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Gram-positive Rhizobacterium *Bacillus amyloliquefaciens* FZB42 Colonizes Three Types of Plants in Different Patterns

Ben Fan[1,2*], Rainer Borriss[2], Wilfrid Bleiss[3], and Xiaoqin Wu[1]

[1]*Institute of Forest Protection, Nanjing Forestry University, Nanjing 210037, P. R. China*
[2]*Institut für Biologie/Bakteriengenetik, Humboldt Universität Berlin, Chausseestrasse 117, D-10115 Berlin, Germany*
[3]*Institut für Biologie/Molekulare Parasitologie, Humboldt-Universität Berlin, Philippstrasse 13, D-10115 Berlin, Germany*

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The colonization of three types of different plants, *Zea mays*, *Arabidopsis thaliana*, and *Lemna minor*, by GFP-labeled Gram-positive rhizobacterium *Bacillus amyloliquefaciens* FZB42 was studied in gnotobiotic systems using confocal laser scanning microscopy and electron microscopy. It was demonstrated that FZB42 was able to colonize all the plants. On one hand, similar to some Gram-negative rhizobacteria like *Pseudomonas*, FZB42 favored the areas such as the concavities in root surfaces and the junctions where lateral roots occurred from the primary roots; on the other hand, we clearly demonstrated that root hairs were a popular habitat to the Gram-positive rhizobacterium. FZB42 exhibited a specific colonization pattern on each of the three types of plants. On *Arabidopsis*, tips of primary roots were favored by FZB42 but not so on maize. On *Lemna*, FZB42 accumulated preferably along the grooves between epidermal cells of roots and in the concave spaces on ventral sides of fronds. The results suggested *L. minor* to be a promising tool for investigations on plant-microbial interaction due to a series of advantages it has. Colonization of maize and *Arabidopsis* roots by FZB42 was also studied in the soil system. Comparatively, higher amount of FZB42 inoculum (~10^8 CFU/ml) was required for detectable root colonization in the soil system, where the preference of FZB42 cells to root hairs were also observed.

Keywords: PGPR, *Bacillus amyloliquefaciens*, *Zea mays*, *Arabidopsis thaliana*, *Lemna minor*

Introduction

It is generally assumed that an efficient colonization of plant roots by plant growth promoting rhizobacteria (PGPR) is a critical step for their further interactions (Chin-A-Woeng *et al.*, 2000; Lugtenberg *et al.*, 2001; Kamilova *et al.*, 2005; Timmusk *et al.*, 2005; Ongena and Jacques, 2008). A number of investigations have been performed on the colonization patterns of Gram-negative PGPR, especially some *Pseudomonas* strains (Lugtenberg and Dekkers, 1999; Lugtenberg *et al.*, 1999; Compant *et al.*, 2005; Lugtenberg and Kamilova, 2009). *Pseudomonas* distributes on plant roots non-uniformly: some areas, including the extreme tips of roots, are practically free from bacteria whereas other areas can be highly colonized (Fukui *et al.*, 1994; Meharg and Killham, 1995; Lugtenberg *et al.*, 2001; Preston, 2004). The heavily colonized areas are usually found at junctions between epidermal root cells, concave parts of the epidermal surface, or sites where side roots appear, all presumed sites of exudation (Bloemberg *et al.*, 1997; Chin-A-Woeng *et al.*, 1997).

Due to various differences in many aspects, Gram-positive rhizobacteria and Gram-negative rhizobacteria may display different ecological behaviour in the rhizosphere. However, compared with a wealth of knowledge about colonization of Gram-negative PGPR, much less is known about that of Gram-positive ones (Timmusk *et al.*, 2005; Liu *et al.*, 2006). Among rhizobacteria species, *Bacillus* spp. draw more and more attention because of their ability to produce heat-resistant endospores, which bestows *Bacilli* an obvious advantage in application as biofertilizer or biocontrol agents (Paulitz and Belanger, 2001; Ryu *et al.*, 2003, 2004; Bais *et al.*, 2004; Idris *et al.*, 2004; Lopez-Bucio *et al.*, 2007; Ongena and Jacques, 2008; Choudhary and Johri, 2009).

Bacillus amyloliquefaciens FZB42 is one of well studied Gram-positive PGPR. In the past several years consecutive studies have been performed with FZB42 in order to elucidate its plant growth-promoting and biocontrol activities (Idris *et al.*, 2004; Koumoutsi *et al.*, 2004, 2007; Butcher and Helmann, 2006; Chen *et al.*, 2007, 2009; Idris *et al.*, 2007; Schneider *et al.*, 2007; Ogata *et al.*, 2009). As a representative of Gram-positive PGPR, however, little is known about its colonization behaviour in plant rhizosphere. In this work colonization of three different types of plants, maize, *Arabidopsis*, and *Lemna*, by GFP-labeled *B. amyloliquefaciens* FZB42 was studied in both gnotobiotic systems and soil system using confocal laser scanning microscopy and electron microscopy.

Materials and Methods

Growth conditions of bacterial strains and plant materials

B. amyloliquefaciens FZB42 was labeled by GFP and tested for stability as previously reported (Fan *et al.*, 2011). The GFP-labeled FZB42 and *Bacillus subtilis* 168 were cultivated routinely in Luria broth at 30°C. *Zea mays* seeds were

*For correspondence. E-mail: fanben2000@gmail.com; Tel.: +86-25-8542-7301; Fax: +86-25-8542-7397

obtained from company Saaten-Union, Germany. The seeds of *Arabidopsis thaliana* ecotype Columbia-0 were obtained from AG genetics, Department of Biology, Humboldt University, Berlin. The duckweed clone *Lemna minor* ST was a courtesy from Institute of General Botany and Plant Physiology, Friedrich-Schiller-University, Jena, Germany. *L. minor* ST was propagated axenically in filter-sterilized Steinberg medium as described previously (Idris *et al*., 2007).

Colonization of maize seedling roots

i) Surface sterilization of maize seeds: Maize seeds were treated with 70% ethanol for 3 min and then with 5% (v/v) sodium hypochlorite for another 3 min before a final rinse of five times with sterile distilled water.
ii) Maize seedlings: After surface sterilization eight maize corn kernels, embryo upside, were placed in a standard 9-cm Petri dish filled with 7 ml sterile water (1:1 distilled water and tap water, V:V) and then incubated in dark at 30°C for overnight. In the second morning 250 μl of water was taken from the Petri dish and spread onto a LB agar in order to check contamination. The seeds continued to be incubated with refreshed water in the same condition. After 40–45 h, when no contamination was found, the germinated maize corns with a root of approximate 2 cm were chosen for the next steps.
iii) Inoculation and incubation: The bacteria strains were grown in Luria Broth till OD_{600} reached 1.0. The cultures were diluted with fresh LB by 1,000 times (~10^6 CFU) and then shaken at 30°C for another 15 min before being used for inoculation. The roots of the maize seedlings were inoculated by dipping into the culture, softly swirling, for two minutes. Finally the inoculated maize were grown in soft agar (0.8%) of basal Murashige-Skoog medium (without sucrose) and incubated in plant growth room at 24°C with a 16-h light regimen. At the same time, some seedlings were transferred to nonsterile greenhouse soil and incubated in the same condition.

Colonization of *Arabidopsis* roots

The seeds of *Arabidopsis thaliana* ecotype Columbia-0 were similarly surface-sterilized as above with reduced treatment time of merging into 70% ethanol for only 30 sec. The sterilized seeds were germinated onto an agar (0.6%) plate of basal Murashige-Skoog medium containing 1% sterile sucrose and grown at 24°C for 7 days. The seedlings were likewise inoculated as described for maize seedlings and subsequently mounted onto another square agar (0.8%) plate (12 cm×12 cm) of basal Murashige-Skoog medium (without sucrose). The plate was kept inclined, standing 30° to the vertical, and incubated in the same condition as for maize seedlings. Simultaneously, some of the inoculated seedlings were transferred to nonsterile greenhouse soil and incubated in the same condition.

Colonization of *L. minor*

Lemna minor ST was grown as previously described (Idris *et al*., 2004) with minor modification. Briefly, one sterile *Lemna* plantlet bearing two fronds were transferred into a well of micro-titer. Each well of 16 mm in diameter contained 2 ml Steinberg medium and was inoculated with 0.2% bacterial culture (OD_{600}≈1.0). The micro-titer plates were incubated at 20°C in a growth chamber with 12-h light and 12-h dark time. Every two days the media were refreshed by pipetting out the old media softly and refilling with new ones.

Specimen preparation for microscopy

For maize and *Arabidopsis* grown in the gnotobiotic systems, the seedling roots of seven days after transferring were sampled for microscopy. For maize grown in soil, the roots of the seedlings seven days after transferring were sampled, while, for *Arabidopsis* grown in soil, roots of the seedlings 14 days after transferring were sampled. For *Lemna*, both the roots and the fronds of one day, five days and nine days after inoculation were observed respectively. While *Lemna* and *Arabidopsis* roots could be observed directly with microscope, maize roots were prepared by scratching a piece of root surface, around 1 cm in length, from different parts of a root with a sterile blade or by cutting a cross section of 50 μm in thickness with a microtome. All specimens were merged into a drop of saline for microscopic observation. At least 10 samples were observed for each planting condition.

For electron microscopy, the primary roots of maize seedlings were taken to prepare some segments of about 10 mm, which were ~25 mm distant below root basis. The segments were divided into two 5-mm segments and processed for TEM and SEM respectively. The ventral surfaces of fronds of *Lemna* nine days after inoculation were sampled for imaging by SEM.

Microscopy

Usually the samples were firstly examined with an epifluorescence microscope Zeiss Axiophot XIOPHOT. GFP fluorescence was examined using a filter set of 450–490 nm excitation filter and LP520 emission filter, while red fluorescence was viewed in the case of *Lemna* by using a BP546 excitation filter and a LP590 emission filter.

Confocal Laser Scanning Microscopy (CLSM) was performed with a Leica DM IRE2&DM IRB system. GFP fluorescence was recorded by using an excitation laser of 488 nm (Argon laser) and collecting the emission of 500–550 nm. Transmission light was collected to visualize root/frond structure and was designated as red color in later image reconstruction in order to manifest the contrast with green color. The images acquired were reconstructed by Leica Confocal Software (LCS 2.6).

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were performed as previously described (Fan *et al*., 2011).

Resluts

Colonization of maize seedlings by FZB42 *in vitro*

In soft agar of Murashige-Skoog basal medium (without sucrose) the primary roots of maize seedlings could reach approximately 20 cm in eight days, at an elongation rate of more than 2 cm/day in average. The overall observation of

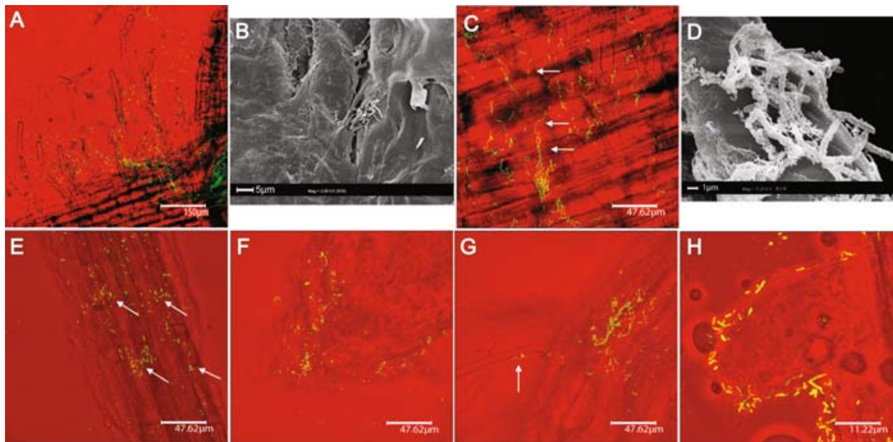

Fig. 1. CLSM micrographs (A, C, and E-H) and SEM micrograph (B and D) of GFP-labeled *B. amyloliquefaciens* FZB42 colonizing maize roots (A-D) and *Arabidopsis* roots (E-H) in gnotobiotic systems. (A) showed that a junction formed between the primary root and the lateral roots was heavily colonized by FZB42. (B) recorded the presence of FZB42 cells in a concavity on the maize root surface. (C and D) showed the bacterial cells colonizing root hairs. Note that the bacterial cells growing along a root hair as indicated by arrows in (C). (E) showed FZB42 cells colonizing *Arabidopsis* root surfaces. Note that the FZB42 cells seemed to locate in the grooves between epidermal cells as indicated by the arrows in (E). (F) indicates bacterial cells colonizing a root tip of *Arabidopsis*. (G&H) showed the FZB42 closely associated with the roots hairs.

primary roots revealed that the segment within 2–8 cm distant from the basal sites was a mostly colonized region by FZB42 (Fig. 4A). On the contrary, few bacterial cells could be observed within the range of 2 cm from a root tip. In general, the green fluorescent FZB42 were decreasingly observed from the upper part of a root down to the root tip.

The highly colonized segments happened to be the region where abundant lateral roots emerged. On the surface of this segment a number of FZB42 microcolonies could easily be detected (Figs. 1A–1D). However, hardly could fluorescent bacteria be observed on the lateral roots except the juncregions formed between the lateral roots and the primary root (Fig. 1A). In many observations a patch of "root surface", where a number of bacterial cells located, turned out to be some root hairs when observed from another angle. Often can be seen that the bacteria grew along (Fig. 1C) or even circling root hairs. In general, root hairs appeared to be one of the most preferred habitats for FZB42.

Scanning electron microscopy confirmed the presence of FZB42 on root hairs, where the bacterial cells were usually associated with a wealth of presumed root exudates (Fig. 1D). Rich nutrients provided by root exudates may account for the high occurrence of FZB42 on root hairs. Another impressive phenomenon shown by SEM was that many FZB42 cells located themselves in some concave parts on the primary root surfaces (Fig. 1B).

Neither cross sections observed with CLSM nor those observed with transmission electron microscopy proved existence of bacterial cells in the epidermis layer of maize roots, suggesting that FZB42 mainly, at least on maize, function as an epiphytic rhizobacterium.

Colonization of *Arabidopsis* by FZB42 *in vitro*

After inoculation the roots of *Arabidopsis* grew along the agar surfaces of MS basal medium (without sucrose). The primary roots reached around 5–6 cm in a week from the original length of 0.5–1.0 cm. The roots could easily be detached off from agar surfaces and observed directly with microscope, without making a section as was done for maize. Like maize, root hairs of *Arabidopsis* were also significantly colonized by FZB42 (Figs. 1G and 1H). Unlike maize, however, primary root tips and lateral roots were other two

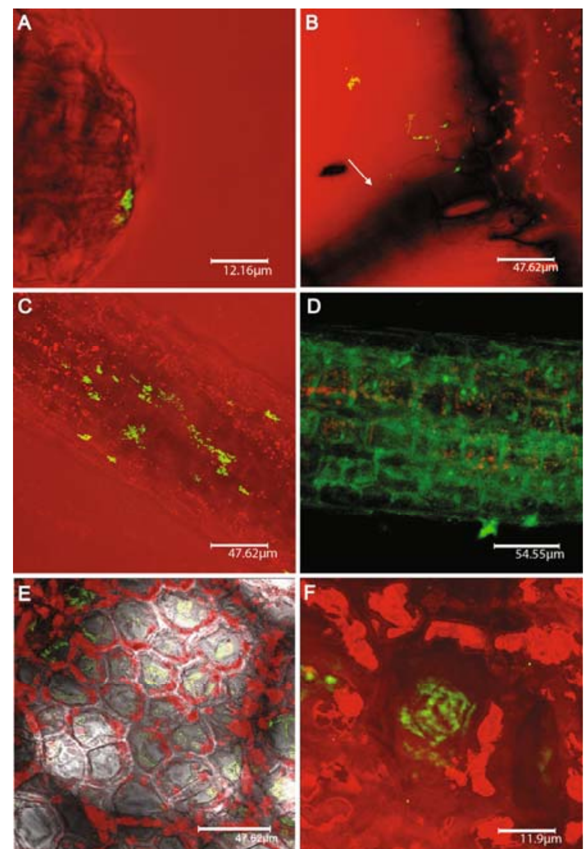

Fig. 2. CLSM micrographs of *B. amyloliquefaciens* FZB42 colonizing *Lemna* minor. The colonization of *Lemna* by GFP-labeled FZB42 one day (A & B), five days (C), and nine days (D-F) after inoculation were shown respectively. Note that one day after inoculation the colonization mainly occurred on a root tip (A) and in the linking regions between roots and fronds (B). In Panel B the dark region indicated by the arrow was the Lemna root. There were more microcolonies on the roots of five days after inoculation (C) than one day after inoculation. A robust biofilm formed on some segments of a root nine days after inoculation (D). In (E) the large sunken spaces are surrounded by layers of chloroplast-bearing parenchyma cells (in red) and most of the sunken spaces in this area accommodated a FZB42 colony (in green). Unlike the other graphs, in Panel E the transmission light was designated in gray in order to illustrate a clear structure of ventral side of Lemna fronts. (F) is a larger view of one sunken space shown in (E).

venues on *Arabidopsis* preferred by FZB42 (Fig. 1F).

Interestingly, it was often detected that FZB42 cells seemed to adapt themselves to the surface shape of root hairs (Fig. 1H). This orientation may lead to an intimate contact between bacterial cells and root hair surfaces so that the bacteria could to maximum extent be merged in the exudates secreted by root hairs. Another scenario which was often observed was that a significant portion of bacteria grew along or inside the boundary regions between epidermal cells, as shown in Fig. 1 (indicated by the arrows in Panel E).

Colonization of maize and *Arabidopsis* by FZB42 in soil system

The colonization studies were also performed in soil system. When planted in soil, maize roots grew differently from those in the nutrient soft agar. In soil the root system was shorter, reaching around 10–12 cm seven days after inoculation; whereas much more secondary roots were produced from the primary root, especially from the region 3 cm below the root base. Further, the primary roots in soil were thinner than and not as obvious as in soft agar system. The primary roots were examined for FZB42; however, scarcely could fluorescent bacteria be detectable. To improve colonization, FZB42 culture of ~10^8 CFU/ml was used for inoculation and the primary roots were then checked. In this condition, fluorescent cells were detected although still much less than in the gnotobiotic system. Relatively, a higher quantity of the bacterial cells was detected in the region within 1–3 cm below the basal sites (Fig. 4B) as well as on some root hairs.

Since the *Arabidopsis* seedlings had not yet rooted well one week after being transferred into soil, the roots of two weeks in soil were sampled for microscopic observation. Like the situation of maize roots in soil, seldom could fluorescent bacteria be observed on the *Arabidopsis* roots when inoculated as in the gnotobiotic system; however, when higher amount of inoculum (~10^8 CFU/ml) was used, some fluorescent cells were detectable on the upper parts of the root systems and some were detected on the root hairs.

Colonization of *L. minor* by FZB42 in the water

L. minor ST is a species of *Lemnaceae* (duckweed family), which occurs broadly in natural environment of still waters from temperate to tropical zones. *L. minor* structurally consists of one, two or three fronds, each with a single root hanging in the water (Fig. 4D). It reproduces primarily by vegetative budding, occasionally by flowering (Armstrong, 2010). Unlike the roots of most other kinds of plants, *Lemna* roots contain rich chlorophyll while have no root hair (Cross, 2002). Due to its rapid propagation rate, *Lemna* has widely been used as an assay plant for many environmental investigations (Lyle Lockhart *et al*., 1989). Here it is reported that FZB42 is able to colonize *Lemna* and form robust biofilms.

One day after inoculation *Lemna* was observed and then imaged by CLSM. Fluorescent FZB42 cells could sporadically be found on *Lemna* fronds and roots, while a relatively higher occurrence of colonization was observed on the root tips (Fig. 2A) and in the connecting regions (Fig. 2B) between roots and fronds. The preference of FZB42 to the two sites may be a suggestion that more nutrients or special compounds were present there, which were specifically recognized by FZB42 cells upon inoculation.

From the first day after inoculation, the Steinberg media were refreshed every other day as described in ‘Materials and Methods’. Five days after inoculation, a number of bacterial microcolonies could easily be observed on *Lemna* roots (Fig. 2C) and fronds. In terms of the quantity of bacteria detected, colonization on the fifth day obviously appeared to be an intermediate phase between the first day and the ninth day after inoculation as described above and below respectively.

Nine days after inoculation, FZB42 cells were found to colonize heavily some areas of the roots or the fronds of older *Lemna* plantlets whereas arise sporadically on those newly-emerged plantlets. In the highly-colonized areas on ventral surfaces of *Lemna* fronds, green fluorescent FZB42 cells formed colonies inside nearly each sunken space surrounded by layers of chloroplast-bearing parenchyma cells (Figs. 2E and 2F). On some segments of old roots the bacteria could even form a robust layer of biofilm (Fig. 2D), the thickness of which was approximately 2 μm according to the analysis with software LCS 2.6.

SEM was also used to study the colonization of nine days after inoculation. The result confirmed the observation with CLSM that many FZB42 cells populated in the concaves formed by sack-like parenchyma cells on the ventral sides of *Lemna* fronds. On some *Lemna* roots, it was clearly demonstrated that FZB42 cells grew along the grooves between epidermal cells (Fig. 3A–3D). There was richer fluffy material in the grooves than elsewhere (Fig. 3B), which were closely associated with the bacterial cells and presumed to be root exudates.

The SEM micrographs also displayed sophisticated biofilms developed on *Lemna*. In the biofilms many FZB42 cells altered their shapes from a smooth rod to a dumpy barrel, which were as approximately twice in diameter as the rod (Figs. 3E and 3F). Meanwhile, the barrel-shaped cells were coated with a rough crust full of swellings or fiber-like structures (Figs. 3E and 3F). While the shorter fibers apparently served to link the nearby bacteria together, the longer ones formed massively weaving the bacterial cells into a complex network or connecting them with *Lemna* surfaces (Figs. 3E and 3F).

In the *Lemna* colonization studies, GFP-tagged *Bacillus subtilis* 168 was also included as a reference strain. Unlike FZB42, nearly no colony of *B. subtilis* 168 could be detected on *Lemna* treated with the same preparation steps, corroborating the earlier reports about the poor capability of domesticated *B. subtilis* to colonize plant roots (Branda *et al*., 2001; Kinsinger *et al*., 2003).

Discussion

In this work the specific colonization patterns of GFP-labeled *B. amyloliquefaciens* FZB42 on three different kinds of plants in gnotobiotic systems and in soil have been described.

The junctions between primary roots and lateral roots were found to be a favored habitat of FZB42, consistent with the results investigated with *Pseudomonas* colonization.

However, the report that root hairs were also colonized as a "hot spot" has not been seen in non-*Rhizobium* PGPR so far. This preference was detected on maize roots and *Arabidopsis* roots in the gnotobiotic systems as well as in the soil system. A main reason for aggregation of FZB42 cells on root hairs may be due to abundant exudates secreted from the regions, as shown in Fig. 1D. According to our microarray results regarding the transcriptomic response of FZB42 to maize root exudates (unpublished), root exudates could trigger a vast array of biological responses of the bacterium; on the other hand, it is known that bacterial activities can affect root developments (Lopez-Bucio *et al.*, 2007). Therefore, it is highly likely that root hairs play an important role in plant-microbe interactions.

Despite favoring root hairs of both maize and *Arabidopsis* seedlings, FZB42 exhibited a distinct preference to their primary root tips. While the tips of *Arabidopsis* were strongly favored by FBZ42 (Fig. 4C), few bacterial cells could be observed on the primary root tips of maize seedlings (Fig. 4A). This difference may be explained in that maize roots grew too fast in the gnotobiotic system, far exceeding the spreading speed of bacteria on root surfaces (Bahme and Schroth, 1987). Nevertheless, other possible reasons can not be excluded. For example, tip structure of the two kinds of primary roots was apparently different. While much exudates were available from the lubricative layers around root tips of *Arabidopsis*, little sloughs were observed nearby maize root tips, possibly due to the tight structure of maize root tips.

On some of *Lemna* roots FZB42 cells accumulated along the grooves between epidermal cells (Figs. 3A–3D). A similar phenomenon seemed to occur on *Arabidopsis* as well (Fig. 1E). It is unlikely that just by chance FZB42 cells favored these niches such as the concavities on maize root surfaces, the bifurcation sites between primary roots and lateral roots of maize and *Arabidopsis*, the grooves between neighbored epidermal cells of *Lemna* root surfaces, and the indented spaces on ventral sides of *Lemna* fronds. Since the morphology of maize roots had changed a lot after innoculation and most of the prefered niches observed had not yet formed when inoculating, the possibility can be excluded that more bacterial cells directly attached to the sites upon inoculation. As to *Lemna*, all parts of root surfaces and ventral sides of fronds should have the same opportunity to contact with FZB42. Therefore, one reason for the "niche phenomenon" could be that there were more exudates available around the niches. Another possible explanation is that the niches provided a relatively isolated microenvironment for bacteria to accommodate, propagate, and finally were transformed into a favored habitat.

Root colonization by rhizosphere bacteria is linked to biofilm formation (Watnick and Kolter, 1999; Bais *et al.*, 2004; Ramey *et al.*, 2004; Reva *et al.*, 2004). Obvious differences existed between biofilms formed by FZB42 on maize roots and those on *Lemna*. Highly structured biofilms formed on *Lemna* roots but not on maize roots, although microcolonies were often seen on the maize roots. This difference may result from the factors such as plant tissue, water availability, and nutrient richness, all which are known to affect biofilm formation strongly (Jones and Blaser, 2003; Kinsinger *et al.*, 2003; Ramey *et al.*, 2004; van de Mortel and Halverson, 2004) but were distinct between the two plant systems.

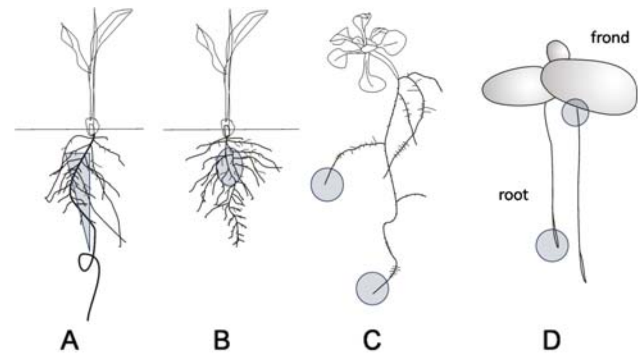

Fig. 4. Scheme of some preferred colonizing regions of maize roots, *Arabidopsis* roots and Lemna by *B. amyloliquefaciens* FZB42. The preferred regions were indicted by the triangle and the circles. Note the root architecture of maize roots in the gnotobiotic system (A) was different from that in the soil system (B). (C) represents the *Arabidopsis* grown in the gnotobiotic system and (D) represents the Lemna grown in the water.

It is not surprising that FZB42 can colonize roots of maize and *Arabidopsis*, since root colonization of these two kinds of plants by other PGPR like *Pseudomonas* has been reported. However, it is quite encouraging to find that FZB42 was also able to colonize *Lemna*, the smallest flower plant in the world, which is suitable for miniaturized micro-titer plate experiment. Moreover, it is shown that FZB42 is able to promote significantly the growth of *L. minor* (Idris *et al.*, 2007). The two facts suggested that *Lemna minor* is a potential tool for investigations of plant-microbe interaction, especially taking into consideration other advantages it has: a simple structure, a rapid propagation speed and the easiness to be inoculated, maintained and observed. Furthermore, *L. minor* contains rich chlorophyll throughout fronds and roots and therefore emits red autofluorescence upon UV-excitation, which has nicely contrasted green fluorescent bacteria and facilitated the monitoring of colonization by them.

Although conducting an investigation in soil system seems ideal to unveil plant-microbe relationship in natural environments, the laborious work required to separate plant roots from soil made it painful to be accepted as a routine method for preparing microscopic specimens. Therefore, it is still very useful to use a gnotobiotic system to study plant-microbial interaction (Kloepper and Schroth, 1981; Lifshitz *et al.*, 1987; Davies and Whitbread, 1989; Conn *et al.*, 1997). However, the findings obtained in a gnotobiotic system must be interpreted with enough prudence due to the limitations of artificial conditions or should be further investigated in soil system. In this work, the preferred colonization sites of maize and *Arabidopsis* were firstly found in the gnotobiotic system and then confirmed in the soil system, but some obvious differences were shown in the two systems. For example, the architecture of maize roots in soil was obviously different from that in the gnotobiotic system (Figs. 4A and 4B). In soil, maize roots grew slower, lacking of a distinctly thicker primary root but with more branching roots. Some of the lateral roots in the soil of approximately

1–3 cm below the root base were aslo colonized by FZB42, but by contrast the lateral roots in the gnotobiotic system were barely colonized. This difference probably because the bacteria were confined by the agar but more motile in soil, especially when watering. In addition, in the gnotobiotic system the tips of *Arabidopsis* roots were universally colonized by FZB42, which was, however, not a tipical phenominon in soil, perhaps due to the competition of indigenous soil microorganisms.

The low detection rate of fluorescent FZB42 cells in soil system strongly suggested that colonization of PGPR could be compromised by indigenous microorganisms. Accordingly, it would always be an important issue to ensure the competitive colonization ability of a PGPR strain when it is going to be applied in soil as biofertilizer or biocontrol agents. From the results obtained in this work, increasing the inoculum amount of a PGPR may be one possible solution.

In this article the colonization patterns of FZB42 on different plants were compared and the colonization development over time on *Lemna* was recorded. Root hairs were demonstrated to be a favored colonization site by the Gram-positive colonizers. Besides, the results suggested that *Lemna minor* could be a promising tool for studying plant-microbe interactions, particularly for investigations of bacterial biofilms formed on plants. This work will deepen our insight into the interactions between PGPR, especially the Gram-positive ones, and their host plants.

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