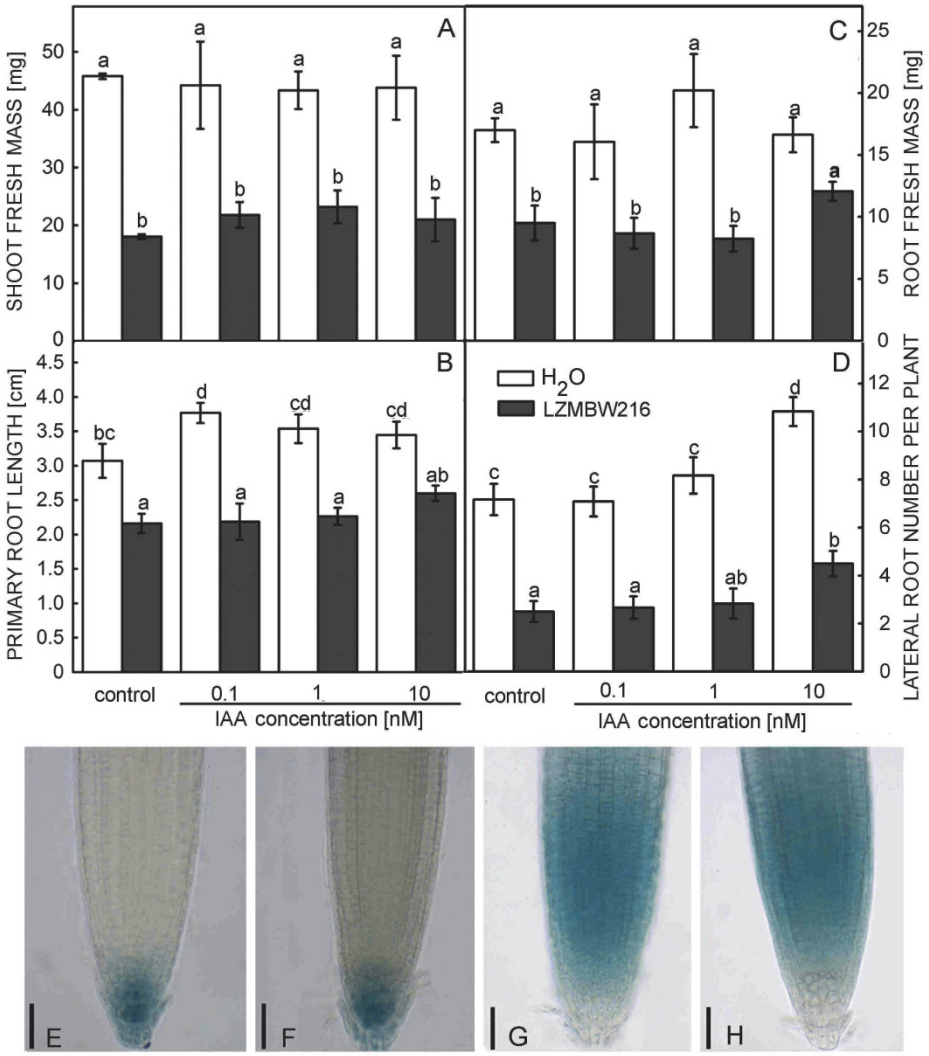

Fig. 4. Effects of IAA on *Arabidopsis* root architecture after LZMBW216 inoculation. 5-d-old *Arabidopsis* Col-0 seedlings were treated with bacterial suspensions (10^7or 10^8 CFU cm^{-3}) and sterile water (controls) for 24 h. Growth parameters: *A* - shoot fresh mass, *B* - primary root length, C - root fresh mass, and D - lateral root number. Means ± SEs of three independent experiments (n = 10). Within each set of experiments, bars with different letters are significantly different at $P < 0.05$. GUS expression: *E* - H_2O inoculated *DR5::GUS*, *F* - LZMBW216 inoculated *DR5::GUS*, *G* - H_2O inoculated *CycB1;1::GUS*, *H* - LZMBW216 inoculated. *CycB1;1::GUS* (*bars* = 30 μm, photographs are representative of at least 20 stained seedlings).

Bacteria producing IAA can stimulate or inhibit root growth depending on their amounts (Persello-Cartieux *et al*. 2001). The IAA is synthesized by plants and by a few microbes (Wooddard and Bartel 2005). In plants IAA plays a key role in root and shoot development. The IAA is key regulator of lateral root development and root hair development (Casimiro *et al*. 2001). A low content of IAA can promote root growth and development, but the application of IAA was not able to rescue the inhibitory effect of the LZMBW216 inoculation (Fig. 4*A-D*).

Auxins are important for plant-microbes interactions (Sukumar *et al*. 2013). For example, *Phyllobacterium brassicacearum* stimulates expression of several auxin biosynthesis pathway enzymes in *Arabidopsis* shoots

(Contesto et al. 2010). When co-cultured with *Populus*, *Laccaria bicolor* induces transcription of *GH3* genes, including *PtaGH3-1*, *PtaGH3-2*, *PtaGH3-7*, and *PtaGH3-8*, which are involved in IAA conjugation (Felten *et al*. 2009). A recent study indicated that ET plays a key role in regulating auxin production and alters root architecture (Stepanova *et al*. 2005). A *Phyllobacterium brassicacearum* strain induces expression of *DR5::GUS* in *Arabidopsis* primary as well as lateral root tips (Contesto *et al*. 2010), but LZMBW216 did not induce the expression of *DR5::GUS* in *Arabidopsis* primary root tips (Fig. 4*E*,*F*).

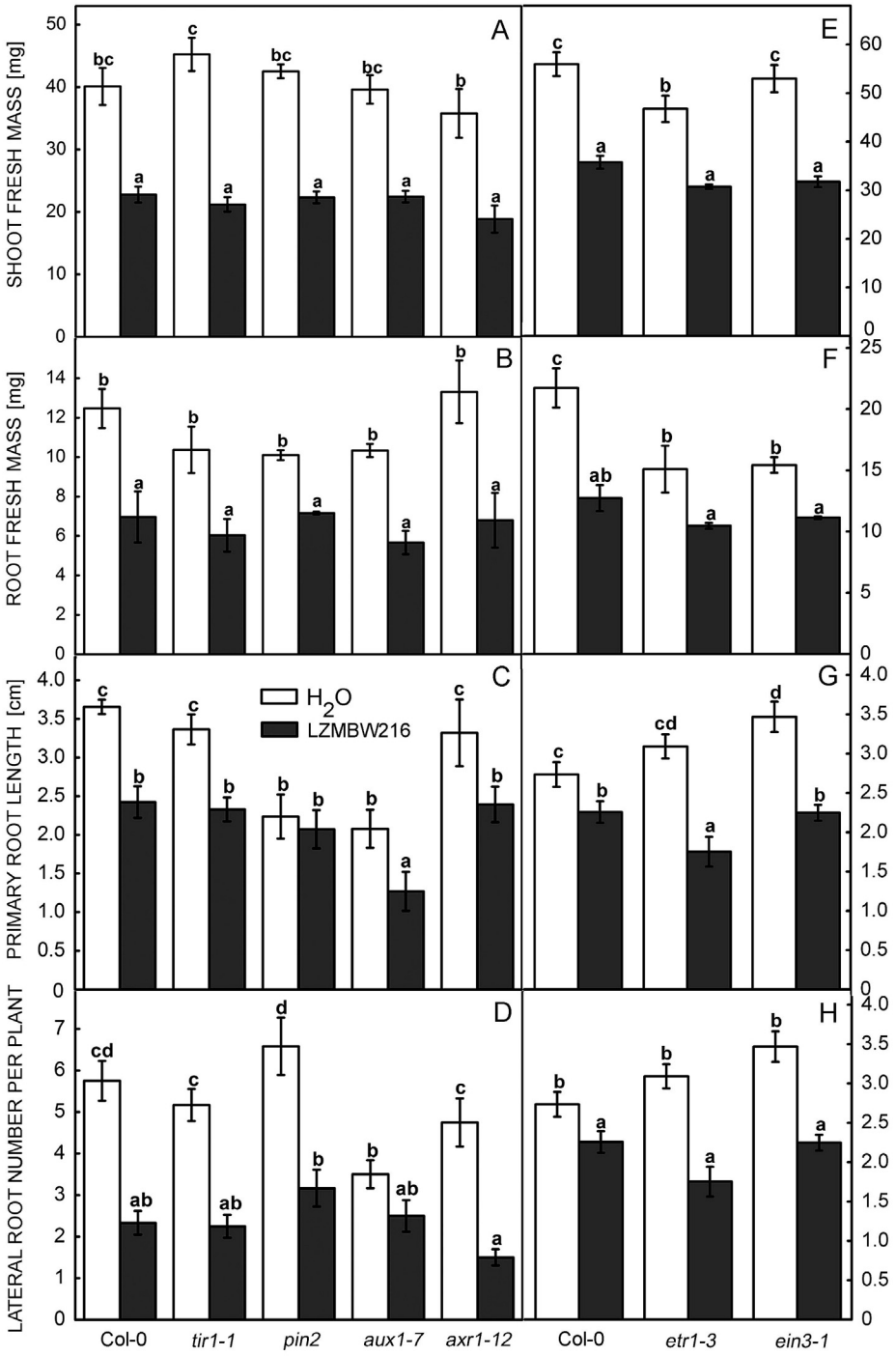

Fig. 5. Five-d-old *Arabidopsis* (Col-0) seedlings and different mutants were inoculated with LZMBW216 suspension (10^8 CFU cm^{-3}) or sterile water (controls) for 24 h and growth parameters were measured after further 4 d on 1/2 MS medium: *A*, *E* - shoot fresh mass, *B*, *F* - root fresh mass, *C*, *G* - primary root length, *D*, *H* - lateral root number per plant. Means ± SEs of three independent experiments ($n = 10$). Within each set of experiments, bars with different letters are significantly different at $P< 0.05$.

Pseudomonas fluorescens can inhibit *Arabidopsis* primary root growth by enhancing expression of *CYCB1;1::GUS* and by decreasing cell length in the elongation zone (Zamioudis *et al*. 2013). Our microscopic examination shows that LZMBW216 did not alter the expression of the *CYCB1;1::GUS* reporter in the meristematic zone of root tips (Fig. 4*G*,*H*). These results imply that LZMBW216 might not disturb cell cycle proteins to affect *Arabidopsis* growth and development. *Arabidopsis* auxin transport mutants *aux1-7*, *doc1*, and *pin2* show a reduced growth compared with wild-type plants when inoculated with *Trichoderma virens* (Contreras-Cornejo *et al*. 2009). Similarly, LZMBW216 did not inhibit the primary root length of the *pin2* mutant but decreased lateral root numbers in the *aux1-7* mutant (Fig. 5*C*,*D*). A literature report indicated that *Bacillus megaterium* can inhibit primary root elongation in *aux1*, *eir1,* and *axr4* mutants (López-Bucio *et al.* 2007). Our previous report indicated that *Bacillus* sp. LZR216 inhibits *Arabidopsis* primary root growth by decreasing PIN1/PIN2/PIN3/AUX1 protein abundance (Wang *et al*. 2015). Inoculation with the *Phyllobacterium brassicacearum* strain STM196 induces a weak increase in transcripts of *PIN1* and *PIN2/EIR1* auxin efflux facilitators in shoots (but not in roots), whereas expression of *AUX1*, encoding for an auxin influx transporter, is not significantly affected in shoots and is down-regulated in roots (Contesto *et al*. 2010). Furthermore, our results show that LZMBW216 inhibited the primary root length of the ET signalling mutants and decreased the lateral root numbers of the ET signalling mutants (Fig. 5*G*,*H*). Similarly, *Bacillus megaterium* can inhibit primary root elongation in *etr1* and *ein2* mutants (López-Bucio *et al*. 2007). *Pseudomonas fluorescens* can inhibit primary root growth in *ein3*/*eil1* and *ein2-1* mutants (Zamioudis *et al*. 2013). These results imply that the roles of ET signalling are not important for LZMBW216 in regulating *Arabidopsis* growth and development. Other studies reported that *Bacillus* sp. B55 inoculation significantly enhances wild-type and 35S-*etr1* seedling growth as clearly seen in the seedling leaf surface area although primary root length of wild-type and 35S-*etr1* seedlings decreases by 36 and 11 %, respectively, after *Bacillus* sp. B55 inoculation (Meldau *et al*. 2012). Several studies demonstrated that ET signalling also plays important roles in communication between plants and mutualistic microbes (Camehl *et al*. 2010, Long *et al*. 2010).

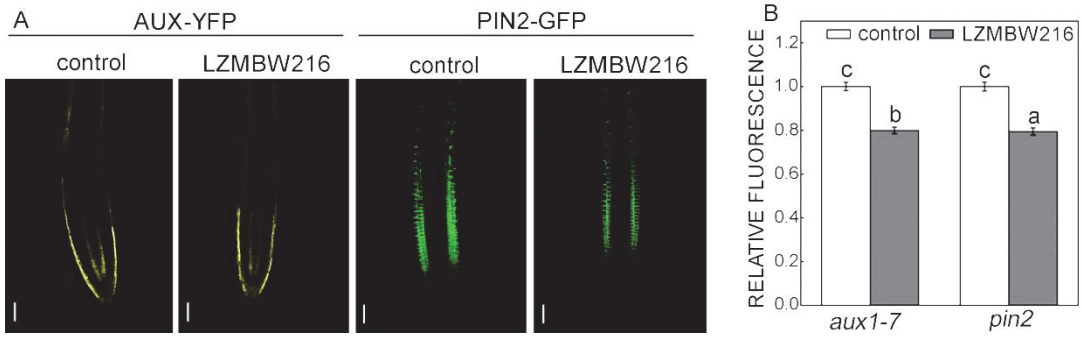

Fig. 6. Effect of LZMBW216 on AUX1 and PIN2 proteins. *A* - 5-d-old seedlings were inoculated with LZMBW216 for 24 h and after transferring on 1/2 MS medium for 2 d, GFP and YFP expression was monitored (images are representative of at least three independent experiments, *bars* = 50 µm). *B* - Quantification of GFP/ YFP fluorescence by image analysis of confocal sections described in *A*. Mean ± SE of three independent experiments (n = 10), *bars* with different letters are significantly different at $P < 0.05$.

In this study, LZMBW216 had a negative impact on *Arabidopsis* growth, and we paid a close attention on the roles of auxin and ET signals in this process. Our results show that LZMBW216 involved polar auxin transport in altering the root system. LZMBW216 also decreased shoot and the root fresh masses of the auxin and ET signal mutants (Fig. 5). *Arabidopsis* as model system to study plant-bacterial symbiosis shows a great promise for investigating molecular mechanisms of plant-growth inhibition by microbes.

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