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NITROGEN FIXATION BY *FRANKIA* IN THE RICE ROOTS

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ABSTRACT

Frankia is an actinomycete symbiont found mainly in the root nodules of non-leguminous tree species such as *Casuarina* spp., *Alnus* spp., *Myrica* spp., and *Elaeagnus* spp. A series of studies were conducted to determine its distribution in the soil and in the rice roots and assess its possible contribution to the yield of rice.

Our results have brought out the following interesting observations:

1. *Frankia* is a widespread soil microorganism. It is equally abundant within as well as in the vicinity of the rice roots. Our results comprise the first successful enumeration of this bacterium in soil and other habitats.
2. *Frankia* isolated from wetland rice soil was shown to nodulate *Casuarina* seedlings thus confirming its identity as *Frankia;*
3. *Frankia* isolates were shown to exhibit nitrogenose activity in aseptically grown rice seedlings in a spermosphere model; and
4. *Frankia* when added to the rice seedlings resulted in a positive contribution to its grain yield under field conditions.

The technology developed in these studies constitutes a major breakthrough never before obtained anywhere. The application of this technology is a positive step toward the development of a sustainable agriculture less dependent on expensive exogenous nitrogen inputs.

Introduction

The genus *Frankia* belongs to the order Actinomycetales and consists of a diverse group of bacteria that often exhibit hyphal growth. Members of the genus *Frankia* are characterized by the ability to fix atmospheric nitrogen in the root nodules of certain woody angiosperms (Becking, 1974). Both the nodules induced by *Frankia* and the species of plants which bear these nodules are termed actinorhizal (Torrey and Tjepkema, 1979).

The ability of *Frankia* to induce nodulation in roots is of considerable importance to forestry, land reclamation, natural ecosystems, and plant genetic en-

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gineering. The role of *Frankia* in food production has not been assessed considering that actinorhizal plants are not important sources of food for people and their domesticated animals. The presence of nodulated *Casuarina* and *Elaeagnus* throughout the Philippines and other actinorhizal plants such as *Alnus, Myrica* and *Coriaria* in high altitude areas indicates the widrespread distribution of *Frankia* in almost all kinds of soils.

Considerable interest in *Frankia* developed after its first successful *in vitro* culture (Callaham *et al.*, 1978). *Frankia* grows *in vitro* predominantly in the form of septate hyphae and under suitable conditions will form *Frankia* vesicles, the site of nitrogen fixation. Our modest contribution in the *in vitro* culture of *Frankia* is the development of an enumeration procedure for *Frankia* in soil as reported in this paper. With this initial success, we chose to undertake studies designed to determine the possible application of *Frankia* in increasing the nitrogen fertility of wetland rice and its possible role in increasing rice yield.

Materials and Methods

A. Enumeration of **Frankia** *in the rice rhizosphere*

To make sure that the sample to be taken would be free from any contamination it was decided that the soil sample be taken from the mountain rice terraces. The selection of this sampling area also provided an advantage in the sense that the soil in this area would be under flooded condition during most of the year.

Core samples of the rice plant with intact root system were collected from the Battad and the Banawe rice terraces. Similar core samples were taken from the UPLB Experiment Station, Azolla Plot along Pili Drive.

The terraces in Battad were under the ratoon crop of *Pinidua,* a native rice variety. The plant was about knee-high at the time of sampling.The Banawe samples were taken from an experimental plot under the BPI located at the back of the Municipal Building. The varieties being grown when sampled were *Pinidua* and *Minangan.* The Plants received the equivalent of 40kg N/ha applied as urea.

The rice rhizosphere was prepared for dilution plating using the modified Quispel medium. The dilution range of up to 10^{-5} was prepared in four replicates. The dilution plates were incubated for four days at 30°C prior to counting of actinomycete colonies.

Soil samples (10 g) were dried in the oven for three days for moisture determination.

B. Enumeration of Frankia-type actinomycetes (FTA) in rice histosphere, rhizosphere from Floridablanca, Pampanga

To test the effect of inorganic N fertilization of FTA levels, core samples of rice plants (IR-64) with intact root systems were obtained in Floridablanca from a

site receiving organic fertilizer and a site receiving inorganic N fertilizer. The plants were brought back to the laboratory and the roots gathered and washed gently under tap water to remove soil particles. Final rinsing with sterile distilled water was done, then the clean roots were cut into 1-2 cm segments and blotted dry with sterile tissue paper. One-gram portions of the segments were placed in 250 ml erlenmeyer flasks contaning 100 ml sterile distilled water and five g glass beads. The flasks were shaken vigorously for 30 minutes. Aliquots taken from these flasks were cosidered containing rhizoplane bacteria (root surface). Histosphere (inner root) microorganisms were obtained by filtering, then washing the shaken root segments. These were then macerated in a Waring blender for 5-10 seconds and the resulting slurry used as a source of aliquots for enumerating the histosphere counts. A series of 10-fold dilutions 10^{-3}, 10^{-4}, and 10^{-5} were prepared for both treatments as well as for the rhizosphere soil 0.1 ml aliquots of the different dilutions were inoculated on plates containing 10% coconut water agar. The plates were incubated for three days before counting of observable colonies. Moisture content of the rhizosphere soil was also determined.

C. Enumeration of FTA in rice, 'talahib', coconut rhizosphere and bare area with no plant growth in Taal Volcano

Core samples of rice plants C_1 and *buluhan* with intact root systems were gathered at the foot of Taal Volcano, at the side of the 1975 eruption. Samples of *talahib* were gathered at the slope. Volcanic ash samples clear from plant growth for a radius of at least 20 meters were also gathered at the slope with a sample taken from the surface and another one at a depth of 20 cms. below the surface. Rhizosphere soil of an isolated coconut plant at the base of the volcano was likewise sampled. All the samples were subjected to a 10-fold serial dilutions, i.e., 10^{-1} to 10^{-5}. 0.1 ml portions of each dilution were plated out on 10% coconut water agar and incubated for three days before counting observable colonies. Moisture content determination of each soil sample was also conducted.

D. Isolation of Frankia-type actinomycetes (FTA) from nodules of Alnus, Casuarina and from rice soil samples

Nodules of *Alnus* and *Casuarina* were washed thoroughly with running water to remove the soil particles. Final rinsing was done with sterile distilled water. Ten grams of root nodules were weighed and added to 90 ml sterile distilled water and then macerated in a Waring blender. A series of 10-fold dilutions from 10^{-1} up to 10^{-5} were prepared for both treatments as well as for the rice soil sample. 0.1 ml aliquots of the different dilutions were plated on Qmod agar medium. The plates were incubated for three days before counting of observable colonies.

E. Acetylene reduction assay (ARA) of selected isolates grown in sterile soil

An attempt to measure the acetylene reduction by selected FTA ioslates was done by growing the isolates in vacuutainer tubes containing soil sterilized at 15

lbs/in^2 for three hours for three consecutive days. The isolates were: FTA from organic soil in Floridablanca (OS4-21), FTA from inorganic soil in Flordablanca (IAR3-11), FTA from Battad which showed the highest ARA in previous tests (SA4-4), and FTA from IRRI experimental plot (C3-2). The isolates were grown for three days at room temperature and then injected with acetylene at the rate of 10% of the tube's volume. Ethylene production was assayed after one day using a Shimadzu gas chromatograph provided with Porapak N column and with FID and N_2 as carrier. After the initial assay, the samples were supplemented with 0.1 ml of 50% (w/v) glucose solution. Ethylene production was again assayed after two days of glucose supplementation.

F. ARA of selected isolates grown in glucose yeast extract soft agar

The isolates tested were: antibiotic-producing isolates from Mayondon lake sediment (aFb-1F) and isolates from *Glomus mosseae* spores, two other *Glomus* spore isolates (FrG1 #1 and 2) an isolate from a compost heap (Frth), an isolate from Battad rice terraces (SA4-4), and a well studied isolate from *Casuarina equisetifolia* (CeBr). All isolates were grown on GYE with 0.0175% agar and assayed for ethylene production after four days.

G. Acetylene reduction assay (ARA) of selected FTA isolates from nodules of Alnus, Casuarina and rice soil in soft-agar

FTA isolates were grown in YME broth for 48 hrs. at 30°C with shaking. The cultures were centrifuged at 7,000 rpm for 20 mins and washed thrice with phosphate buffer. After each washing, cells were centrifuged for five minutes at 7,000 rpm. 0.1 ml of the cells were inoculated onto soft-agar in vacuutainer tubes incubated at 30°C for two days. Ater incubation, acetylene was injected at the rate of 10% of the tube's volume. Ethylene production was assayed after one day using a Shimadzu gas chromatograph provided with Porapak N column and with FID and N_2 as carrier.

The isolates tested were: FTA from *Casuarina* nodule (Cnl and Cn2), FTA from *Alnus* nodule (Anl and An2), and FTA from rice soil (Rsl and Rs2).

H. Growth and acetylene reduction in the spermosphere system

Seed sterilization and Germination. Rice seeds (IR42, Peta and Elon-elon) were dehulled, then sterilized in a succession of disinfectants: 70% ethyl alcohol for five minutes, undiluted commercial hypochlorite bleach preparation for 20 minutes and acidified mercuric chloride (0.1%) for 2-3 minutes. Prior to transfer into each succeeding agent, the seeds were washed of the preceding disinfectant two to three times with sterile distilled water, and after the last agent, 20 times with the same. The seeds were then transferred to cotton plugged sterile culture tubes (150 x 10 mm) containing 4.5 ml of Dobereiner's medium. The seeds were allowed to germinate in the dark at 30°C until coleoptiles and radicles were 1-2 cm long.

Preparation of Inoculum. Frankia-type actinomycetes (FTA) were grown in YME broth in 250 ml flasks. Cultures were incubated at 30°C without shaking. Cells were harvested by centrifugation at 7,000 rpm for 20 minutes and washed thrice with 0.9% normal saline solution. After each washing, cells were centrifuged for five minutes at 7,000 rpm.

One loopful of culture was inoculated into the root zone of the plant. After inoculation, 5% of the air inside the tube was evacuated (1.3 ml) and replaced with acetylene.

The isolates tested were a well-studied isolate from *Casuarina equisetifolia* (CeBr), an isolate from Battad rice terraces (SA4-4), an isolate from Taal (BU4-7) an isolate from a garden soil in Canlubang GS^{3}5), an antibiotic producing FTA (afg-15D), and recent isolates from rhizosphere soil from Taal ($BIRS_1$, $BIRS_3$ and $BIRS_4$).

Growth and acetylene reduction assay of rice seedlings under light. The rice seedlings previously germinated in water-agar plates were transferred aseptically with the aid of a pair of forceps into sterile cotton-plugged test tubes containing 4-5 ml of semi-solid (0.2% Noble agar, Difco) modified Quispel medium. Two seedlings were transferred into each tube. Inoculation with FTA was made two days later by delivering 0.1 ml aliquots of the cell suspension near the roots. The tubes were placed in a growth chamber provided with illumination from fluorescent light bulbs on a 12-hour cycle for seven days.

After one week, nitrogen fixation associated with the rice plant was measured by the acetylene reduction assay. The tubes were rubber stoppered and one ml of the air was withdrawn and replaced with acetylene (99% pure, CIGI). The tubes were incubated at 30°C and ehtylene produced was measured by gas chromatography after one and then four days of incubation.

I. Effective on Frankia on nodule formation of Casuarina equisetifolia

Seed sterilization and Germination. Seeds of *Casuarina* were sterilized in a succession of disinfectants as follows: 95% ehtyl alcohol for five minutes, mercuric chloride (0.1%) for three mins. Prior to transfer into each succeeding agent, the seeds were washed of the preceding disinfectant two to three times with sterile distilled water, and after the last agent 10 times with the same. The sterilized seeds were allowed to germinate into soil sterilized at 15 lbs/in^2 for three hours for three consecutive days.

Preparation of Inoculum. FTA isolates (CeBr, SA4-4, afg-2, afg-15P) were grown in yeast malt extract broth for 48 hours at 30°C with shaking. Cells were harvested by centrifugation at 7,000 rpm for 20 minutes and washed thrice with phosphate buffer. Cultures were centrifuged after each washing for five minutes at 7,000 rpm. The cells were suspended in phosphate buffer and 0.1 ml was plated on YME agar to determine the cfu/ml.

Two seedlings of *Casuarina* were transferred to each sterile pot, and was inoculated with two ml of cell suspension. The pots were watered daily with sterile water.

J. Effect of Frankia on rice yield based on two plots of 4m x 4m

Actinomycete SA4-4, an isolate from Battad rice terraces was inoculated into coconut water broth. The culture was in cubated at 30°C with shaking for three days.

The inoculant was broadcast on a slightly raised but moist or wet rice seedbed prior to sowing pre-germinated rice seeds. The rate was about one bag of inoculum for 20 to 25 sq. meters seedbed area. No effort was made to incorporate the organism into the soil (as it appeared inconvenient to go over the prepared seedbed without threading on it). Except for some rain, the bed was kept dry for about 10 days, after which alternate drying and flooding was followed until the rice seedlings were ready for transplanting. The plants treated with *Frankia* were grown in plots separated from the untreated rice seedlings. The plants were observed in the seedbed and in the field.

Results and Discussion

A. Previous Years

In the first year, the investigation concentrated on the enumeration of *Frankia* in flooded soil environment. Initial determination of their nitrogen-fixing ability *in vitro* presents tremendous opportunity for ascertaining their role in rice culture. Indication of decrease in number and reduced nitrogenase activity under continuous application of inorganic fertilizer nitrogen presents its potential use as a biological indicator for the injudicious application of commercial N fertilizer.

The actinomycete population enumerated from the sample by the plate dilution technique ranged from 1.27×10^7 to 3.72×10^7 cfu (colony-forming units)/g dry soil (Table 1). The highest actinomycete count was obtained from samples from Battad rice terraces. The soil from a levee in Battad rice terraces also had a high actinomycete population. The actinomycete population of the samples from Banaue was a little lower than that obtained in Battad. It must be pointed out that the samples were taken from an experimental plot which received nitrogen fertilization at the rate of 40 kg N/ha.

The population of actinomycete in the Los Banos sample approximated the one from the rice terraces. The soil had been on azolla basal application for the last three years. The rate of azolla application used provided the equivalent of 60 kg N/ha.

Several actinomycete isolates were obtained and purified prior to the nitrogenase test. Of the isolates tested, those from sites that did not receive any N

amendment had higher percntage of the population still active in nitrogen fixation (Table 2). The population from the uncultivated area in Battad was mostly active in nitrogen fixation. The lowest percentage was obtained from the azolla plot.

It would seem that nitrogen fertilization had a depressing effect on the nitrogen-fixing activity of actinomycetes. However, there is need to increase the number of isolates and the number of soil samples to be studied to strengthen the validity of this observation.

There was no significant difference in FTA counts between the area receiving organic fertilizer and the one receiving inorganic fertilizer (Table 3). An interesting point however is that the organic rhizosphere yielded the highest FTA count thus far in all our studies (8.4×10^7 as compared to 3.72×10^7 in Battad.

Table 1. Enumeration of actinomycete population in the rice rhizosphere taken from various sites (Pour plate method: 4 replicates)

Sample Site	*Actinomycete cfu/g dry soil ($x10^7$)*
Battad Rice Terraces	
Pinidua rice variety	3.72
Soil from levee	1.96
Banaue Rice Terraces[1]	
Pinidua rice variety	2.09
Minanga rice variety	1.27
UPLB Experiment Station[2]	
Azolla plot along Pili Drive (IR62)	2.07

[1]Plants were taken from the BPI experimental plot that received commercial inorganic N fertilizer at the rate of 40 kg N/ha.

[2]Azolla plot received 0-30-30 per cropping; the plot has been on 3 years of Azolla basal application equivalent to 60 kg N/cropping.

pH of fresh sample (1:1 soil water ratio)

Battad Rice Terraces
- Pinidua – 5.75
- Soil from levee – 5.60

Banaue Rice Terraces
- Pinidua – 5.35
- Minangan – 5.60

UPLB Azolla Plot – 6.40

Table 2. *In vitro* acetylene reduction assay (ARA) conducted on actinomycete isolates from various rhizosphere samples

Sampling Site	*No. of isolates positive ARA* / *Total no. of actinomycetes Tested*	*%*
Battad Rice Terraces		
Pinidua rice variety	7/11	63
Soil from a levee	10/12	83
Banaue Rice Terraces[1]		
Pinidua rice variety	3/5	60
Minangan rice variety	4/7	57
ULB Experiment Station[2]		
Azolla plot along Pili Drive (IR62)	4/10	40

ARA was done 1 day after injection of tubes with acetylene. Data are based on duplicate readings.

[1]Plants were taken from the BPI experimental plot that received 40 kg/ha of inorganic N.

[2]Azolla plot received 0-30-30 per cropping; the plot has been on 3 years of azolla basal application equivalent to 60 kg N per cropping.

Table 3. Population distribution of FTAs in rhizosphere of rice plants grown in field plots receiving organic and inorganic N fertilizers, Floridablanca (ave. of 4 repliates)

Type of N Fertilization	*cf/g soil*
Organic	8.4×10^7
Inorganic	7.1×10^7

Although the Floridablanca experiment showed a definite association between rice plants and FTA, it was considered necessary to show further a definite relationship between the plant and *Frankia.* The island of Taal was chosen as the best site to conduct further FTA enumeration since by virtue of its recent eruption, it contained in one geographical location: areas not yet colonized by plants, areas already colonized, and areas used for agricultural purposes, i.e., planted to rice and other crops.

The results showed high FTA count in rice rhizosphere growing at the foot of the volcano and in the rhizosphere of *talahib* (*Saccharum spontaneum* L.) growing

on the volcano's slopes (Table 4). No FTA was found on the upper slopes where no plants were growing. Microbial activity was present however in this area as shown by the presence f non-FTAs and other bacteria. The predominant actinomycete observed present was a filamentous, but non-*Frankia* type, present mostly on the surface volcanic ash. Another plant tested was an isolated young coconut tree growing in the residential area of the island at the edge of the lake. It also showed the presence of FTA, less numerous than those found in the rice fields. All these results confirm that FTA are indeed associated with the root system of plants including rice.

Table 4. Population distributions of FTAs in various samples taken from Taal Volacano (ave. of 5 replicates)

Sample	*cfu/g soil*
Rhizosphere of rice: 'Buluhan'	2.12×10^7
Rhizosphere of rice: C_1	1.33×10^7
Rhizosphere of 'talahib'	2.08×10^6
Rhizosphere of coconut	5.46×10^6
Volcanic ash sample, bare area:	
Surface layer	0
Sample at 30 cm. depth	0

Previous findings described above demonstrated the presence of *Frankia*-type actinomycetes (FTA) in irrigated rice filed soils. The associated between *Frankia* and the rice plants was likewise established. At this point we are raising the question of how important is the presence of FTAs to the plant. But first we wanted to know the FTAs in rice soil compared to the population is root nodules of *Casuarina* and *Alnus*.

Although *Frankia* is a recognized micro-symbiont in the nodules of non-legumes such as *Casuarina equisetifolia* and a *Alnus maritime*, the results indicate that *Frankia* could be existing in free-living forms in large numbers (Table 5). It is not known at this point however, if these populations are helpful to crops such as rice.

Table 5. Population distribution of *Frankia* from nodules of *Alnus*, *Casuarina* and rice soil samples (ave. of 5 replicates)

Samples	*cfu/ml X* 10^7
Alnus nodule	1.65
Casuarina nodule	1.47
Rice soil	2.13

To demonstrate the nitrogenase activity of *Frankia* isolates in rice roots under aseptic conditions, the spermosphere model was used. Results showed mostly positive ARA but with highest values obtained from CeBr X Peta, and BU4-7 X IR42 (Table 6). The term spermosphere refers to the zone immediately adjacent to the germinating seed and developing radicle, characterized by a high concentration of organic substrates for growth of the inoculated bacteria. This sytem was used to enrich and select nitrogen-fixing strains occurring in the rhizosphere of field-grown rice (Bally *et al.*, 1983). In the spermosphere system, the sole source of carbon would be exudate from the seed and developing radicle which better approximates the composition and rate of exuddation of organic matter into the plant rhizosphere by the mature rice root, and the nitrogen-deficient conditions, which encourage nitrogen-fixing activity of microorganisms present in the root or soil sample, and is maintained by the continuous uptake of the fixed nitrogen by the developing seedling. Nitrogen fixation is then concentrated in the area adjacent and along the seed and developing radicle. ARA in the spermsphere system thus includes the activity of bacteria proliferating in the medium upon diffusion of substrates from the seed and roots.

The results presented in Table 7 clearly show that the selected FTA isolates had high nitrogen fixation activities and the readings taken after 24 hours from the introduction of acetylene could be taken as indicative of maximum for a particular variety. After choosing the most suitable strain of FTA we would then embark on the nitrogen fixation test involving a number of rice varieties to determine the suitability of this technology in indicating the varieties suitable for breeding so that the resulting crosses could be monitored for the ability to support high nitrogen fixation in their roots.

Table 6. Nitrogen fixation (ARA) associated with the spermosphere of IR42 and Peta seedlings inoculated with FTA isolates (ave. of 10 replicates)

Treatment	*ARA (log nmoles ethylene per tubes)*
Blank	1.39
SA4-4	1.51
BU4-7	1.39
CeBr	1.57
SA4-4 x IR42	1.43
SA4-4 x Peta	1.52
BU4-7 x IR42	1.70
BU4-7 x Peta	1.47
CeBr x IR42	1.44
CeBr x Peta	1.87
IR42	1.51
Peta	1.52

Table 7. Nitrogen fixation (ARA) associated with the spermosphere of Elon-elon after 1 and 4 days of incubation underlight

FTA Isolate	*log nmoles of ethyle produced (ave. of 10 replicates)*	
	24-hr.	96-hr.
Control	0.00	0.00
CeBr	2.26	2.23
SA4-4	2.19	2.08
BU4-7	2.30	2.30
$BIRS_1$	2.57	2.69
$BIRS_3$	2.48	2.73
$BIRS_4$	2.20	2.30
afg-15D	2.56	2.61
GS^35	2.00	2.36

To establish the association of *Frankia* with nodulated non-legumes among families of woody dicotyledons, *Casuarina equisetifolia* was used as the test plant for nodule formation. FTA isolates were inoculated into the roots of the seedlings grown in sterile pots. Results presented below the presence of nodules on the inoculated plants 83 days from the start of inoculation.

Table 8. Effect of *Frankia* on nodule formation of *Casuarina equisetifolia* seedlings grown aseptically in soil

Treatment	*Inoculum size used cfu/ml*	*Nodule formation*
Control	0	–
CeBar	1.67×10^6	+
afg-2	7.45×10^5	+
afg-15P	5.76×10^4	+
SA4-4	4.0×10^6	+

The nodulated roots of *C. equisetifolia* was placed in a sterile vacuutainer tubes and injected with acetylene (10% of the tube's volume). Ethylene production was assayed after four hours. Results showed that SA4-4 gave the highest ARA activity followed by CeBr (Table 9). SA4-4 was an isolate from Battad rice terraces, and CeBr, an isolate from *Casuarina equisetifolia*.

Table 9. Acetylene reduction assay of nodulated roots of *Casuarina equisetifolia* (Ave. of duplicate readings)

Isolate used	*ARA (log nmoles ethylene produced)*
Control	1.37
SA4-4	2.76
CeBr	1.82
afg-15P	1.64
afg-2	1.38

It has been established in the results presented earlier that *Frankia* is capable of nitrogenase activity *in vitro* in the presence of mineral nutrients and in the presence of the rice seedlings in the spermosphere. The field experiment was conducted to determine the contribution of *Frankia* to rice yield (Table 10). The field experiment, conducted under the suspices of the Masipag Project of which Dr. Aspiras is a participating microbiologist, involved seven rice cultivars: Puro-puro, Binato, Elon-elon, Borong, Milagrosa, UPL Ri5, and Japanese 2. The first five are traditional varieties and the last two, improved varieties.

Table 10. Effect of *Frankia* treatment on rice yield (based on 2 replicates of 4 m^2 each), Wet season 1987

Yield Measurement	*Treatment*	
	No Frankia	*With Frankia*
	(a) *Puro-puro*	
Grain wt (g)/10 hills	147.5	175
Grain wt (g), remaining area of plot	1105	1288
Total grain wt (g)/4 m^2	1252	1463
Yield, kg/ha	3130	3658
Yield, cav/ha	69.6	81.3
Effect of treatment on yield (%)	+17	
	(b) *Binato*	
Grain wt (g)/10 hills	97.5	130
Grain wt (g), remaining area of plot	647.5	740
Total grain wt (g)/4 m^2	745	870
Yield, kg/ha	1862	2175
Yield, cav/ha	41.4	48.3
Effect of treatment on yield (%)	+17	

Table 10 (Continued)

Yield Measurement	*Treatment*	
	No Frankia	*With Frankia*
	(c) *Elon-elon*	
Grain wt (g)/10 hills	155	162.5
Grain wt (g), remaining area of plot	944.5	1260
Total grain wt (g)/4 m^2	1109.5	1422.5
Yield, kg/ha	2773.5	3556
Yield, cav/ha	55.3	71.0
Effect of treatment on yield (%)	+28	
	(d) *Borong*	
Grain wt (g)/10 hills	172.5	157.5
Grain wt (g), remaining area of plot	1240	1262
Total grain wt (g)/4 m^2	1412	1420
Yield, kg/ha	3531	3550
Yield, cav/ha	78.5	78.9
Effect of treatment on yield (%)	1.0	
	(e) *Milagrosa*	
Grain wt (g)/10 hills	117.5	82.5
Grain wt (g), remaining area of plot	655	645
Total grain wt (g)/4 m^2	7725	7275
Yield, kg/ha	1931	1819
Yield, cav/ha	38.5	36.5
Effect of treatment on Yield (%)	−5.2	
	(f) *UPL Ri5*	
Grain wt (g)/10 hills	132	158
Grain wt (g), remaining area of plot	1162	1180
Total grain wt (g)/4 m^2	1294.5	1338
Yield, kg/ha	3237	3345
Yield, cav/ha	64.5	66.5
Effect of treatment on yield (%)	+3.1	
	(g) *Japanese 2*	
Grain wt (g)/10 hills	1185	187.5

Table 10 (Continued)

Yield Measurement	*Treatment*	
	No Frankia	*With Frankia*
Grain wt (g), remaining area of plot	1101	1002
Total grain wt (g)/4 m2	1286	1190
Yield, kg/ha	3215	2974
Yield, cav/ha	64.3	59.5
Effect of treatment on yield (%)	−7.5	

An isolate of *Frankia* SA4-4, obtained from the Battad rice terraces, was used as inoculant. The actinomycete was added on the rice seedbed prior to the sowing of pre-germinated seeds at the rate of 1 bag of the inoculum (0.5 kg) per 25 sq. meter seedbed.

At maximum tillering stage, there was no visual difference in height and color due to treatment. At flowering stage, however, the plants treated with *Francia* flowered three to four days later than the untreated ones.

The yield data presented in Table 10 show the favorable effect of *Frankia* on rice yield. In three of the seven varieties (*Puro-puro, Binato* and *Elon-elon*), *Frankia* definitely had a positive effect on yield. The other cultivars had yields that were either not affected by *Frankia* or were slightly the last two varieties were heavily infested by the stem-borer resulting in vary significant white head count of 17% for *Milagrosa* and 21.4% for *Japanese 2.* The results on the last 2 varieties were therefore deemed unreliable.

Summary and Conclusion

Frankia is an actinomycete symbiont of nodulated non-leguminous tree spcies such as *Alnus* and *Casuarina.* Because of the unique ability to conduct nitrogen fixation this organism could also be important in supporting the needs of other plants such as rice. Hence, the studies were designed to determine the possible contribution of *Frankia* to rice.

The results of our studies have confirmed the following:

a) *Frankia* is a common soil organism. Our results on enumeration of this organism comprises the first successful attempt on direct enumeration of this organism in soil and other habitats.

b) *Frankia* is abundant both in well-drained and in wetland soil.

c) *Frankia,* isolated from soil, was shown to nodulate *Casuarina* seedlings, confirming the identity as *Frankia.*

d) *Frankia* could conduct nitrogen fixation in the presence of rice roots in a spermosphere model.

e) *Frankia* treatment on rice seedlings have resulted in positive contribution on rice yield under field conditions.

There is also an indication of possible use of this technology in screening rice breeding materials toward obtaining rice varieties that support high nitrogen fixation in their roots.

The technology demonstrated here constitutes a major breakthrough never before demonstrated anywhere. The technology can be developed further to make it available to rice farmers in the hope of making rice farming more self-sustaining.

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