Beneficial effects of inoculated nitrogen-fixing bacteria on rice

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Nitrogen fixation in two rice varieties—Super Basmati and Basmati 385—was studied by the acetylene reduction (AR) assay and the ^{15}N dilution technique. In the roots and submerged shoots of field-grown plants of both varieties, a higher acetylene reduction activity and population of diazotrophs were detected at the grain-filling stage than at the panicle initiation stage. Nitrogen-fixing bacterial isolates from rice and other plants were differentiated by using ERIC- and BOX-PCR. A unique banding pattern of the PCR products was obtained from each isolate, confirming that distinct bacterial strains have been isolated. The beneficial effects of the bacterial inoculations were studied on rice plants grown in pots filled with nonsterile soil. Super Basmati showed a better response (plant biomass and grain yield) to bacterial inoculations than did Basmati 385. Maximum fixation (59%Ndfa) in plants of Super Basmati was obtained when *Azospirillum lipoferum* N-4 was used as inoculum, whereas *Herbaspirillum* RR8 showed maximum fixation (39%Ndfa) in Basmati 385.

High interest in nitrogen-fixing bacteria associated with rice and other graminaceous plants has been shown during the past few years to minimize the use of expensive chemical fertilizers in agriculture. One of the reasons for considering rice as a more promising candidate for research on nitrogen fixation is the observation that nitrogen status of wetlands under rice cultivation is sustained due to the activity of nitrogen-fixing bacteria that find the environment favorable for their growth in the submerged rice system (App et al 1980, Ladha et al 1993, Roger and Ladha 1992). Using the acetylene reduction method, nitrogen fixation in rice roots was for the first time detected by Yoshida and Ancajas (1971). Nitrogen balance studies (App et al 1980, Ventura and Watanabe 1983) and long-term fertility studies (Watanabe et al 1981) confirmed nitrogen gains in wetland rice soils, mostly from N_2 fixation. Nitrogen fixation associated with wetland rice includes N_2 fixation that occurs on or in the leaf sheaths, the basal portion of the shoot, and the root zone (Watanabe et al 1981, Yoshida and Rinaudo 1982). A large percentage of the aerobic heterotrophic bacteria in wetland rice roots are N_2-fixing (Watanabe et al 1979).

From rice and other members of the family Gramineae, several diazotrophic bacteria belonging to the genera *Azospirillum*, *Herbaspirillum*, *Enterobacter, Acetobacter, Azoarcus*, *Alcaligenes*, *Pseudomonas, Zoogloea*, and others, have been isolated (Baldani et al 1986, 1996, Barraquio et al 1983, Bilal and Malik 1987, Cavalcante and Dobereiner 1988, Dobereiner 1992, Hassan et al 1998, Khammas et al 1989, Ladha et al 1983, 1987, Malik et al 1994, Reinhold-Hurek et al 1993, Reinhold et al 1987, Tarrand et al 1978, Watanabe et al 1979, You et al 1991). Some newly identified N_2-fixing genera such as *Acetobacter*, *Azoarcus*, and *Herbaspirillum* have been called endophytes because of their occurrence mainly within plant tissues (James and Olivares 1998). Endophytic diazotrophs have been isolated from several grasses in which significant biological nitrogen fixation has been demonstrated, particularly Brazilian sugarcane varieties, but also in rice, maize, and sorghum. Endophytic diazotrophs have been linked with the high N_2 fixation (up to 80% of the N incorporated) reported particularly in sugarcane where the bacteria are found in high numbers

(Boddey et al 1991, 1995a,b, Dobereiner et al 1995a,b). Endophytic diazotrophs colonize the interior rather than the surface of the plants and hence are better placed to exploit carbon substrates supplied by the plants and avail a low pO_2 environment necessary for the expression and operation of nitrogenase (Baldani et al 1997, Gallon 1992, James and Olivares 1998, Patriquin et al 1983).

Incorporation of biologically fixed N and enhancement of crop yields of cereals by inoculation with nitrogen-fixing bacteria have been observed in many field experiments (Baldani et al 1983, 1987, Boddey et al 1986, 1991, Dobereiner et al 1993, Kapulnik et al 1981a,b, 1987, Lima et al 1987, Sarig et al 1984, Urquiaga et al 1992). Yield increases obtained in inoculated plants, however, have been attributed to biological N fixation and also to the production of plant growth substances by the root-colonizing bacteria. Increases in yields of wheat by inoculation with *Azospirillum* strain Cd have been consistent in field experiments, mainly in Israel and other semiarid regions (Kapulnik et al 1981a,b, 1987, Okon 1985, Smith et al 1984). In two cultivars of grain sorghum, inoculation with three *Azospirillum* strains, but not with a strain of *Herbaspirillum,* led to an increase in plant dry weight and total N in grain (Pereira et al 1989). Pereira et al (1989) have also reported that, in rice, *A. lipoferum* Al 121 and *A. brasilense* sp 245, isolated from rice and wheat roots, respectively, when used as inoculum, did not affect plant growth or grain yield. A field study carried out by Ali et al (1998), in which N_2-fixing bacteria were used as inoculum for rice, indicated that a low input of chemical N fertilizer was useful for increasing rice yield, fertilizer-N-use efficiency, and BNF in rice grown under flooded soil conditions.

To reduce dependency on the use of chemical fertilizers, research efforts have also attempted to identify rice genotypes that naturally support high nitrogen-fixing activity or show the best response to inoculations with diazotrophs. Several studies indicate that significant genotypic differences exist in ability to support nitrogen fixation (App et al 1986, Hirota et al 1978, Ladha et al 1987, 1988, Lee et al 1977, Shrestha and Ladha 1996, Yoshida and Ancajas 1971). Ladha et al (1986) have suggested that these genotypic differences may be due to specificity of plant-bacterial associations, differences in root exudation, and gaseous diffusion efficiency. Therefore, rice genotypes showing high N_2-fixing activity in the presence of indigenous populations of diazotrophs or showing the best response to bacterial inoculations may be selected on the basis of their lower requirement for chemical N input.

In the present study, ARA activity was measured in two field-grown Basmati rice varieties to detect the presence of diazotrophic bacteria. The morphologically highly related bacterial isolates were differentiated by using BOX- and enterobacterial repetitive intergenic consensus (ERIC)-PCR (polymerase chain reaction). The main objective of the study was to evaluate the performance of diazotrophic strains isolated from rice and other grasses when used as inoculum for two rice varieties, Super Basmati and Basmati 385.

Materials and methods

Detecting N_2-fixing activity in roots and submerged shoots of rice

The acetylene reduction assay was used to detect the presence of nitrogen-fixing activity associated with rice varieties Super Basmati and Basmati 385 growing in farmers' fields at Ghakar, Gujranwala District, Pakistan. Roots and shoots of rice submerged under water (5-cm pieces of shoots near the base) were collected from the field and washed with sterile water. Approximately 5 g of fresh weight root or shoot samples were transferred to 16-mL-capacity glass tubes and incubated in 10% acetylene at 30 °C for 24 h. Triplicate samples of

roots and shoot were used for ARA. The tubes with plant material (roots and shoots) but without C_2H_2 were used as a control. Another set of tubes containing only 10% C_2H_2 and no plant material was also used as a control. Ethylene production was measured on a gas chromatograph (Gasukuro Kogyo model 370) using a Porapak N column. Root and shoot samples were dried in an oven at 70 °C to a constant weight.

Detecting bacterial populations in roots and submerged shoots of rice

The roots and lower parts of shoots submerged under water were collected from the field and thoroughly washed with sterile water to remove adhering soil. Roots or shoots (1 g) were homogenized in sterile water and serial dilutions were prepared. These dilutions were used to inoculate semisolid combined carbon medium (CCM; Rennie 1981) and incubated at 30 °C for 48 h. Acetylene (10% V/V) was injected to the vials showing bacterial growth and acetylene reduction activity was measured to determine most probable number (MPN) counts on a gas chromatograph (Gasukuro Kogyo model 370) using a Porapak N column. For plate counts, the serial dilutions prepared from roots and shoots of rice were also used to inoculate Luria broth (LB) plates.

Characterizing bacteria by ERIC- and BOX-PCR

Bacterial cells were grown in LB for 24 h at 30 °C and centrifuged at 13,000 rpm for 5 min. The cell pellets from 1.5-mL cultures were washed with TE buffer (10 mM TRIS Cl, 1 mM EDTA, pH 8) and then dissolved in 200 µL of TE. Cell lysis was obtained at 37 °C for 30 min with lysozyme (2 mg mL^{-1}, final concentration) and by using sodium dodecyl sulfate (SDS) (1%). The lysate was extracted twice with phenol/chloroform followed by two extractions with chloroform/isoamyl alcohol (24:1). After adding 1/10 volume of sodium acetate (3 M, pH 5.2) and 0.5 volume of isopropanol, the supernatant was incubated at –20 °C for 30 min. The nucleic acids were then precipitated by centrifugation at 13,000 rpm for 20 min and the pellets were washed with 70% ethanol before drying under a vacuum. The nucleic acid pellets were then dissolved in TE and the DNA concentration of the samples was adjusted between 50 and 100 ng μL^{-1}. One µL of this DNA solution was used as a template for PCR. The PCR reaction was carried out in a 25-µL volume containing 50 pmole (1 µL) for each primer, 1.25 mM deoxynucleoside triphosphate, and 2 units of *AmpliTaq* polymerase. For ERIC primers (ERIC1R 5'-ATGTAAGCTCCTGGGGATTCAC, ERIC2-5' AAGTAAGTGACTGGGGTGAGCG; Versalovic et al 1991), the first cycle at 95 °C for 7 min was followed by 30 cycles at 94 °C for 1 min, at 52 °C for 1 min and at 65 °C for 8 min, 1 cycle at 65 °C for 16 min, and a final soak at 4 °C. For BOX-PCR (BOX primer 5'-CTACGGCAAGGCGACGCTGACG-3'; Louws et al 1994), the following cycles were used: 1 cycle at 95 °C for 7 min; 30 cycles at 90 °C for 30 s, at 53 °C for 1 min, and at 65 °C for 8 min; one cycle at 65 °C for 16 min and a final soak at 4 °C.

After the reaction, 10 µL of the ERIC- and BOX-PCR products were used for electrophoresis on 1.5% agarose gels. The gels were stained with ethidium bromide to visualize the banding patterns of PCR products and then the gels were photographed.

For the analysis of the bacterial fingerprints, product moment-correlations and Dice coefficients software programs were used.

Inoculation of rice with bacterial isolates

For this experiment, rice plants (varieties Super Basmati and Basmati 385) were grown in

nonsterilized soil filled into 25-cm diameter fiberglass pots. To fill each pot, 10 kg of air-dried soil was used to make about a 25-cm depth in the pot. The paddy soil used in this experiment was a sandy loam collected from the top 30 cm of a rice-growing area of the Institute and it had an electrical conductivity of saturation extract (ECe) 2.5 dS m^{-1}, pH 8.2, organic matter 0.60%, and total N 0.059%. The pots were kept flooded with canal water until 2 wk before the rice harvest.

In the 2nd week of July, 5-wk-old rice seedlings were transplanted. Ten seedlings were grown in each pot. At the time of transplanting, the seedlings were inoculated by keeping the root system submerged in liquid bacterial cultures (approximately 10^9 cells mL^{-1}) for 30 min. Two weeks after transplanting, ^{15}N-labeled ammonium sulfate of 5 atom % excess (0.72 g pot^{-1}) was added as a tracer to quantify nitrogen fixation. The plants were harvested at maturity and dried in an oven at 70 °C until no change in weight was noted. The dried plant samples were analyzed for ^{15}N excess on a double-inlet Mass Spectrometer (MAT GD 150). Uninoculated plants were used as a nonfixing reference to estimate ^{15}N dilution.

Results and discussion

With the ARA technique, nitrogen-fixing activity was detected in roots as well as in submerged shoots of field-grown rice varieties Super Basmati and Basmati 385 (Table 1). This indicates colonization of rice roots as well as shoots by diazotrophic bacteria. The presence of nitrogen-fixing activity in lower parts of the shoot, in addition to the roots, has also been reported by other researchers (Watanabe et al 1981, Yoshida and Rinaudo 1982). In the aerial parts of rice plants above the level of floodwater, no AR activity was found. In the roots and submerged shoots of both varieties, more AR activity was detected at the grain-filling stage than at the panicle initiation stage. Relatively higher AR activity was detected in roots than in submerged shoots of both varieties at the panicle initiation stage. At the grain-filling stage, more activity was found in submerged shoots than in roots. The population of diazotrophs as estimated by AR assay-based MPN at the grain-filling stage was also higher in submerged shoots than in roots (Table 2). The high AR activity detected in shoots and the presence of diazotrophs in high numbers may be of practical significance as the isolation and use of these bacteria as biofertilizers along with root-colonizing bacteria may enhance the efficiency of such inocula.

We used the PCR technique with primers corresponding to repetitive DNA sequences, namely, the 124–127-bp ERIC and 154-bp BOX elements to generate specific DNA fingerprints of the nitrogen-fixing isolates from rice and other hosts with two objectives. The first was to differentiate morphologically highly related *Azospirillum* isolates and to compare these isolates with standard strains of this genus. The second was to make available for comparisons specific DNA fingerprints of bacterial strains for use in future ecological studies requiring reisolations of the inoculated bacteria. Five locally isolated nitrogen-fixing and plant growth hormone-producing strains—*Azospirillum* N-4, *Azospirillum lipoferum* ER-20, *Azoarcus* K-1, *Pseudomonas* 96-51, and *Zoogloea* Ky-1—were used for ERIC- and BOX-PCR fingerprinting. Banding patterns generated after amplification of genomic DNA with BOX and ERIC primers are given in Figures 1 and 2, respectively. PCR products with both types of primers were obtained from all the strains tested. Several strong and weak bands (some of the weak bands were visible on the gel but are not clearly visible in the photographs) were observed with both types of primers. Each strain showed its own specific banding pattern of PCR products. These unique banding patterns could be useful in future field experiments for comparisons with those obtained from reisolates for confirmation as the

respective inoculated strain. The BOX and ERIC-PCR patterns of morphologically highly related *Azospirillum lipoferum* strains N-4 and ER-20, isolated from rice and wheat, respectively, were different from each other. The amplified products of these two strains ranged from less than 450 bp to more than 4 kb with BOX primers and from less than 400 bp to more than 3.5 kb with ERIC primers. With BOX primers, 18 PCR bands were obtained from the template DNA of *A. lipoferum* N-4 and 19 PCR bands were obtained when the template of *A. lipoferum* ER-20 was used for amplifications. In *A. lipoferum* N-4, the size of the products ranged from about 600 bp to 2 kb, whereas in *A. lipoferum* ER-20, the size ranged from about 410 bp to 4 kb. When ERIC primers were used for amplification of DNA from *A. lipoferum* N-4, four strong and six weak bands of PCR products ranging from about 370 bp to 1.4 kb were visible on the gel. With these primers, four strong and seven weak bands were formed from the template of *A. lipoferum* ER-20 and the size of the bands ranged from about 550 bp to 3.5 kb. These different banding patterns of ERIC- and BOX-PCR obtained in this study indicate that *A. lipoferum* N-4 and ER-20 are different isolates and not the reisolates of the same strain.

To find out the degree of relatedness, the banding patterns of the PCR products of the local isolates were compared with the DNA fingerprints of standard *Azospirillum* strains by using Gel Compar and calculated using Pearson's software correlations and Dice coefficients. When the analysis was carried out using Pearson's correlations, BOX-PCR fingerprints produced by *Azospirillum* N-4 and ER-20 showed about 74% similarity (Fig. 3A). When compared with the standard *Azospirillum* strains, the local isolates *Azospirillum* N-4 and ER-20 showed more similarity to *A. lipoferum* sp USA5, which was found to be 76% and 74%, respectively. Similar observations of 77% similarity have been reported between different *Rhizobium meliloti* strains when repetitive extragenic palindromic sequence (REP)-PCR-generated genomic fingerprints were compared (Rossbach et al 1995). The remaining standard *Azospirillum* strains showed less than 60% similarity. Standard strain *A. halopraeferens* was not closely related with either our local isolates or with other standard *Azospirillum* strains. The same BOX-PCR patterns of the local isolates were also analyzed by using Dice coefficients (Fig. 3B). This analysis showed 67% similarity between *Azospirillum* N-4 and ER-20 strains. All the local and standard *Azospirillum* strains except one (*A. halopraeferens*) formed a cluster, which showed similarity values ranging from less than 60% to more than 85% among various strains. The analysis of ERIC-PCR banding patterns by using Pearson's correlations showed very low similarity values between local *Azospirillum* strains and between most standard *Azospirillum* strains (Fig. 3C). Similarity values of more than 70% were observed only between two *A. brasilense* strains—sp7 and sp13. Our results, especially when ERIC-PCR patterns were compared, support suggestions by Versalovic et al (1994) that these PCR-based methods are more important for differentiating among bacterial strains belonging to the same species than for studying relatedness at the species or genus level.

Beneficial effects of inoculations with nitrogen-fixing and plant growth hormone-producing bacteria on various plant growth parameters have been reported. In this study, four *Herbaspirillum* strains and one *Azospirillum* strain (N-4) isolated from rice, *Azoarcus* K-1, *Zoogloea* Ky-1, and *Pseudomonas* 96-51 isolated from Kallar grass, and *Azospirillum* wb3 isolated from wheat were used to inoculate plants of two rice varieties—Super Basmati and Basmati 385—grown in unsterilized soil. All bacterial strains showed beneficial effects on root weight, shoot (straw) weight, and grain weight of Super Basmati (Table 3). Inoculation with *Azospirillum* N-4 isolated from rice resulted in the highest root weight, whereas

Zoogloea Ky-1, an isolate from Kallar grass, proved to be the best strain as a higher increase in straw weight and grain weight was recorded in plants inoculated with this strain. These results clearly show that some nitrogen-fixing isolates from other hosts may show a better performance with rice than the isolates from the homologous host. The *Pseudomonas* strain used in this study as a nonfixer inoculant also resulted in increased plant biomass (root, shoot, and grain weight) over the control. As this strain is a phytohormone producer like all N_2-fixing strains included in this study, beneficial effects observed in this study may be due to combined effects of nitrogen fixation and growth hormone production by the inoculated strains. It has been reported in the literature that improved growth of plants inoculated with bacteria may be due to the effect of growth substances produced by inoculated strains (Tien et al 1979, Umali-Garcia et al 1980).

The response of Basmati 385 to bacterial inoculations was poorer than that observed in Super Basmati because the difference between the shoot weight and grain weight of inoculated and uninoculated plants was not statistically different. In Super Basmati, *Azospirillum* Wb3, an isolate from wheat, had the most beneficial effect on grain weight. The *Zoogloea* Ky-1 strain in this variety as well was the most promising strain as it showed a 20% increase in straw weight and 13% increase in grain weight over the control.

Quantification of nitrogen fixation was carried out by the ^{15}N isotopic dilution method in the two rice varieties. The effect of bacterial inoculation was more prominent in Super Basmati than in Basmati 385 (Table 4). In Super Basmati, *Azospirillum* N-4 and *Herbaspirillum* RR8 showed maximum fixation, where 58.9% and 58.2% Ndfa were recorded, respectively. In Basmati 385, *Herbaspirillum* strain RR8 was the most efficient, where 38.7% Ndfa was observed. Thus, the performance of *Herbaspirillum* strain RR8 was excellent with both varieties. *Herbaspirillum* strains are expected to live endophytically in a protected, energy-rich environment and fix more nitrogen than diazotrophs (e.g., *Azospirillum*), which occur predominantly in the rhizosphere (James and Olivares 1998). For plants inoculated with a phytohormone producer, nonfixer *Pseudomonas* strain 96-51, Ndfa values of 44% and 24.3% were observed for Super Basmati and Basmati 385, respectively. This indicates stimulation of growth of diazotrophs or their nitrogen-fixing activity or enhanced root colonization when this growth hormone producer is used as inoculum. No prominent increase in root weight of plants inoculated with *Pseudomonas* 96-51 was observed, which suggested that a modification in root morphology, such as the formation of thin roots or more root hair resulting in more colonization by an indigenous diazotrophic bacterial population, might have occurred. Effects of plant growth hormones produced by inoculated strains on root growth and root morphology have been reported (Okon 1985, Tien et al 1979, Umali-Garcia et al 1980).

The results of this study suggest that genotypic differences among rice varieties Super Basmati and Basmati 385 exist as Super Basmati shows the best response to inoculated strains, which is reflected in an increase in plant biomass and high Ndfa values compared with those of the control obtained with this variety. From the performance of *Zoogloea* Ky-1, an isolate from Kallar grass, it can be concluded that in some cases the isolates from nonhomologous hosts may be even better than the inoculants obtained from the same host.

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Notes

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Figure legends

Fig. 1. Genomic fingerprinting of different bacterial strains using BOX primers in PCR. Lane 1 = 1-kb ladder; lane 2 = 100-bp ladder; lane 3 = *A. lipoferum* N-4; lane 4 = *Azospirillum* ER-20; lane 5 = *Pseudomonas* 96-51; lane 6 = *Zoogloea* Ky-1; lane 7 = *Azoarcus* K-1; lane 8 = *Azospirillum* sp. 4ATR; lane 9 = 1-kb ladder; lane 10 = 100-bp ladder; lane 11 = *Azospirillum* sp. AZLS12; lane 12 = *A. lipoferum* USA5; lane 13 = *A. brasilense* Sp7; lane 14 = *A. brasilense* Sp13; lane 15 = *A. lipoferum* 4T; lane 16 = *A. halopraeferens* AU4; lane 17 = 100-bp ladder; lane 18 = 1-kb ladder.

Fig. 2. Genomic fingerprinting of different bacterial strains using ERIC primers in PCR. Lane 1 = 1-kb ladder; lane 2 = 100-bp ladder; lane 3 = *A. lipoferum* N-4; lane 4 = *Azospirillum* ER-20; lane 5 = *Pseudomonas* 96-51; lane 6 = *Zoogloea* Ky-1; lane 7 = *Azoarcus* K-1; lane 8 = *Azospirillum* sp. 4ATR; lane 9 = *Azospirillum* sp. AZLS12; lane 10 = *A.lipoferum* USA5; lane 11 = *A brasilense* Sp7; lane 12 = *A.brasilense* Sp13; lane 13 = *A. lipoferum* 4T; lane 14 = *A. lipoferum* 34 H; lane 15 = *A. halopraeferens* AU4; lane 16 = 100-bp ladder; lane17 = 1-kb ladder.

Fig. 3. (A) Dendrogram showing relationships between diazotrophic bacteria based on BOX-PCR fingerprints using product moment-correlations. (B) Dendrogram showing relationships between diazotrophic bacteria based on BOX-PCR fingerprints using Dice coefficients. (C) Dendrogram

showing relationships between diazotrophic bacteria based on ERIC-PCR fingerprints using product moment-correlations.

Table 1. Detection of acetylene reduction activity[a] in roots and submerged shoots of field-grown rice.

Stage	Basmati 385		Super Basmati	
	Root	Shoot	Root	Shoot
	(Mean ±SD)[b]		(Mean ±SD)	
Panicle initiation	20 ± 6	15 ± 7	35 ± 9	25 ± 6
Grain filling stage	75 ± 13	110 ± 21	95 ± 11	113 ± 17

[a]n.mole C_2H_4 g^{-1} dry weight 24 h^{-1}. [b]SD = standard deviation.

Table 2. Detection of the bacterial population (in the grain-filling stage) associated with roots and submerged shoots of field-grown rice.

Technique	Basmati 385		Super Basmati	
	Root	Shoot	Root	Shoot
	(Mean ±SD) x 10^7		(Mean ±SD) x 10^7	
AR assay-based MPN[a]	0.43 ± 0.12	0.06 ± 0.01	3.3 ± 1.1	0.05 ± 0.01
Plate counts on LB	820 ± 80	29 ± 7	770 ± 140	67 ± 10

[a]AR = acetylene reduction, MPN = most probable number, LB = Luria broth.

Table 3. Effect of bacterial inoculations on rice variety Super Basmati.

Treatment	Root wt	Shoot wt (g plant^{-1})[a]	Grain wt
Control	3.2 b	17.8 b	8.3 c
Herbaspirillum RS1	3.7 ab	24.3 ab	13.0 bc
Herbaspirillum RS4	5.2 ab	25.7 ab	15.7 ab
Herbaspirillum RR3	5.0 ab	25.7 ab	15.8 ab
Herbaspirillum RR8	3.3 b	19.2 b	12.0 bc
Azoarcus K1	3.3 b	17.7 b	11.8 bc
Azospirillum lipoferum N-4	5.7 a	28.3 ab	15.0 ab
Azospirillum brasilense Wb3	4.7 ab	24.5 ab	14.5 b
Zoogloea Ky-1	5.2 ab	30.3 a	20.0 a
Pseudomonas 96-51	3.7 ab	20.0 ab	13.5 bc

[a]Average of 8 plants collected from 4 different pots.
Means followed by the same letter are not statistically different at the 5% level.

Table 4. Quantification of nitrogen fixation (% Ndfa) in rice varieties Super Basmati and Basmati 385 inoculated with bacterial strains.

Treatment	Basmati 385[a]	Super Basmati
Herbaspirillum RS1	19.5 bc	38.1 ab
Herbaspirillum RS4	28.0 abc	51.6 a
Herbaspirillum RR3	28.5 ab	50.7 a
Herbaspirillum RR8	38.7 a	58.2 a
Azoarcus K-1	10.5 c	21.6 b
Azospirillum lipoferum N-4	20.0 bc	58.9 a
Azospirillum brasilense Wb-3	19.9 bc	47.1 a
Zoogloea Ky-1	22.3 abc	46.8 a
Pseudomonas 96-51	24.3 abc	44.0 ab

[a]Means followed by the same letter are not statistically different at the 5% level.
The uninoculated control, used as a nonfixing reference for the estimation of ^{15}N dilution, has 0%Ndfa for both varieties.