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Transcriptional Analysis of *Pseudomonas stutzeri* A1501 Associated with Host Rice

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Abstract

Pseudomonas stutzeri A1501, associative and endophytic nitrogen-fixing bacterium showed the capacity of colonization in the rice roots and considered as the good colonizer in the rice plant. The experiment was conducted to study the expression of genes potentiality relevant to the association of nitrogen fixing *Pseudomonas stutzeri* with host rice and reveal the molecular mechanism by which underlying interaction between bacteria and host rice. The bacteria were shown to be uniformly distributed on the rhizoplane of the root and the density of bacteria was found at the intercellular junction and micro colony developed on the surface of the epidermal cells and on the cellular junctions. Root exudates of rice were the major components of carbon and energy sources for bacteria. RT-PCR analyses of *pilK*, *metE*, *rpoN* and *fdhE* genes expression of *P. stutzeri* A1501 were performed at positive and negative (control) conditions. After 1 h, it was found that *pilK*, *metE* and *rpoN* transcription were increased 5.7, 6.4 and 3.4-fold, respectively, whereas in the *fdhE* gene has no expression. Consequently, after 4 h *pilk*, *fdhE*, *metE* and *rpoN* were decreased −1.9, −4.4, −0.2 and −0.8-fold, respectively. The gene *pilK*, expression was up-regulation after 1 h and down-regulation after 4 h that has twitching motility to convey the bacterial cell to point of attachment in to host plant. The gene expressions of the bacteria, *pilK*, *metE*, *rpoN* and *fdhE* were up- and down-regulated during the influence of root exudates which regulated the colonization of bacteria during plant-microbe interaction.

Keywords

1. Introduction

The establishment of beneficial interactions between microbes and their host plants, molecular signaling plays a

fundamental role between the partners of bacteria and host. Root exudates of host plant represent an important source of nutrients for microbes and act as signals influenced the ability of microbial strains to colonize the roots and to survive in the rhizosphere [1]. The rhizosphere is a natural microbial environment in which the activity of the microorganisms is maintained at a high level owing to a continuous release of organic substances by the plant roots. Simulation of microorganisms present in the rhizosphere seems to be due to the presence of organic compounds released by the roots and representing up to 20% plant dry weight [2]. This material includes flanked cells of root cap, mucellage and soluble and non soluble exudates, which may contain free amino acids, proteins, carbohydrstes, alcohols, vitamins, or hormones [3]. In cereals, it has been estimated that 4% - 29% of the photosynthetes can be transferred to the rhizosphere which is readily available for consumption for microbes [4]. Chemotactic response of bacteria towards amino acids, sugars and organic acids is a fundamental for bacterial behaviour both *in vitro* and *in situ* and represents, probably the first step in root colonization. The capacity of colonization in the rhizosphere of a host plant could be favoured and even increased several components of root exudates, which could induce some temporary modifications in the structure of bacterial lipopolysaccharide [5]. Microbial communities on plant selection are depending on the activation of specific patterns of gene expression in the microbe in response to molecular signals secreted from the plant. The plant attracts nitrogen-fixing bacteria to invade the cells in the root and provides them with carbohydrates as a food source while the bacteria reduce nitrous compounds in the soil that are then used by the plant [6]. The interactions of rhizosphere microbes with plants depend on the establishment of intimate associations between the two partners. Theses intimate cooperation between plant and bacteria display a high level of host specificity. There is also a growing body of evidence suggesting that many other associations between plants and microbes show similar degrees of specificity; different plant species, and even different cultivars of the same plant species, establish distinct microbial populations in their rhizosphere when grown in the same soil. The formation of these communities depends, at least in part, on the activation of specific programs of gene expression in the microbe in response to chemical signals secreted from the plant. These signaling processes have profound implications for the design of new strategies to promote those interactions of benefit to the host plant. So far, our knowledge of bacterial endophytes has come from the study of model PGPR, via genomic and functional analysis of candidate genes. As reviewed by Reinhold-Hurek and Hurek [7], several genomes of endophytes are now available, including *Azoarcus* sp. BH72 [8], *Klesiella pneumoniae* 342 [9], *Pseudomonas stutzeri*A1501 [10], *Gluconacetobacter diazotrophicus* Pal5 [11], *Azospirillum* sp. B510 [12] and *Herbaspirillum seropedicae* SmR1 [13]. The exploration of these genomes revealed a number of characteristics that are important for rhizosphere competence. Gene-encoding products with relevant functions linked to plant-microbe interactions were identified, such as nitrogen fixation, production of hormones and degradation of ethylene intermediate, iron transport, flagella, pili and quorum sensing that modulate functions related to rhizosphere competence and adaptation. The global approaches based on bacteria in to the plant havetargeted plant transcriptomics [14] [15], proteomics [16] and metabolomic approaches [17]. First results of "omics" approaches were reported with the model plant *A. thaliana*, followed by studies in rice (*Oryza sativa*), the model plant for monocot species, and more recently in maize. However, very little is known about the effects of root exudates on patterns of bacterial gene expressions involving the establishment of plant-microbe interactions. The objectives of this study are the expression of genes potentiality relevant to the association of nitrogen fixing *Pseudomonas stutzeri* with host rice and reveal the molecular mechanism by which underlying interaction between bacteria and host rice.

2. Materials and Methods

2.1. Bacterial Strains and Media

The wild-type bacterial strain of *P. stutzeri* A1501 used in this study. The media were used LB broth and minimal lactate medium broth (A15) for bacterial growth and; Hoagland's solution and semisolid nutrient solution for rice culture.

2.2. Sterilization and Germination of Rice Seeds, and Cultivation of Rice Seedlings

Rice seeds (approximately 15 g) were washed with sterile water twice. Seeds soaked by sterile water about 10 min, then used 75% ethanol to shake the seeds about 2 min. 50% NaClO was used to shake the seeds about 10 min and then used 100% NaClO to shake them about 20 min. 75% ethanol used to shake the seeds about 5 min.

Then the seeds were washed by sterile water about 5 - 6 times in the laminar flow cabinet. The seeds were put on the petri dish with 8 layers gauge and ensure that the seeds were not too dense. The petri dish was put on 30°C in incubation chamber to sprout of seeds and watered the seeds by sterile water when required (**Figure 1**).

For root exudates collection, twelve axenic rice plantlets (3 - 5 days) cultivated in a sterile hydrophonic system. The system consisted of a beaker with nutrient medium and gauze on which the seedlings were placed. Rice roots were kept in half-strength Hoagland's solution [18] containing 50 mg/l nalidixic acid to avoid development of microorganisms. Rice plants were put on the growth chamber with constant temperature of 26°C, 12-h day, and 70% relative humidity [19] for 14 days, adding nutrient solution periodically, under axenic conditions [2] (**Figure 2**). The components of Hoagland's solution were presented in **Table 1**.
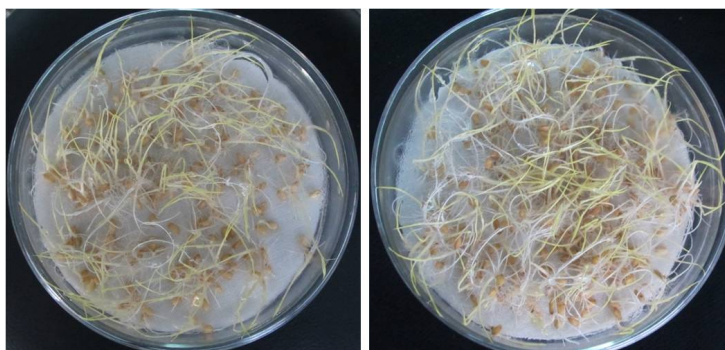

Figure 1. Germinated seedlings of rice.

Figure 2. Cultivation of rice in Hoagland solution (left) and semi-solid nutrient solution (right).

Table 1. Component of Hoagland's solution.

Components	Stock solution	mL stock solution/1 L
2 M KNO$_3$	202 g/L	2.5
2M Ca(NO$_3$)$_2$·4H$_2$O	236 g/0.5 L	2.5
Iron (Sprint 138 iron chelate)	15 g/L	1.5
2 M MgSO$_4$·7H$_2$O	493 g/L	1
1 M NH$_4$NO$_3$	80 g/L	1
Minors:		
H$_3$BO$_3$	2.86 g/L	1
MnCl$_2$·4H$_2$O	1.81 g/L	1
ZnSO$_4$·7H$_2$O	0.22 g/L	1
CuSO$_4$	0.051 g/L	1
H$_3$MoO$_4$·H$_2$O or	0.09 g/L	1
Na$_2$MoO$_4$·2H$_2$O	0.12 g/L	1
1 M KH$_2$PO$_4$ (pH to 6.0 with 3 M KOH)	136 g/L	0.5

And the components of semisolid nutrient solution (**Figure 2**) were 50 mg NaH_2PO_4, 88 mg K_2SO_4, 110 mg $CaCl_2$, 202 mg $MgSO_4 \cdot 7H_2O$, 1 mg $MnSO_4 \cdot H_2O$, 1 mg $FeSO_4$, 1 mg Na_2MoO_4, 150 mg $(NH_4)_2SO_4$ and agar 0.25% for 1 litter with pH 6.8.

2.3. Root Exudates Collection

Plants were transferred from the culture solution beaker to jars, where their roots were rinsed consecutively with distilled water and trap solution (1 mM NH_4Cl and 0.1 mM $CaCl_2$). Finally, the root mass was carefully immersed in a 1 L dark bottle containing trap solution for 24 h to collect root exudates. The pH was monitored every 8 h during the 24 h collection period and re-adjusted to 5.6 ± 0.2 with HCl or NaOH if necessary. After 24 h, the root and shoot tissue were saved for dry matter determination. The trap solution was evaporated to dryness using a rotary evaporator at 40°C, then re-suspended in 100% methanol and re-evaporated twice. Finally, the concentrated sample was suspended in 1.0 mL of 100% methanol and passed through a syringe-driven 0.22 mm membrane filter paper. The filtrate was completely evaporated in a centrifuge evaporator, re-suspended in 50 or 100 mL of dimethyl sulfoxide (DMSO). Control for the trap solution was subjected to the same procedure as for the sample. Other alternative method used as rice plants were placed at top of a paper bottle containing 250 mL of sterile deionised water. Bottle was aerated and placed in a dark room for 12 hours (modified [20]). Root exudates were filter-sterilized on Whatman no.42 filter paper and 0.22 µm filter Millipore and stored at −20°C until use.

2.4. Determination of Low Molecular Weight Organic Acid from Root Exudates by HPLC

The root exudation was poured into anion exchange (DEAE) column (1.2 cm × 8 cm, 9 ml DEAE32). The column was washed with 25 ml of deionised water and the organic acids were eluted with 15 ml of 1 MHCl. The exudate was dried with rotary evaporator (40°C). The residue was dissolved with 1 to 2 ml of the HPLC (high-performance liquid chromatography) mobile phase solution of 0.5% KH_2PO4. The mixture was filtered through 0.45 µm filter to remove suspended material prior to injection into the HPLC. Separation was conducted on a 150 × 4.6 mm reverse phase column (Extend Zorbax). Sample solutions (20 µl) were injected into the column with a flow rate of 0.6 ml·min^{-1} at 50°C and UV detection at 210 nm. 0.5% KH_2PO4 solution was used for isocratic elution. Identification of organic acids was performed by comparing retention times and absorption spectra with those of known standards of different organic acids.

2.5. Selection of Genes Responsible for Bacteria-Rice Interactions

The genes were selected from the *P. stutzeri* A1501 genome (**Table 2**) which has interactions and colonization capacity to the host plant [1] [10] [19]. For the identification of genes fragments, the primers were designed by using Perl Primer software. The selected primers mentioned at **Table 3**.

Table 2. List of selected genes used in this study.

Gene	Functions	Organism	Reference
fdhE	FdhE protein (Energy metabolism)	*P. stutzeri A1501*	This study
metE	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase (Amino acid biosynthesis and metaboloism)	,,	,,
pilK	methyltransferase PilK (Twitching motility)	,,	,,
rpoN	RNA polymerase sigma-54 factor (amino acid biosynthesis)	,,	,,

Table 3. List of primers used in this study.

Gene name	Primer	Thermo Tm
fdhE	F: ACCAAGAAGCTCGACTACC	54.5°C
	R: GCCAATTCGAGATAGAGCTG	57.7°C
metE	F: TTCGATACCAACTACCACTATCTG	57.4°C
	R: GATCTGTACCCACTCGACTC	54.5°C
pilK	F: CTGTTGATCTACTTCCGCC	55.8°C
	R: GAACCTGATCATTGGCAACC	60.3°C
rpoN	F: TGGCTGGAGAAGAAGTATTTGAG	55.0°C
	R: AACGAAACACTGATGAAGGTC	55.0°C

2.6. Establishment of Bacterium into Root Exudates

Overnight 10 ml bacterial culture of *Pseudomonas stutzeri* was inoculated in to the 50 ml of A15 medium and then added 40 ml root exudates in the conical flask, mixed it thoroughly and shaking the flask by the shaker with 150 rpm. Bacterial samples were collected after 1 hour and 4 hours. At the same time control sample was run and followed the same procedures and then all collected samples of bacteria were stored at −80˚C.

2.7. Colonization of Rice Roots by *Pseudomonas stutzeri* Strain

Three days aged rice seedlings of similar root length were selected and inoculated by 20 mL of a bacterial suspension. This bacterial suspension was made by suspending bacteria in sterile water to OD 600 of 0.10, then diluting 1:20 with sterile water, giving a bacterial cell count of about 4×10^6 cells per mL. Seedlings were then placed into the appropriate plant growth chamber (**Figure 3**). The seedlings were examined at 14 days after inoculation to determine the success of the association of the bacteria with the root and the level of invasion into root tissues. To obtain cross sections of the root samples, fresh root segments were fixed in 3.6% formaldehyde solution in phosphate buffer (0.5 M, pH 7.0) for 2 days, and then rinsed three times with phosphate buffer. Fixed root segments were embedded in 5% agarose and then sectioned using a Lancer Vibratome Series 1000.

2.8. Scanning Electron Microscopic Study

Seedlings of 14 days inoculation were randomly selected for scanning electron microscopic examination. Tissue samples from inoculated seedling roots of rice were fixed in 2% glutaraldehyde (made up in 0.1 Mcacodylate buffer) in the refrigerator (8˚C) for 1.5 hr. Samples were washed two times in the same buffer for 10 min, post-fixed in 1% OsO_4 for 4 hrs, and dehydrated as follows: 30%, 50%, 70%, 85%, and 95% ethanol for 15 min; 100% ethanol, two times for 15 min each. For the scanning electron microscopy, the Critical Point Drying (CPD) method, sputter coating, and an Amray 1600 scanning electron microscope operating at 20 kv were used. Root vascular systems and rhizobacteria colonization patterns were observed by SEM.

2.9. Isolation of RNA

The bacterial samples which were stored at −80˚C took it out and transferred to a 1.5 micro centrifuge tubes and it were centrifuged for 2 minutes at $14000 \times g$. The supernatant removed carefully and the pillet left as dry as possible. The pillet was re-suspended in 100 µl fleshy prepared TE containing lisozyme and mixed it gently. The re-suspended pillet was incubated at room temperature at 3 - 5 minutes and added 3 - 5 µl of RNA lysis buffer. It was added 350 µl RNA dilution buffer and mixed it by inversion and added 200 µl ethanol (95%) to the clear lysate; and mixed by pipeting 3 - 4 times, mixture was transferred to the spin column assembly; and centrifuge at 12,000 - 14,000 × g for one minute. The spin basket was taken from the spin column assembly, discarded spin basket in the collection tube and put back into the collection tube, it was added 600 µl of RNA wash solution to the spin column assembly and centrifuged at 12000 - 14000 × g for 1 min. The collection tube did the empty as before and placed it in a rack. It was incubated for 20˚C - 25˚C. After this incubation, 200 µl of DNase stop solution was added and centrifuged at 12000 - 14000 × g for 1 min. Then 600 µl of RNA wash solution with ethanol was added and centrifuged at 12000 - 14000 × g for 1 min. The collection tube did the empty and added 250 µl RNA wash solution and centrifuged at high speed for 2 minutes. The spin basket was transferred from the
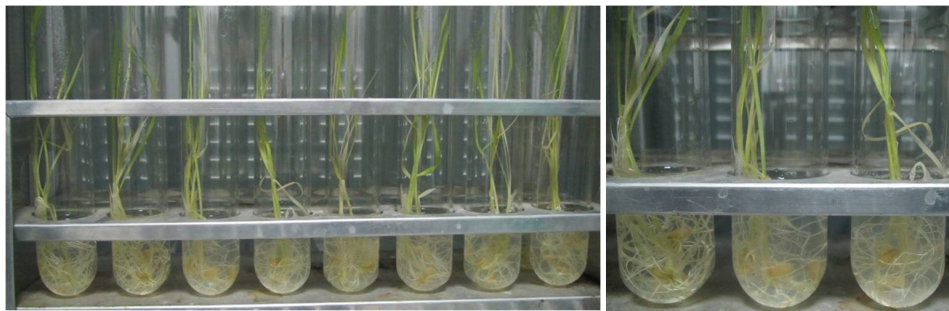

Figure 3. Inoculation of rice roots by *Pseudomonas stutzeri*.

collection tube to the elution tube and added 100 µl nuclease free water to the membrane. The spin basket assemblies placed in the centrifuge with lids of the elution tube facing out and centrifuged at 12000 - 14000 × g for 1 min. The collected RNA was done gel electrophoresis (**Figure 4**). The spin basket was removed and discarded. The elusion tube was capped containing the purified RNA and stored at −80˚C.

2.10. Conversion to cDNA

A control reaction without reverse transcriptase to be examined the DNA contamination in the sample. RNA sample and primer d (T) 23 VN mixed in two sterile Rnase-free micro centrifuge tubes. The mixture total volume were 8 µl where total RNA was 1 - 6 µl (10 pg - 1 µg), d (T) 23 VN (50 µM) was 2 µl and nuclease free H_2O was variable. The RNA was denatured for 5 minutes at 70˚C, spinned briefly and put promptly on ice. Then the components of 10 µl M-MuLV Reaction Mix and 2 µl M-MuLV Enzyme Mix were added to one tube. For the negative control, 10 µl M-MuLV Reaction Mix and 2 µl H_2O were contained another tube. 20 µl cDNA synthesis reactions were incubated at 80˚C for one hour. For the enzyme inactivation, reaction was done at 80˚C for 5 minutes. The reactions were dilute to 50 µl with 30 µl H_2O for PCR. The cDNA product was stored at −20˚C.

2.11. Reverse Transcription (RT)-PCR

The PCR reaction was carried out with 70 ng DNA as template 1 µM forward and reverse primers. The reaction was cycled 25 times with a 55˚C annealing temperature and a 2 - 4 min extension time at 72˚C, depending on the size of the expected product. The same RNA was used in the PCR reaction as a negative control to test for genomic contamination.

3. Results

3.1. Organic Acids of Root Exudates

Rice root exudates were provided to HPLC for determination of two organic acids as acetic acid and tartaric acid and it found that the concentrations were 19.84 and 324.12 µmol·g^{-1} dry weight roots, respectively (**Table 4**). Generally, amino acids, monosaccharides, and organic acids were considered as the major exudates compounds to evaluate the importance of these components for proliferation and root colonization in respect of plant-bacteria interaction.

Figure 4. Isolation of total RNA.

Table 4. Low molecular weight organic acid concentrations of rice root exudates (µmol·g^{-1} dry weight root).

Name of low molecular acids	µmol·g^{-1} dry weight root
Acetic acid	19.84
Tartaric acid	324.12

3.2. Colonization of Rice Roots by *P. stutzeri* A1501

Establishment of endophytic colonization of rice plants by *P. stutzeri* A1501 was demonstrated with a gnotobiotic system, using axenic rice plantlets. Interaction between rice roots from 14 dpi (days post inoculation) plantlets and *P. stutzeri* obtained under gnotobiotic conditions was observed by SEM. Rod-shaped bacteria were observed, mostly in pairs or in small tri-dimensional microcolonies covering the root surface (**Figure 5(C)**). Bacteria were particularly accumulated in the basal portion of the root hair, forming micro aggregates on disrupted areas of the epidermal surface (**Figures 5(D)-(E)**). Such damage of the epidermal surface on heavily colonized areas suggested an active invasion mechanism, probably associated to a high density of bacterial population as compared to the non infected control plant (**Figure 5(A)**). The Bacteria were also found in disrupted zones of the mucigel (**Figure 5(F)**) in to the cells.
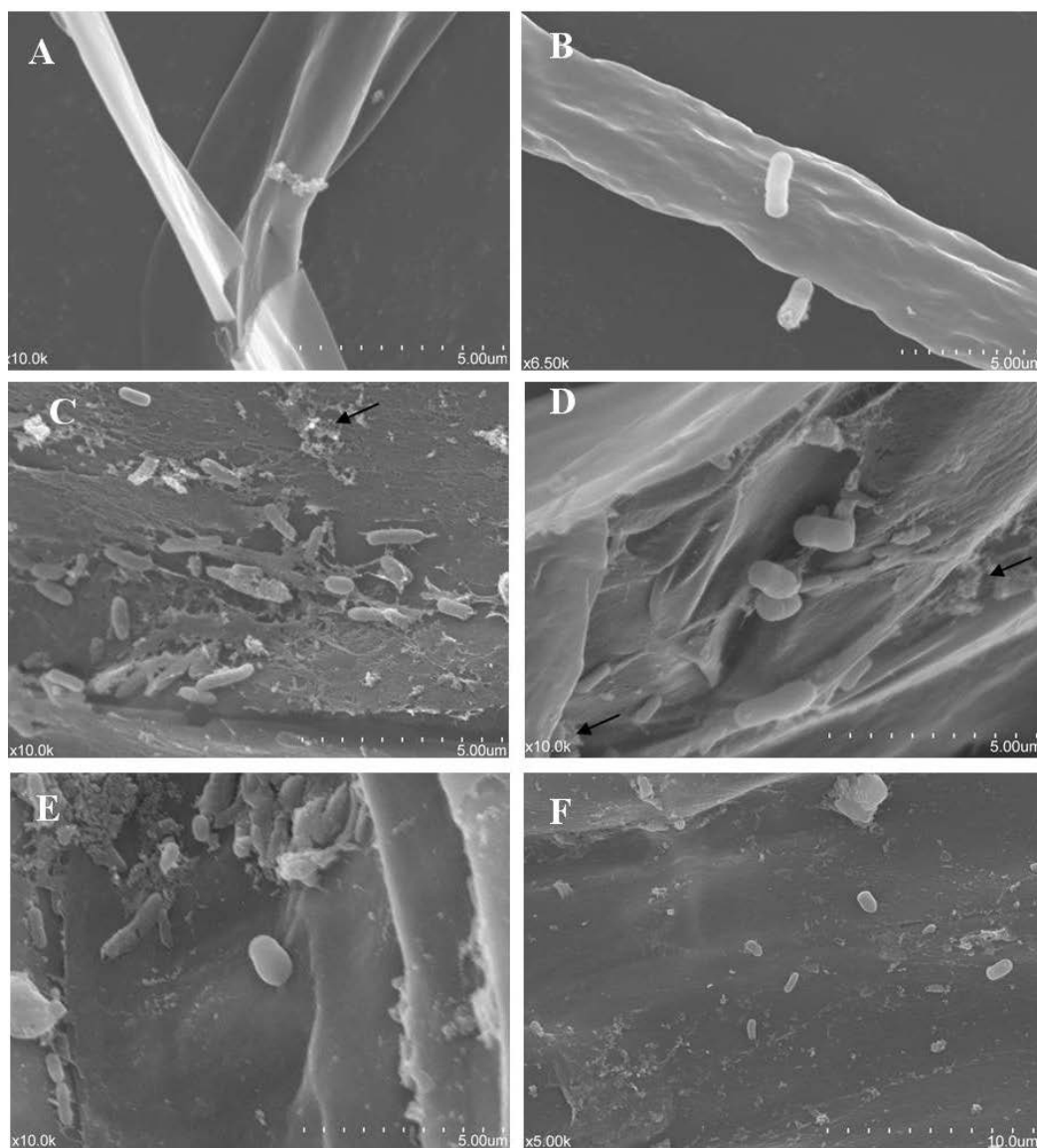

Figure 5. SEM study of root surface in rice seedlings of 14 days after inoculation of *P. stutzeri*. (A) Root surface of non-inoculated seedlings free of bacteria; (B) Attachment of the bacteria in root hair; (C) Root surface covered by endophytic bacteria, which showed adhesion filaments (arrow head) among them, and with the rhizoplane; (D) *P. stutzeri* micro colony consistently distributed on the surface of root; (E) High magnification of bacteria between the mucigel coat and the secondary roots; (F) Bacteria found in disrupted zones of the mucigel covering the grooves formed by the junctions among the epidermal cells.

3.3. Gene Expressions

RT-PCR analyses of *pilK*, *metE*, *rpoN* and *fdhE* genes expression of *P. stutzeri* A1501 were performed A1501 with positive (with root exudates) and negative (control) conditions. After 1h, it was found that *pilK*, *metE* and *rpoN* transcription were increased 5.7, 6.4 and 3.4-fold (**Table 5**), respectively, whereas in the *fdhE* gene has no expression. Consequently, after 4 h *pilk*, *fdhE*, *metE* and *rpoN* were decreased −1.9, −4.4, −0.2 and −0.8-fold, respectively. After 1 h it was noted that genes expression were up regulated, there were found no down regulated expression but after 4 h all genes expression were down regulated. These results indicated that up-regulated and down-regulated genes were important for bacterial root colonization. The relative expression levels of these genes were presented in **Figure 6**.

4. Discussion

4.1. Organic Acids of Root Exudates

The low molecular organic acids were tested in to the root exudates like acetic acid and tartaric acid; it was found that the concentrations were 19.84 and 324.12 µmol g^{-1} dry weight root, respectively (**Table 4**). These results had a similarity of low molecular weight organic acid concentrations of root exudates in acetic acid from *Solanum nigrum* L. was 36.56, and *Solanum lycopersicum* L. was 28.23; in tartaric *Solanum nigrum* L. was 280.12 and *Solanum lycopersicum* L. was 265.41 [21]. It was indicated that the quality of the root exudates remained unchanged in to the studied samples.

4.2. Colonization of Rice Roots by *P. stutzeri* A1501

The Gramineae rice (*Oryza sativa* L.) was chosen as a plant model in this study with the aim of investigating nitrogen-fixing *P. stutzeri* A1501 ability to colonize rice roots. A1501 is a versatile soil bacterium which has capacity of nitrogen fixation and endophytic association with rice plants. This association of A1501 with plants is a promising model system for the study of plant-microbe-soil interactions in the rhizosphere [22]. In the present

Table 5. Change of expression of genes during influence with root exudates.

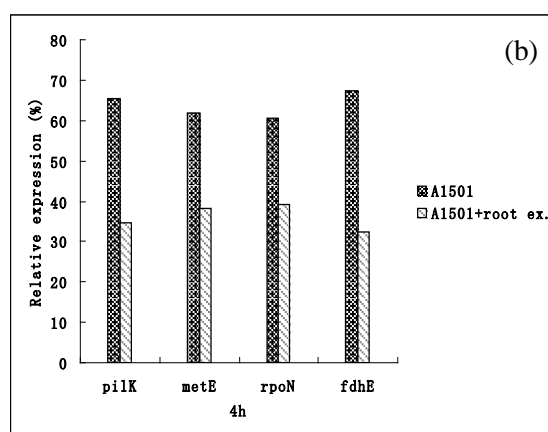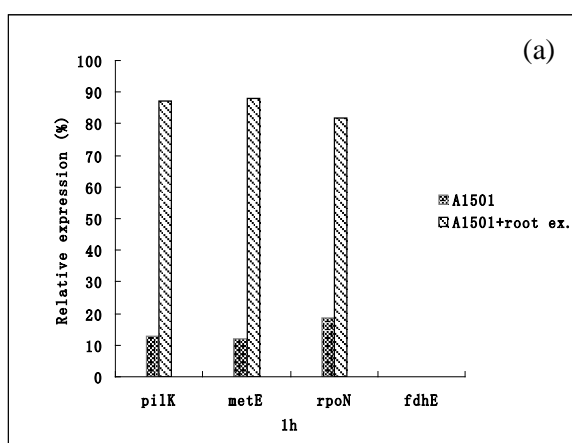| Gene	Change (*n*-fold)	
	1 hour	4 hours
pilK	5.7	−1.9
metE	6.4	−0.2
rpoN	3.4	−0.8
fdhE	-	−4.4

Figure 6. RT-PCR analysis of the *P. stutzeri pilK*, *metE*, *rpoN* and *fdhE* genes expression after 1 h (a) and 4 h (b) induction of root exudates.

study, it was demonstrated that *P. stutzeri* can invade rice roots, spreading rapidly and systematically through the root tissues and colonize rice roots. According to the results, endophytic *P. stutzeri* A1501bacteria were seem to be uniformly distributed on the rhizoplane of the root (**Figure 5(C)**); although, it identified the greatest density of bacteria at the intercellular junction (**Figure 5(E)**). This finding is probably due to the fact that inter-cellular regions represent more space and opportunity for the movement of endophytes; besides, very probably the mucilaginous layer, which covers the epidermis of the root, has a lower tension in these regions [23]. Previous reports indicated that, at the intercellular regions, there was an important increase in the concentration of carbon as a source of energy, thus explaining the preference of bacteria for this part of the root [24]. It has been suggested that microcolonies could develop on the surface of the epidermal cells and on the cellular junctions [23]. In addition, these results distinguished consecutive stages of *P. stutzeri* invasion in rice roots. The initial step consisted of the attachment of bacteria onto epidermal cells of the root surface, where root hair zone represent one of the major sites of primary colonization, mainly on the basal region of emerging hairs. At this stage, filamentous structures were observed in the microscopic analysis, apparently promoting bacteria-to-bacteria and bacteria-to-plant surface cross-linking (**Figure 5(C)** & **Figure 5(D)**). Similar observations have been described in other endophytic interactions, resembling bacterial polysaccharide-like structures that may point to crucial stages of ligand-receptor apparatuses with significant potential to modulate the plant-bacteria interactions [25]-[27]. As observed in different plant-bacteria associations [28], surface polysaccharides such as exopoly-saccharides and lipopolysaccharides (LPS) were involved in the colonization of roots. In a Tn5 mutant of *A. brasilense* affected in the biosynthesis ofd TDP-rhamnose, LPS composition was modified and resulted in impaired attachment of the mutant to maize roots and reduced root colonization [29]. Additional studies recently were undertaken on *H. serepedicae* by Balsanelli *et al*. [30] confirmed these data. In addition to LPS, a major outer membraneprotein from *A. brasilense* strain Cd was purified and shown, by in vitro adhesion assays, to bind to roots of wheat, corn and sorghum seedlings [31]. In addition to its involvement in root adsorption, this protein acted on cell aggregation of *Azospirillum*. Results indicated that the presence of many filaments cross-linking the endophytic bacteria and with the rhizoplane, suggesting a structural compatibility between endophytes and the vegetal cell wall. Evidence of a specific interaction of cyanobacteria with plant roots has been found with *Nostoc* 259B. This bacterium specifically interacts with wheat roots through a sequence of three neutral sugars and glucuronic acid; this interaction allows for an efficient colonization and exclusion of other colonizing cyanobacteria [32]. It were also identified high densities of endophytic bacteria in emerging zones from the lateral roots and, particularly, in the basal parts. This finding agrees with other studies indicating that these parts of the roots are highly susceptible to disruption, causing the release of endophytes [33]. Several investigators stated that the root colonization involves migration of bacteria towards the plantroots, adsorption and anchoring onto the root system, as well as microbial proliferation and the formation of microcolony/biofilm structures at the surface of roots [7] [34]-[37] that is consistent in our study.

4.3. The Regulation of Gene Expression by Root Exudates

In the present study, results indicated that *P. stutzeri* A1501, the expression of the number of genes were up regulated and down regulated in response to root exudates after 1h and 4h when compared with the control (**Table 5** & **Figure 6**). The genes of known function whose expression were up-regulated after 1h in response to the root exudates were involved in aspects of metabolism such as aromatic compound catabolism, energy generation, and amino acid biosynthesis (**Table 2**). This finding is more expectable because organic acids, amino acids and monosaccharides is the major components of root exudates [38] that contribute to the influence of genes that may be important for plant-bacteria interactions. Lugtenberg *et al*. [38] showed that utilization of organic acid by *Pseudomonas* is the nutritional basis conducting the ability of colonization of this organism in tomato roots. The organic acids of root exudates are citric, acetic, tartaric, malic, lactic, and succinic acid which have the major exudates carbon sources helped to grow high bacterial population. In addition, the up-regulation of genes of bacteria involved likely to be colonization process [39].

Study on gene expressions, the gene *pilK*, up-regulated after 1h and down-regulated after 4 h (**Table 5**), involved in twitching motility, encoding methyltransferase was a unique type of locomotion governing the ability of colonization of plant. This result consistent with *P. aeruginosa*, type 4 pili mediated the locomotion and involved in colonization of plant by associative nitrogen fixing of *Azoarcus* [40] [41]. Three genes *metE*, involved in amino acid biosynthesis and metabolism, encoding 5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase, *rpoN* involved in amino acid biosynthesis, encoding RNA polymerase sigma-54 factor and

fdhE involved in energy metabolism, encoding FdhE protein; these effects are unknown in root exudates for root colonization. These results consistent with Mark *et al.* [1] described that *pilK* and *metE* acted as twitching motility and; amino acid biosynthesis and metabolism for these reasons the effects of the genes are unknown. It was found that the diazotrophic employ an array of distinct mechanisms to colonize successfully the plant roots and compete with other soil microorganisms. Among the mechanisms, chemotaxis resulting from the presence of flagella allowed the bacteria to get into contact with roots, together with type IV pili and twitching motility. Twitching motility was based on a mechanism which includes pilus extrusion, surface attachment of the pilus tip, and pilus retraction to convey the bacterial cell to the point of attachment [42]. In *Azoarcus*, type IV pili were shown to be involved in adherence to plant surfaces and the *pilA*, *pilB* and *pilT* genes were essential for root-surface colonization and for infection of plant tissues in rice [8] [41] [42]. The expressions of the genes after 1 h and 4 h, *metE* and *rpoN* were up-regulated and down-regulated, respectively but *fdhE* expression after 4 h was down-regulated and in 1 h, there had no expression. It was noted that the identification of genes that are both up-and down-regulated irrespective of the level of expression in minimal lacted medium because it was plausible that down regulated of certain genes required for successful colonization. In Sugar beet, some genes expression in root exudates were down-regulated that were indicated as successful plant-microbe interaction [1].

5. Conclusion

It is concluded that *P. stutzeri* A1501 showed the ability of successful colonization of rice roots. The bacteria were uniformly distributed on the rhizoplane of the root and the density of bacteria was found at the intercellular junction. The genes, with known functions expression were up- and down-regulated, which indicated the plant-microbe interaction in bacterial colonization in the rhizosphere. Being influenced in root exudates after 1 h, it was found that *pilK*, *metE* and *rpoN* transcription were increased 5.7, 6.4 and 3.4-fold, respectively, whereas in the *fdhE* gene had no expression. Consequently, after 4 h *pilk*, *fdhE*, *metE* and *rpoN* were decreased −1.9, −4.4, −0.2 and −0.8-fold, respectively. The gene *pilK*, expression was up-regulated after 1 h and down-regulated after 4 h that had twitching motility to convey the bacterial cell to point of attachment in to host plant.

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