investigation, efforts have been made to unravel the mechanism of resistance to Zn in this hitherto unexplored organism as a step towards effective biofertilizer application. This study also fulfils the need to understand the effect of arbitrary application of metal-containing pesticides on numerous non-target microorganisms. The efficacy of Zn^r *A. brasilense* when applied to maize seeds in *in vitro* studies has already been established in our laboratory[21].

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Ammonia volatilization and denitrification losses from nitrogen fertilized flooded soil as affected by the addition of iron pyrites

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Results of a laboratory study indicated that iron pyrites reduced nitrous oxide (N_2O) and NH_3 evolution from flooded soil fertilized with KNO_3 or urea. Total gaseous ($N_2O + NH_3$) loss was reduced by 47.3% with the addition of pyrite compared to the urea alone treatment.

GASEOUS N loss through NH_3 volatilization and denitrification is responsible for the low N use efficiency of rice under flooded conditions[1]. Nitrification[2] and urease inhibitors[3] and slow-release fertilizers[4] are proposed as strategies to reduce N loss and enhance NUE. Nitrification inhibitors reduce losses through denitrification[5,6] but have been found to accentuate NH_3 loss[7]. Iron pyrites, a mineral occurring in plenty as sedimentary deposits in parts of Bihar, India, have been found to inhibit nitrification[8] and reduce NH_3 loss from surface-applied urea[9]. Since pyrites retard nitrification, it could possibly reduce denitrification. Hence, an incubation study was conducted in the laboratory to find out the effect of pyrite on denitrification and NH_3 volatilization from urea applied to a flooded soil.

Surface soil (0–15 cm) from cultivated fields of Duernast, Freising, Germany, having pH 6.8 (soil:$CaCl_2$ 1:2.5), organic C 1.12%, 4.1 mg NH_4^+–N and 15.6 mg NO_3^-–N kg^{-1} soil, total N 0.12% was used in the study. Air-dry soil (200 g) was placed in 500 ml jars. The soil was flooded to a depth of 2 cm and pre-incubated for two weeks. To enhance the reduced conditions, glucose solution equivalent to 500 mg C kg^{-1} soil was applied. The mineral-N status of soil after pre-incubation was 6.3 mg NH_4^+–N and 0.1 mg NO_3^-–N kg^{-1} soil. After the pre-incubation, the soils were treated with N at the rate of 100 mg kg^{-1} soil in the form of KNO_3 and urea solution, separately, with or without pyrite. Pyrites, obtained from Pyrites and Phosphates Chemicals Limited (PPCL) New Delhi, was added equivalent to 100 mg S kg^{-1} soil. Each treatment combination was replicated thrice. A no N control was also maintained. The jars were covered with a lid provided with a rubber septum. The sides of the lid and rubber septum were sealed with silicone to prevent gas leaks. After 1, 2, 3, 5 and 7 days, gas samples were taken and analysed for N_2O–N by gas chromatography (Varian 3400) using an electron capture

Table 1. Effect of pyrite on NH_3 loss from urea applied to a flooded soil (20 mg urea–N 200 g $soil^{-1}$ jar^{-1})

	Days			
	1	2	7	Total
	(mg NH_3–N jar^{-1})			
Urea	0.62 ± 0.078*	0.46 ± 0.024	0.41 ± 0.019	1.49 ± 0.104
Urea + pyrite	0.53 ± 0.016	0.36 ± 0.046	0.054 ± 0.0034	0.94 ± 0.062

*Standard error.

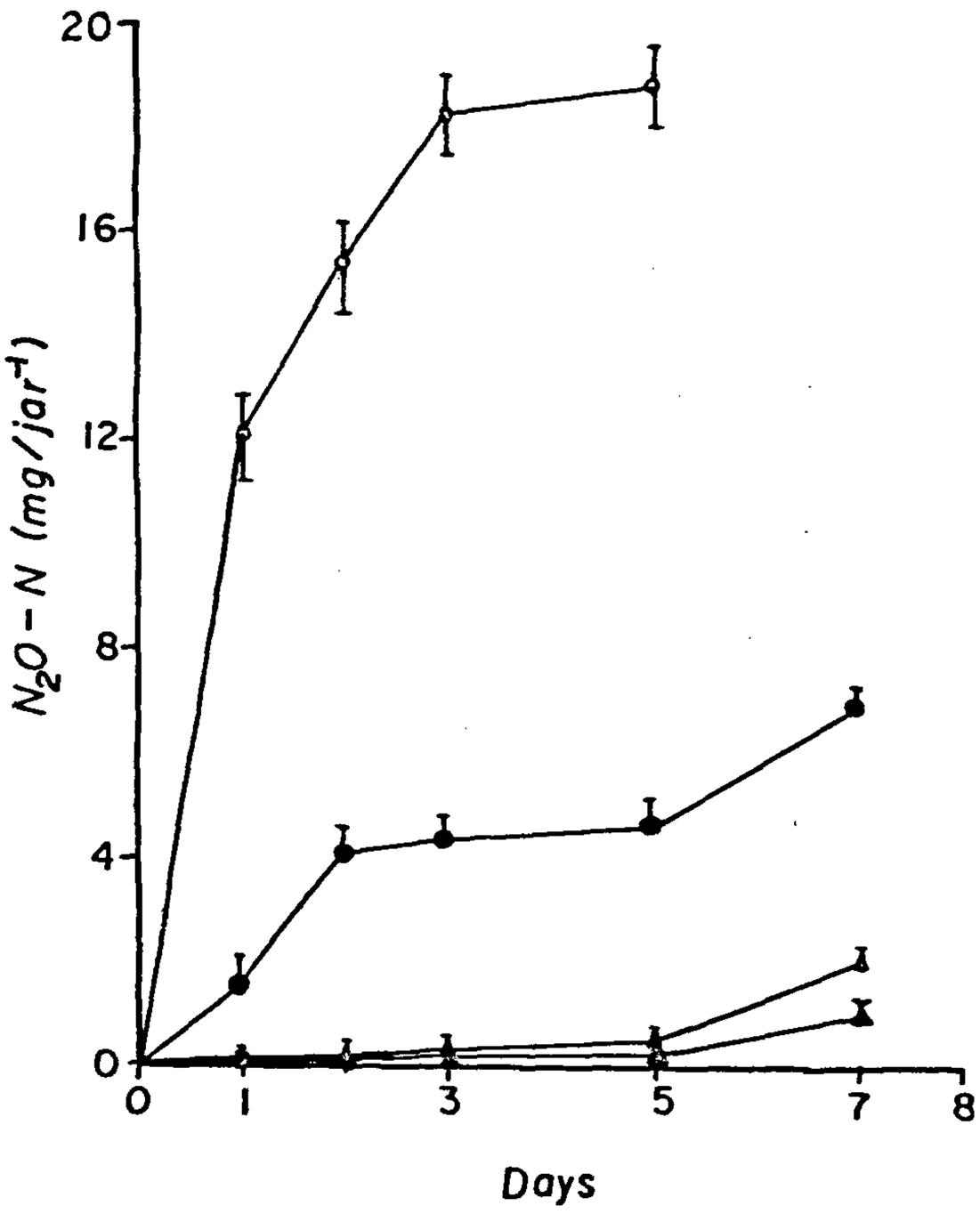

Figure 1. Effect of iron pyrite on nitrous oxide evolution from flooded soil (○, KNO_3; ●, KNO_3 + pyrite, Δ, urea; ▲, urea + pyrite). Vertical bars indicate standard error; LSD ($P < 0.01$) = 0.73 for day seven.

detector. For estimating NH_3 loss, a separate set of jars of each treatment in triplicate was set up similar to the previous one. The jars in this case were covered with parafilm provided with a few micropores for aeration. The NH_3 evolved was trapped in 4% 10 ml boric acid-mixed indicator solution contained in vials placed at the soil surface[9]. The trapped NH_3 was estimated by titrating with H_2SO_4 (0.005 N).

By day 5, almost the entire applied N was recovered as N_2O–N with KNO_3-treated soils (Figure 1). Addition of pyrite resulted in lower N_2O evolution. At the end of 7 days, amount of N_2O–N recovered was 34.5% of applied N with KNO_3 + pyrite treated soils compared to KNO_3 alone treatment. Under reduced conditions, nitrate is known to be rapidly denitrified[10]. On the other hand, urea-treated soils had low N_2O evolution. Most loss occurred between fifth and seventh day. The slow urea hydrolysis[11] and nitrification[12,13] processes under flooded conditions are responsible for the initial lag period observed with the urea-treated soils. Urea + pyrite-treated soils had less than half the N_2O evolution compared to urea alone treatment. This could be due to the nitrification inhibitory effect of pyrite[8]. But we observed that the inhibitory effect of pyrite is rather very low in flooded soils. The most plausible reason appears to be that pyrite is directly involved in inhibiting nitrite- or nitric- or $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ nitrous oxide reductases. This is very much evident from the low N_2O evolution observed with KNO_3 + pyrite treatment. Kowalenko[14] reported S anions to reduce denitrification loss, which suggests that the sulphides in iron pyrites could play a major role in the process. The other possible explanation could be that as NO_3^- becomes limiting, N_2O is used as an electron source reducing it to N_2 (ref. 15).

Ammonia volatilization was observed only in the urea-treated soils (Table 1). NH_3 evolution was observed right from day one, which declined later possibly due to it being nitrified or adsorption by the soil[13]. Addition of pyrite reduced NH_3 loss from urea by 37%. This is attributed to the acidic nature of pyrite[16] preventing the rise in floodwater pH[9]. Total gaseous–N (N_2O + NH_3) loss was 3.49 mg jar^{-1} for the urea alone treatment which was brought down to 1.85 mg jar^{-1} with the addition of pyrite, a reduction of 47.3%. The results also indicate the NH_3 volatilization and denitrification losses to be interdependent[17]. The possibility of losses beyond 7 days cannot be eliminated. However, earlier studies[18,19] showed maximum loss to occur within first 10 days.

From the results of this study it is not clear whether pyrite reduces total denitrification as the other NO_x components could not be analysed. The effect of pyrite on denitrification, therefore, needs to be probed further. However, it does offer a benefit by way of reducing NH_3 loss and N_2O evolution which is implicated in the depletion of stratospheric ozone layer.

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Biological control of blast disease of fingermillet (*Eleusine coracana* L.) and an analysis of fertility of *Magnaporthe grisea*

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Six strains of fluorescent pseudomonads which belong to *Pseudomonas fluorescens* and *P. putida* were screened in the laboratory for their ability to inhibit the ragi blast fungus *P. grisea*. The bacterial strains showed fungal inhibition in dual plate assays in the laboratory and reduced ragi blast severity in the field. Leaf and neck blast were reduced by 45.3 to 65.2% and 24.3 to 54.9% in moderately resistant ragi cv. CO-7 and by 59.1 to 72% and 33.3 to 63.3% respectively in the susceptible cv. PR-202. *P. fluorescens* strains 7–14 was most effective amongst the bacterial treatments. For the first time, perithecial formation occurred in 11 of the 96 *P. grisea* combinations when they were mated in the laboratory with known testers of rice blast fungus which were used as a parent.

FINGERMILLET (*Eleusine coracana* L.) commonly known as ragi ranks second in importance among the millets in India and is widely cultivated in several parts of Tamil Nadu, Andhra Pradesh, Karnataka and Maharashtra. Blast incited by *Pyricularia grisea* (Cke) Sacc, is one of the major destructive diseases causing excessive damage to this crop from seedling to earhead-forming stages. The disease occurs during all growing seasons and on almost all ragi varieties cultivated.

Considering the hazards of chemical applications, biological control has emerged as an important alternate strategy for disease control in recent years. There has been much success in biological control of crop diseases by using antagonistic bacteria of the fluorescent pseudomonad group[1–3]. Studies have revealed the potential of antagonistic microorganisms in inhibiting pathogens at the root–soil interface, thereby protecting perennial and annual plants such as cotton, potato, tobacco, wheat and rice[4–7]. Thomoshow and Weller[8], Weller and Cook[9] and Schippers *et al.*[10] found these microorganisms to inhibit pathogens by producing antibiotics, siderophores (compounds that chelate biologically available iron) and plant growth-stimulating substances. Earlier studies from our laboratory have shown that, when carefully selected strains of *Pseudomonas fluorescens* or *P. putida* were used as bacterial treatments, such treatments resulted in the reduction of blast, sheath-rot and sheath blight severities in rice[11–15].

The discovery of the sexual stage[16] in *P. grisea* has broadened the understanding of genetic variation in the genus *Pyricularia* and is important for effective blast disease management, as resistant breeding strategies are based on the clonality of the pathogen. Complexity of fertility in this fungus is partially responsible for incompatibility amongst isolates. The purpose of the present fertility studies is to determine the sexual compatibility of the Indian isolates of *P. grisea* when mated with fertile testers. We have evaluated a selected set of fluorescent pseudomonads for the suppression of ragi blast in the laboratory and in the field.