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Microbiological Management of Wetland Rice Fields

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I. INTRODUCTION

About 75% of the 143 million ha of rice land are wetlands where rice grows under flooded conditions during part or all of the cropping period. Flooding is beneficial to rice cultivation by (1) bringing the soil pH near to neutrality; (2) increasing availability of nutrients, especially P and Fe; (3) stimulating biological N_2-fixation; (4) depressing soilborne diseases; (5) supplying nutrients from irrigation water; (6) decreasing weed incidence, especially those of C_4 plants; and (7) preventing water percolation and soil erosion (Watanabe et al., 1988).

Flooding changes the chemistry, microbiological properties, and nutrient supply capacity of soil. It leads to the differentiation of macro- and microenvironments differing in redox state, physical properties, and light and nutrient status that allows a wide range of microorganisms to be active. In particular, all kinds of N_2-fixing microorganisms (aerobes, facultative and strict anaerobes, heterotrophs, phototrophs, free-living, and symbiotic) can and do grow in wetland rice fields, resulting in a unique agroecosystem in which moderate, but constant, yields have been obtained after continuous cropping for centuries without N fertilizer addition. Biological N_2 fixation contribution, estimated from N_2 balance studies, is 15–50 kg N per crop (Roger and Watanabe, 1986).

The microbiology of rice soils was reviewed by Sethunathan et al. (1983), Watanabe and Furasaka (1980), and Yoshida (1975), who highlighted the interactions between pesticides and microbial activities in rice fields. This review deals with the microbiological management of rice fields and emphasizes agricultural practices that can increase rice production through direct manipulation of soil and

water microflora. The first section presents a brief summary of the major environments of the wetland rice field ecosystem and the major microbial activities they host, and a short overview of research on microbiological management of rice fields. The next sections discuss in detail potential and adopted practices, including (1) the utilization of symbiotic and free-living N_2-fixing blue-green algae as biofertilizers, (2) the inoculation of rice with heterotrophic bacteria and the potential for rhizosphere microflora management, and (3) the utilization of bacterial and algal inhibitors to decrease N fertilizer losses.

II. THE WETLAND RICE FIELD ECOSYSTEM AND ITS MICROBIOLOGICAL MANAGEMENT

A. Major Environments and Microbial Activities

Diffusion of air into soil is reduced about 10,000 times when a rice field is flooded and O_2 supply cannot meet the demand of soil aerobic organisms. Facultative and strict anaerobes proliferate, using oxidized soil substrates for respiration, reducing the soil in a sequence predicted by thermodynamics, and creating anaerobic conditions in a reduced layer a few millimeters beneath the soil surface (Yoshida, 1975).

Flooding and crop growth lead to the differentiation of macroenvironments differing in physicochemical and trophic properties: floodwater, surface-oxidized soil, reduced soil, rice plants (submerged parts and rhizosphere), plow layer, and subsoil (Fig. 1). Although those environments can be macroscopically differentiated, they are more or less continuous. In particular, continuous exchanges take place between floodwater and oxidized soil (Watanabe and Furasaka, 1980). Macroenvironments might also be heterogeneous in their oxidation–reduction status at the microsite level because the activity of soil fauna creates microaerophilic sites in the reduced layer, while organic matter debris and aggregates might provide anaerobic microsites in the oxidized soil layer and the water.
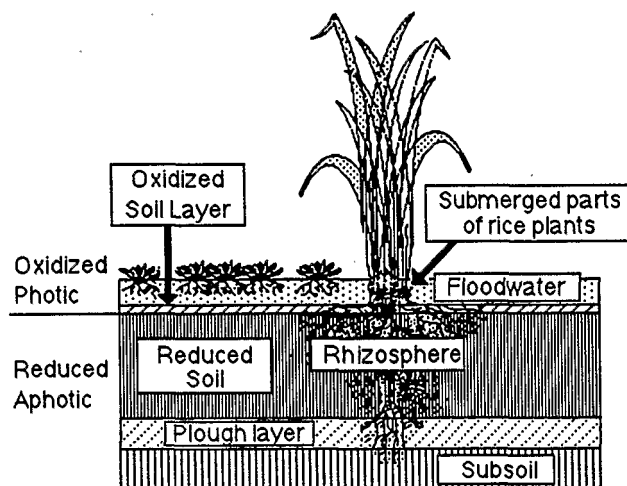

Figure 1 Macroenvironments of the wetland rice field ecosystem.

Floodwater

The floodwater is a photic, aerobic environment in which chemotrophic and photo-synthetic producers (bacteria, algae, and aquatic weeds), primary consumers (graz-ers), and secondary consumers (carnivorous insects and fish) recycle nutrients and provide organic matter to the soil. The floodwater is subject to large variations in insolation, temperature, pH, O_2 concentration, and nutrient status (Roger and Kurihara, 1988). Because of the photosynthetic activity of algae and aquatic plants, O_2 content and pH exhibit marked diurnal variations. During daytime, pH may increase above 10 and O_2 may be oversaturated by 200%.

The photosynthetic aquatic biomass that develops in floodwater is composed of planktonic, filamentous, and macrophytic algae and vascular macrophytes the standing crop of which is usually a few hundred kilogram dry weight per hectare and rarely exceeds 1 t/ha (10–20 kg/ha of N). Reported productivity ranges from 0.5 to 1 g/m^2 per day of C (Roger and Kurihara, 1988). Populations of heterotrophic bacte-ria in the floodwater may attain 10^5–10^6/ml, with variation correlated with the quantity of soil particles in suspension in water (Baldensperger, 1981). Major activi-ties in the floodwater include photosynthesis and respiration by the photosynthetic aquatic biomass, and photodependent biological N_2 fixation by free-living and sym-biotic blue-green algae.

Oxidized Soil Layer

The oxidized soil layer is a photic aerobic environment, with a positive redox potential (Yamane, 1978), in which NO^{-3}, Fe^{+3}, SO_4^{-2}, and CO_2 are stable, and algae and aerobic bacteria predominate. The depth of the oxidized layer, which is usually between 2 and 20 mm, depends on the quantity of O_2 dissolved in floodwater, the reducing capacity of the soil (C content), the water percolation, and the activity of the soil and water fauna (Neue, 1988). After land preparation, algae develop at the soil surface and support grazing populations. Later in the crop cycle, organic matter accumulates at the soil surface and supports populations of invertebrates that recy-cle the nutrients (Roger and Kurihara, 1988).

The oxidized soil layer is microbiologically very active. Major activities include (1) aerobic decomposition of organic matter, (2) photodependent biological N_2 fixation by algae and photosynthetic bacteria, (3) nitrification by ammonium and nitrite oxidizers, and (4) methane oxidation (Watanabe and Furasaka, 1980).

Reduced Soil Layer

The reduced soil layer is a nonphotic, anaerobic environment for which the soil E_h is predominantly negative (Yamane 1978), and the E_h of the soil solution is lower than 300 mV. The reduction processes predominate; E_h and pH are low enough to allow the reduction of iron oxides (Neue, 1988); NH_4^+, S^{-2}, Mn^{+2}, and Fe^{+2} are stable chemical forms, and microbial activity is concentrated in soil aggregates containing organic debris. Although the reduction of flooded soils proceeds stepwise in a thermodynamic sequence, oxidation–reduction reactions are only partially applica-ble to field conditions under which their catalysis is mediated by microbial popula-tions (Neue, 1988). The equilibrium depends strongly on microbial growth and behavior and on the degree to which reacting products diffuse and mix.

Major activities in the reduced soil layer include (1) anaerobic decomposition of organic matter, (2) heterotrophic biological N_2 fixation mostly associated with

organic debris, (3) denitrification, (4) manganese reduction, (5) iron reduction, (6) sulfate reduction, (7) methanogenesis, and (8) H_2 production (Watanabe and Furasaka, 1980).

Rice Plant: Submerged Parts and Rhizosphere

From a microbiological point of view, the rice plant provides two environments for the microflora: submerged plant parts and the rhizosphere.

Submerged portions of rice shoots (and aquatic plants) are colonized by epiphytic bacteria and algae. Epiphytes are ecologically important in deep-water rice for which the submerged plant biomass, including nodal roots, is very large. Epiphytic biological N_2 fixation can be agronomically significant in deep-water rice (Kulasooriya et al., 1981).

The rhizosphere is a nonphotic environment in which redox conditions are determined by the balance of the oxidizing and reducing capacities of rice roots, and production of C compounds by roots provides energy sources for microbial growth. The rice plants ability to transport O_2 from the stem to the root and the diffusion of this O_2 in the adjacent soil layer lead to the differentiation of an oxidized–reduced interface. Because rice roots can occupy a large volume, a significant fraction of the planted soil can be aerobic, and the soil solution can maintain a high redox potential.

Major activities in the rhizosphere include (1) associative heterotrophic biological N_2 fixation, (2) nitrification–denitrification, and (3) sulfate reduction (Watanabe and Furasaka, 1980).

Plow Pan and Subsoil

The plow pan exhibits low permeability and a higher bulk density and mechanical strength than other soil layers. It acts to reduce water and nutrient losses by leaching and percolation. The soil below the plow pan is aerobic in well-drained soils and anaerobic in poorly drained soils. It is microbiologically active in its upper layer, and its role in providing nutrients to rice, especially N, should not be underestimated (Ventura and Watanabe, 1984).

B.   Overview of the Microbiological Management of Rice Fields

Nitrogen is the key nutrient in rice production. Most actual and potential microbiological soil management of wetland rice refers to the N cycle. Emphasis has been on biological N_2 fixation and inoculation with N_2-fixing microorganisms.

The oldest technology of employing N_2-fixing microorganisms in rice fields is utilization of *Azolla*. The use of *Azolla* as a green manure dates back to the 11th century in Vietnam and at least to the 14th century in China (Lumpkin and Plucknett, 1982). The causative agent of the beneficial effect of *Azolla* inoculation was identified by Strasburger (1873) as a N_2-fixing blue-green alga (*Anabaena azollae*) but progress in *Azolla–A. azollae* biotechnology, in particular recombination and sexual hybridization, is very recent (Lin et al., 1988; Lin and Watanabe, 1988; Wei et al., 1986).

The agronomic potential of N_2-fixing blue-green algae was recognized in 1939 by De, who attributed the natural fertility of wetland rice fields to biological N_2 fixation by blue-green algae. Research on blue-green algae inoculation of rice fields was initiated in Japan by Watanabe et al. (1951).

Although the presence of N_2-fixing bacteria in rice roots was reported as early as 1929 by Sen, the study of the potential of N_2-fixing heterotrophs started in 1971, when Rinaudo and Dommergues, and Yoshida and Ancajas, using the acetylene reduction assay (ARA), demonstrated that some N_2-fixing activity is associated with wetland rice roots. Early inoculation trials were reported by Dobereiner and Ruschel (1962) with *Beijerinckia,* and by Sundara et al. (1962) with *Azotobacter.* Most of the reports on bacterial inoculation of rice fields (40 of 44), however, have been published since 1976. Between 1976 and 1981, most of the trials were with *Azotobacter.* Since 1983, most trials have been with *Azospirillum.* Recent studies on bacterial inoculation and N_2 fixation in the rice rhizosphere have shown that most yield increases observed after inoculation with N_2-fixing bacteria could not be explained solely by increased biological N_2 fixation and were probably due to a growth-promoting effect of the inoculum. Therefore, a possible new approach to inoculation is to select strains producing plant growth regulators (PGR). The potential of inoculation with P-solubilizing bacteria has also drawn some attention.

Research on associative biological N_2 fixation in the rice rhizosphere has also revealed differences in the ability of rice genotypes to stimulate associative biological N_2 fixation and N uptake (Ladha et al., 1988). This suggests that N utilization by rice can be improved by selection and breeding of varieties that can stimulate the development of a more efficient associated microflora.

Numerous studies have shown that N fertilizer recovery in wetland rice is seldom more than 30–40% and rarely exceeds 60–65%, even with the best agronomic practices and strictly controlled conditions (De Datta, 1981). In wetland soils, nitrogen is the "escape artist" among plant nutrients (Hauck, 1980), having a great potential for loss through denitrification, NH_3 volatilization, leaching, and runoff. Loss through denitrification is mostly a microbiological process. Loss through NH_3 volatilization results from a chemical process caused by a marked increase in floodwater pH as a consequence of algal activity. Therefore, control of microbial activities or microbial populations directly or indirectly responsible for N losses holds potential for microbiological management of wetland rice fields.

III. *AZOLLA*

A. The Symbiosis

Azolla Lam. is an aquatic, heterosporous fern that occurs in a broad latitudinal range on five continents. This unique freshwater pteridophyte lives in symbiosis with the diazotrophic cyanobacterium *Anabaena azollae* Strasb. (1873). There are seven known species of *Azolla* in two extant sections (Table 1), taxonomically separated by their secondary reproductive structures, including the number of float corpuscles (homologous to massulae) per megasporocarp and the form of glochidia that extend from the microsporic massulae. Several species are also distinguishable by the branching patterns or growth habits of their sporophytes (Fig. 2). The taxa of section Azolla are believed to have originated evolutionarily before those of section Rhizosperma (Follieri, 1977).

The endophytic *A. azollae* resides within a basal cavity of the dorsal lobe of each leaf of *Azolla* (Fig. 3), but its cells are also associated with the terminal meristem and the megasporocarp. Filaments of the cyanobacterium have heterocyst

Table 1   Taxonomy and Continental Range of Extant *Azolla* Species

Species	Indigenous distribution
Section Azolla	
A. filiculoides Lamarck	North and South America
A. mexicana Presl	North and South America
A. microphylla Kaulfuss	North and South America
A. caroloniana Willdenow	North and South America
A. rubra R. Brown	Asia, Australia
Section Rhizosperma	
A. pinnata R. Brown	
var. pinnata R. Brown	Australia, Africa
var. imbricata Roxburgh	Asia
A. nilotica DeCaisne	Africa

Figure 2   Dorsal view of immature small fronds of (1) *A. filiculoides,* (2) *A. pinnata* var. pinnata, (3) *A. nilotica,* and (4) *A. caroliniana;* bar scale = 1 cm.

frequencies of 20–30% when actively fixing N_2 in the leaf cavities (Hill, 1975). By inducing N excretion by the cyanobacterium through the repression of its glutamine synthetase levels the N needs of *Azolla* are also met (Ray et al., 1978). The entire association may grow prolifically without mineral N (Fig. 4). *Azolla* N is released into the external environment upon decomposition and mineralization of the fern.

B.   Growth and Biofertilizer Potential

The use of *Azolla* has been a part of rice cultivation in Vietnam and China for centuries and has been applied or tested more recently in other rice-growing countries (Roger and Watanabe, 1986). In addition to N supply, the benefits of *Azolla* as

Figure 3 Filaments and cells of *Anabaena azollae* lining the cavity of the dorsal leaf lobe of *Azolla* cross section); bar scale = 0.1 mm. (Courtesy of B. Rosen.)
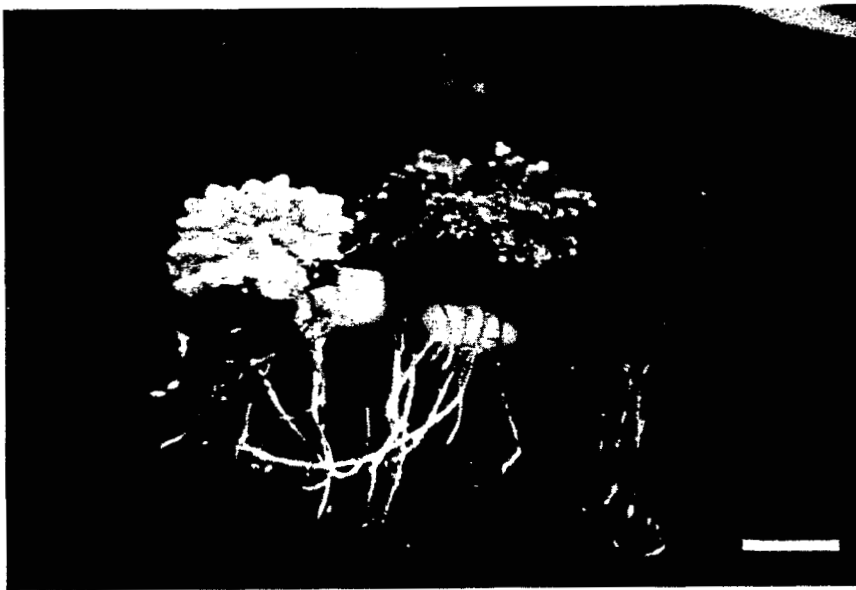

Figure 4 Two fronds of *Azolla filiculoides* grown in a nitrogen-free medium (right) and in a nitrogen-free and cobalt-free medium (left) (Johnson et al., 1966). The plant grown in a N-free medium is healthy, whereas that grown in a N-free and cobalt-free medium lacks its cyanobiont and is necrotic from N deficiency; bar scale = 1 cm.(Courtesy of H. Evans.)

a green manure include provision of other mineral nutrients and organic matter to the soil. When established in rice fields, *Azolla* also reduces water evaporation and NH_3 volatilization (Rains and Talley, 1979). However, the realizable potential of *Azolla* as a green manure is restricted by climatic factors, water availability and quality, soil factors, mineral nutrition, and the need for labor-intensive managment.

The key limiting nutrient for *Azolla* is P (Tung and Shen, 1981; Tung and Watanabe, 1983; Watanabe et al., 1980). Growth rates have been reported to exceed an N content of 1 g/m^2 when available P (Olsen P) is greater than 25 mg/kg soil; optimum soils have a P-absorbing capacity of less than 4.4 g/kg soil (Watanabe and Espinas, 1976; Watanabe and Ramirez, 1984). Potassium, Ca, Mg, and some trace elements are also important for growth and biological N_2 fixation (Espinas et al., 1979; Kondo et al., 1989; Watanabe et al., 1977; Yatazawa et al., 1980).

The optimum ambient temperatures for growth of *Azolla* are between 20 and 30°C, except for *A. rubra,* which thrives at cooler temperatures (Singh, 1977; Watanabe and Berja, 1983). Several species can tolerate temperatures of 30–35°C (Peters et al., 1980; Talley et al., 1977). Excessive irradiance (>50% full sunlight with moderate temperatures) may be alleviated by partial shading of *Azolla* mats by a rice canopy, but growth may still be good if other conditions are favorable (Achtnich et al., 1986).

Azolla grow well at pH 4–9; therefore, pH is not a limiting factor for *Azolla* in most submerged rice fields (Holst and Yopp, 1979). However, salinity tolerance is minimal and *Azolla* is not resistant to desiccation (Ge et al., 1980; Haller et al., 1974; Zimmerman, 1985a). Stable growth is also enhanced when physical disturbance by wind or water action is averted (Ashton and Walmsley, 1976; Singh, 1977). Water levels of 3–5 cm provide for good mineral nutrition for *Azolla* because of direct root contact with the soil. However, deeper water, which attenuates variation in temperature, is an advantage in summer when temperature is high (Watanabe, 1982).

When all of these conditions are optimized and biological interactions such as grazing or competition from other vascular plants and algae are controlled, *Azolla* can double its fresh weight in 2 days (Peters et al., 1980; Tung and Watanabe, 1983). Even when certain environmental factors are not optimal, the doubling time for *Azolla* might be 10 or fewer days (Becking, 1979; Tung and Shen, 1981; Zimmerman, 1985b).

C. Management Technology

Current management of *Azolla* consists of three stages: (1) the maintenance of inocula between rice-growing seasons, (2) cultivation to obtain sufficient quantities in the field, and (3) its agronomic application as a green manure. These stages are preceded by the selection of appropriate *Azolla* germplasm.

Germplasm

Azolla classification is often tentative because it is morphologically plastic, especially the taxa of section Azolla. Accessions in germplasm collections tend not to develop into mature populations with differentiating features. Ecophysiological traits may be helpful in identification, and progress has been made in biochemical fingerprinting (van Hove et al., 1987; Zimmerman et al., 1989a,b). Scientists at the

International Rice Research Institute, (IRRI), which houses the world's largest germplasm collection of *Azolla,* expedite selection of desireable accessions for agronomic use by conducting trials to test characteristics such as stress tolerance and decomposition rate.

Shipping fresh *Azolla* to farmers is possible if the containers (plastic bags or petri dishes) are sealed to prevent desiccation. There should be no standing water, which promotes mechanical damage and deterioration, and the biomass should be free of plant and animal epiphytes. The packaged *Azolla,* although preferably kept cool (5°C), can survive for at least two weeks in transit and may be shipped internationally through routine airmail services. When available in sufficient quantities, spores are the preferable form of germplasm for transport.

Maintaining the Inoculum

Maintaining inocula of *Azolla* between cropping season is a major problem for rice farmers. The plants are usually cultivated in greenhouse nurseries or in outdoor ponds or fields. Because *Azolla* must be overwintered or oversummered, protecting the plants against extreme temperatures is a constant task. They must also be guarded against mechanical injury, fungal infection and grazers.

Enclosed greenhouses ameliorate the effects of cold temperature, and an available source of warm water provides additional protection (Ye and Wu, 1964), or the live *Azolla* may even be insulated with moist rice straw (Anwei Academy of Agricultural and Forestry Science, 1974; IRRI, 1982). Sites with cool water, partial shade, and good ventilation are necessary for oversummering *Azolla.* The amount of live biomass to be maintained depends on the season and the local agroecosystem. In parts of China, for example, 400–600 kg of inoculum must be overwintered for 1 ha of transplanted spring rice, whereas a summer rice crop with a longer period between growing seasons requires only 7.5–15 kg of inoculum (Lumpkin, 1987).

The problem of maintaining *Azolla* vegetatively would be eliminated if mass quantities of spores could be reliably obtained. Technologies to induce mass sporulation and efficiently harvest the spores have not been developed, although some experimental work has been conducted (Ke et al., 1981; Li, 1984; Toia et al., 1987; Wang et al., 1981). Attempts have been made to induce mutations in *Azolla* spores with ^{60}Co irradiation (Fujian Academy of Agricultural Sciences, personal communication).

Spore germination is slow, which may conflict with the rice-transplanting schedules. In China, spore germination and maturation take at least a month (Wang et al., 1981; Lumpkin, 1987); residual *Azolla* spores in Philippine rice fields require 40–60 days for germination (Payawal and Paderon, 1986).

Field Inoculation

Field cultivation of *Azolla* entails a coordinated management to obtain rapid multiplication of inocula in rice fields or in adjacent bodies of water. By either means, the initial density of *Azolla,* when applied to the field, directly affects its successful establishment. Suboptimal densities encourage the presence of weeds. The amount of inoculum to be applied varies between 300–500 kg and 2–5 tons/ha of fresh biomass (Roger and Watanabe, 1986; Singh, 1979; Watanabe, 1982).

Phosphorus amendments to the soil are generally required. Split applications are more efficient for rapid inoculum growth than are single basal applications. One

kilogram of P_2O_5 per hectare every 4 days has been recommended, although this may be a minimum requirement (Watanabe et al., 1980). In Vietnam, field application of *Azolla* is combined with split applications of P, K, and farmyard manure. Ash is sometimes substituted for the chemical amendments (IRRI, 1982). Vietnamese farmers utilize the half-saturation method of cultivation. *Azolla* is first grown in small sections of the field until the surface of the water is covered, then half of the biomass is transferred to new sections. The process is then repeated. The mat is intentionally fragmented to increase vegetative propagation. Alternatively, the harvested half of the azolla biomass may be incorporated into the soil (Watanabe, 1982; Roger and Watanabe, 1986).

Agronomic Practices

Three different cropping systems are possible with *Azolla* (Lumpkin, 1987; Metting et al., 1990). One is to cultivate *Azolla* as a monocrop in the field and then incorporate it into the soil before transplanting rice. A variation of this method is to grow *Azolla* beforehand and to incorporate it as dried biomass. However, the mineralization rate is slower and the N input is only half of that derived from live plants (Kaushik and Venkataraman, 1981; Ito and Watanabe, 1985).

A second method is to grow the fern as a cover crop with rice to either decompose naturally or to be mixed into the soil as a topdressing (Fig. 5). An inorganic N fertilizer may be added with the topdressing or further intercroppings of *Azolla* may be grown and incorporated into the field. Nitrogen is consequently available for the rice crop during growth and maturation.

The third system, which makes N continually available to the crop, is to combine monocropping and intercropping. The labor required for management of this complete utilization of Azolla is that much greater.
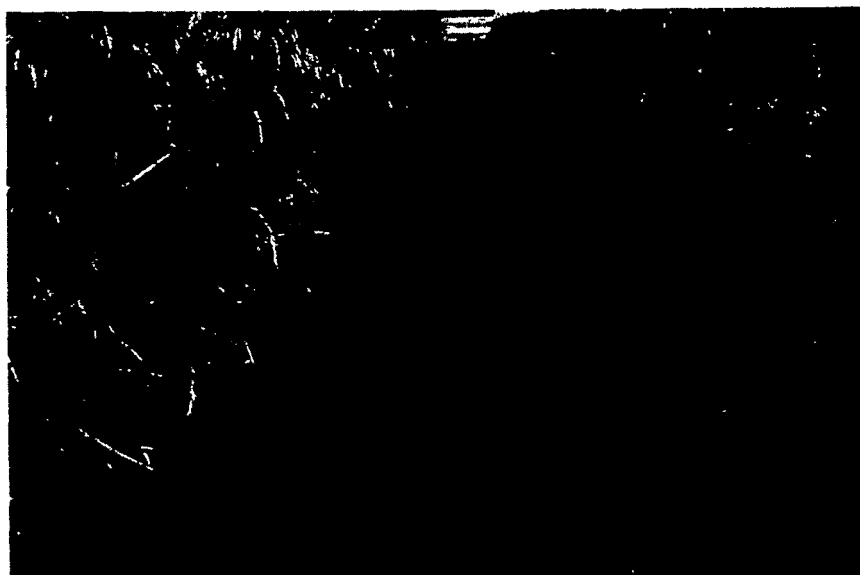

Figure 5 *Azolla* grown as a cover crop with irrigated rice in the Cauca Valley, Colombia, South America.

The decomposition rate of *Azolla* can be significant in its effect on rice plants, as extravagant green manuring may result in lodging and increased grazing or disease. Even the thickness of the *Azolla* mat must be considered, relative to its effect on the water temperature and C/N ratio (Lumpkin and Plucknett, 1982; Watanabe, 1982). The timing and method of incorporating into the soil also affect *Azolla* effectiveness as green manure.

The estimated fresh weight for a mat of *Azolla* is 10 tons/ha, which is calculated to release 20–30 kg N. Successive croppings of *Azolla* yield at least 100 kg/ha of N, which can produce a high-yielding rice crop. *Azolla* incorporated before rice planting or as an intercrop, plus 30 kg/ha of N as urea can give similar or greater rice yields than 60 kg/ha of N alone (Singh and Singh, 1986a,b).

A summary of field trials with *Azolla* has shown that rice grain yields ranged from 2.6 to 5.6 tons/ha (Kikuch et al., 1984), which is about the same yield when 30–40 kg/ha of chemical N fertilizer is applied. The efficiency of uptake of mineralized N from *Azolla* by rice plants is generally similar to that of $(NH_4)_2SO_4$ or urea (IRRI, 1987; Ito and Watanabe, 1985; Kikuchi et al., 1984; Watanabe and Berja, 1983).

D. Future Technology

The key economic costs in *Azolla* utilization are those of P, labor, and pest control. Rice-growing areas, such as Vietnam and parts of the Philippines, where the fern is easily cultivated and local phosphate deposits are found, are conducive to use of *Azolla* (Kikuchi et al., 1984), although there may be exceptions (Rosegrant et al., 1985).

However, with the advent of available, cheap sources of urea and potash, the area devoted to *Azolla* technology decreased dramatically during the 1980s in areas where it has been traditionally used. In Fujian Province, China, use of *Azolla* has decreased to 5–10% of the rice hectarage from 20% at the beginning of the 1980s. This has also been observed for green manures such as *Astragalus* currently used in 10–20% of the land, down from a maximum of about 30% (Liu Chung, Chu, personal communication). This trend is less extreme in Vietnam. The reduction of *Azolla* use in China also stems from the changing governmental economic policy that has led to the disbanding of many agricultural communes and the reallocation of labor. World use is now a fraction of the estimated 2 million ha of rice that were fertilized with *Azolla* in just China and Vietnam in the late 1970s (Dao and Tran, 1979; IRRI, 1982; Liu, 1979; Roger and Watanabe, 1986).

Economic calculations should also consider the long-term benefits of *Azolla* as an organic fertilizer, with the concomitant increase in soil organic matter and fertility, in addition to those costs directly comparable with commerical N fertilizer prices. Nevertheless, improvements in international research coordination and management–extension are essential for expanding *Azolla* utilization in rice-growing countries. One example is the recent Chinese practice of rotating *Azolla* spp. with successive rice crops to meet season environmental constraints (Cheng et al., 1981).

Biological advances, specifically the creation of improved *Azolla* hybrids, recently achieved by researchers in the Philippines, Thailand, and China, and an understanding of the mechanism for inducing sporulation are also essential. Other

innovations requiring further exploration include the artificial reconstitution of the symbiosis using stress-tolerant varieties of both host and cyanobiont (Watanabe et al., 1989). Secondary achievements include the continuing selection for superior germplasm. An increased practical knowledge will also result from the present efforts of scientists to understand the molecular genetics of the *Azolla* symbiosis.

IV. FREE-LIVING BLUE-GREEN ALGAE

Blue-green algae (cyanobacteria) constitute the largest, most diverse, and most widely distributed groups of photosynthetic prokaryotes (Stanier and Cohen-Bazire, 1977). The N_2-fixing ability of several genera has implications for the maintenance of the fertility of natural and cultivated ecosystems and many trials have been conducted to increase rice yield by blue-green algae inoculation. These aspects have been discussed in several reviews (Roger, 1990a; Roger and Kulasooriya, 1980; Roger and Reynaud, 1982; Roger and Watanabe, 1986).

A. Ecology of Blue-Green Algae in Rice Fields

Environmental Factors

Blue-green algae are ubiquitous in rice fields, contrary to early reports (Watanabe, 1959; Venkataraman, 1975). Quantitative estimates of N_2-fixing forms in rice soils range from 10^1 to 10^7/g dry soil, with a median of 2×10^4 from 396 measurements. Their abundance is positively correlated with soil pH and available P (Roger et al., 1987). In arid tropics, abundant light and high temperatures may inhibit blue-green algae growth (Reynaud and Roger, 1978). In wet monsoonal zones, they may develop early in the crop cycle during the dry–warm season (Gupta, 1966). During the wet season, light deficiency and disturbance by heavy rain may limit their growth (Ichimura, 1954; Roger, 1990a; Watanabe, 1961). High water temperatures (30–35°C) favor blue-green algae versus eukaryotic algae, but temperatures higher than 40°C may be inhibitory (Roger and Kulasooriya, 1980). Spore-forming blue-green algae can withstand long periods of desiccation, which results in their higher relative abundance after a dry period (Roger and Kulasooriya, 1980) and explains the higher prevalence of *Nostoc* in dry rice field soils (80%) than in wet ones (47%) (Roger et al., 1987).

Biotic Factors

The major biotic factor limiting cyanobacterial growth is grazing by invertebrates such as cladocerans, copepods, ostracods, mosquito larvae, and snails. (Grant et al., 1986). Diet preferences are exhibited, with strains that form mucilaginous colonies being less susceptible to grazing than noncolonial strains. Grazing thus leads to the dominance in rice fields of mucilaginous blue-green algae, especially *Nostoc* spp. which are often less active in biological N_2 fixation than are the noncolonial forms (Antarikanonda and Lorenzen, 1982; Grant et al., 1985; Roger et al., 1987).

Cyanophages (Singh, 1973) and myxobacteria pathogenic to blue-green algae (Huang, 1982) have been isolated from rice fields. Antagonisms among blue-green algae and other algae or aquatic weeds have been observed, but the mechanisms involved are unknown (Kulasooriya et al., 1980; Saito and Watanabe, 1978).

Effects of Agrochemicals on Blue-Green Algae

Mineral N is known to inhibit N_2 fixation by cultures of blue-green algae. Roger and Kulasooriya (1980) listed seven references reporting inhibition of blue-green algae growth by N fertilizer in rice fields. Recent data confirm that broadcast N fertilizer often strongly inhibits photodependent biological N_2 fixation (75% of 60 cases studied) and show a negative correlation between photodependent biological N_2 fixation and N use efficiency (kilogram rice produced per kilogram of N applied) (Roger, 1990b). Broadcast application of N fertilizer, which is widely practiced by farmers, not only inhibits photodependent biological N_2 fixation, but also causes fertilizer losses by NH_3 volatilization. In contrast, deep placement of N fertilizer decreases N losses by volatilization and reduces the inhibitory effect of N fertilizer on blue-green algae (Roger, 1991; Roger et al., 1980).

Phosphorus is a key nutrient for rice field blue-green algae, which are often P deficient (Roger et al., 1986). Its application stimulates photodependent biological N_2 fixation and blue-green algae growth, especially in acidic soils (Cholitkul et al., 1980). Split application increases growth more efficiently than basal application (IRRI, 1986).

Blue-green algae seem to be more resistant to pesticides than other algae and frequently tolerate levels higher than those recommended for application (Chinnaswamy and Patel, 1984). Insecticides have an indirect stimulatory effect by decreasing grazer populations (Roger and Kulasooriya, 1980).

B.   Potential of Blue-Green Algae as a Biofertilizer for Rice

The extent to which blue-green algae contribute to the N requirement of the crop is determined by the algal standing biomass and turnover time, the rate of N_2 fixation, and the extent to which N from blue-green algae becomes available to the plant. In addition, blue-green algae might benefit rice by mechanisms other than biological N_2 fixation.

Blue-Green Algae Biomass and Nitrogen Fixation

Blue-green algae can develop impressive blooms in rice fields. Standing crops of 5–20 tons/ha fresh weight are usually recorded for growth visible to the naked eye. But because of the low and wide range of dry matter (0.2–14%) and high ash (31–71%) content of fresh blue-green algae, a bloom usually represents less N than 10 kg/ha (Roger et al., 1986). The median of 400 weekly biomass measurements in 65 plots on the IRRI farm when blue-green algae were visible was 4 kg/ha of N, and the maximum was 17 kg/ha of N (Roger, 1990a). Nitrogen contribution by blue-green algae is largely the result of nutrient turnover of the standing biomass, for which no data are yet available. However, the observation that blue-green algae usually do not bloom more than twice during a crop cycle indicates a rough N potential of 30 kg/ha per crop.

Estimates of biological N_2 fixation by blue-green algae in rice fields published before 1980 range from a few to 80 kg/ha of N per crop, with an average of 27 kg (Roger and Kulasooriya, 1980). Figure 6 presents the distribution of 190 estimates of the average ARA during a crop cycle in experimental plots subjected to various cultural practices. The histogram is biomodal: the left part corresponds to a log-normal distribution of low ARA values measured mostly in plots where photo-
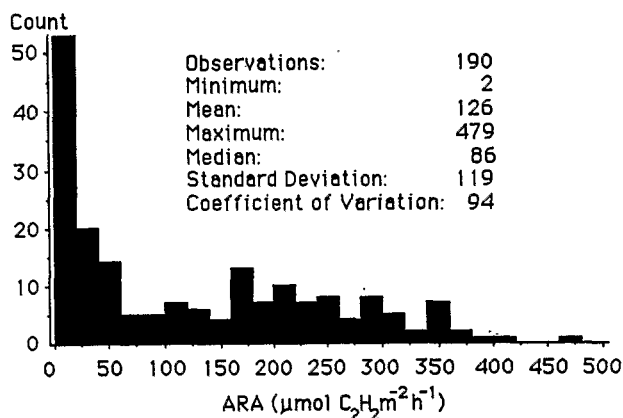
Figure 6 Distribution of 190 estimates of the average ARA during a crop cycle in experimental plots at IRRI. (From Roger et al., 1992, and unpublished data.)

dependent biological N_2 fixation was inhibited by N fertilizer application; the right part is a more or less bell-shaped distribution of activities recorded mostly in plots with deep placement or no N fertilizer. If one assumes an acetylene reduced/N_2 fixed ratio of 4, biological N_2 fixation expressed in kilograms N per hectare per crop is roughly one-tenth the value of the average ARA over the crop cycle, expressed in micromoles C_2H_2 per square meter per hour. Extrapolated values for N average about 19 kg/ha per crop in no-N control plots, 7 kg in plots with broadcast urea, and 12 kg in plots where N was deep-placed (Roger, 1991).

Field Experiments on Blue-Green Algae Inoculation

Field inoculation experiments provide indirect information on the overall potential of blue-green algae. In experiments summarized by Roger and Kulasooriya (1980), the relative increase in yield over the control averaged 14% (450 kg/ha). A recent compilation of 634 field experiments showed a very large variability of the difference in yield between inoculated and noninoculated plots (CV > 100%) (Roger, 1991). Because of the asymmetric data distribution, the median grain yield (257 kg/ha) was considered a better index of the average effect of inoculation than the mean (337 kg/ha). Although the difference in average yield between inoculated and noninoculated plots was significant (p < 0.01), only 17% of the 634 individual observed differences were statistically significant. This indicates a small and variable response of yield to algal inoculation and also an experimental error frequently larger than the response. When interpreting data from the literature, it should also be remembered that unsuccessful trials have often not been reported.

Utilization of Blue-Green Algae Nitrogen by the Rice Plant

No information is available on *in situ* N exudation by blue-green algae, but because algal photosynthesis increases floodwater pH, it is foreseeable that during the buildup of the bloom, most of the excreted N will be either reimmobilized or lost by NH_3 volatilization (Roger and Kurihara, 1988). Therefore, most N originating from blue-green algae is probably made available to the crop through mineralization after the death of the algae. Late decomposition of the bloom during the cycle and the resulting late availability of N to rice might increase N content of the grain

without increasing yield (Grant et al., 1985). Depending on the nature of the material (fresh vs dried), the method of application, and the presence or absence of soil fauna, recovery of blue-green algae N by rice averages 30% and varies 13–50% (Roger 1992). Recovery was highest with fresh blue-green algae incorporated into a soil depleted of fauna. It was lowest with dried blue-green algae applied on the surface of a soil rich in tubificid worms, which reduce the recovery of algal N by rice by making more soil N available through mineralization (Grant and Seegers, 1985).

Beneficial Effects Other Than Nitrogen Supply

The N_2-fixing ability of blue-green algae is the principal, but probably not the only reason for increased rice yields. Other possible beneficial effects summarized by Roger and Kulasooriya (1980) include (1) competition with weeds, (2) increased soil organic matter content and improved soil aggregation, (3) excretion of organic acids that increase P availability to rice, (4) inhibition of sulfide injury in sulfate reduction-prone soils by increased O_2 content and plant resistance to sulfide, and (5) production of plant growth regulators (PGR) that enhance rice growth.

There have been many claims that blue-green algae can benefit rice plants by producing PGRs. Roger and Kulasooriy (1980) cited 12 references reporting that presoaking seeds or seedlings in blue-green algae cultures or extracts enhanced germination, growth, or yield. However, when Pedurand and Reynaud (1987) studied the effect of 133 unialgal strains on rice germination and growth, 70% had a negative effect on germination, and only 21% had a stimulatory effect. The PGR-like effects of blue-green algae cultures were likened to those of vitamin B_{12}, gibberellins, and amino acids; but, as Metting and Pyne (1986) pointed out, despite the numerous reports on algal PGR effects, none showed the isolation and characterization of a microalgal PGR. The beneficial effects of algal inoculation in rice fields might be partially due to PGRs, but the relative importance of their contribution to rice productivity is still unknown.

C.   Management Technology

Algal Inoculation Technology and Its Current Status

Applied research on blue-green algae inoculation is conducted mostly in India where the All-India Coordinated Project on Algae was initiated in 1977 and, to a lesser extent, in Burma, Egypt, and China. A similar technique of growing blue-green algal inocula in shallow open-air ponds is used in India, Egypt, and Burma (Venkataraman, 1981). A multistrain starter inoculum produced from laboratory cultures is propagated, on the spot, in shallow trays or microplots with 5–15 cm of water, about 4 kg/m^2 of soil, 100 g/m^2 of superphosphate, and insecticide. When necessary, lime is added to adjust soil pH to 7.0–7.5. In 1–3 weeks, an algal mat develops that is then allowed to dry. Algal flakes are scraped off and stored for further use at 10 kg/ha (Venkataraman 1981).

Blue-green algae inoculation is currently used on a trial-and-error basis. Methods to estimate the chance of success of inoculation in a given agroecosystem are unavailable because the factors underlying yield increases associated with successful algal inoculation are not clearly understood or quantified. Most experiments measured only grain yield and did no estimation of inoculum quality or establishment. No published study reporting a significant increase in yield

after algal inoculation includes biological N_2 fixation or blue-green algae biomass estimates.

Reports on the adoption of algal inoculation are somewhat controversial, but even with the most optimistic evaluations, adoption seems to be restricted to a limited area in a few Indian states, in Egypt, and possibly in Burma. In 1985, Roger and associates reported that algal inoculation was adopted in only two states of India (Tamil Nadu and Uttar Pradesh) where inoculated fields constituted a small percentage of the total area planted to rice. Farmers' limited acceptance of algal inoculation probably reflects the low and erratic increases in yield obtained.

Reconsidering Agronomic Utilization of Blue-Green Algae

Methods for utilizing blue-green algae in rice cultivation need to be reconsidered in view of recent studies showing that (1) N_2-fixing blue-green algae are ubiquitous in rice soils, (2) their growth in rice fields is most commonly limited by low pH, P deficiency, grazing, and broadcasting of N fertilizer; (3) nonindigenous strains inoculated in various soils rarely establish themselves; and (4) indigenous N_2-fixing blue-green algae are frequently more numerous than blue-green algae contained in the recommended dose of 10 kg of soil-based inoculum (Bisoyi and Singh, 1988; Grant et al., 1985, Reddy and Roger, 1988; Reynaud and Metting, 1988; Roger et al., 1987). These findings suggest that more attention should be paid to cultural practices to alleviate factors that limit growth and N_2 fixation by indigenous strains already adapted to the environment. Practices known to enhance blue-green algae growth include liming of acidic soils, P split application, grazer control, and deep placement of N fertilizer (Roger and Watanabe, 1986). These practices might suffice to realize more of the potential for blue-green algae and are a prerequisite for establishing inoculated strains, if and when inoculation is needed.

The ubiquity of heterocystous blue-green algae in rice soils does not mean that inoculation is unnecessary. Inoculation with desired strains might be useful because the accumulation of P by the propagules of the inoculum (produced with high levels of P) gives them an initial advantage over the propagules of the indigenous blue-green algae, which are usually P-deficient (Roger et al., 1986). Because spore germination is photodependent (Reddy, 1983), inoculated propagules applied on the soil surface should germinate more readily than the indigenous propagules mixed with the soil. The effect of inoculation is likely to be more important after an upland crop grown before rice, or after a long dry fallow, when the density of indigenous blue-green algae population may be low at the beginning of the rice season. Inoculation might also permit early establishment of an N_2-fixing bloom and, thereby, the availability of more fixed N to rice.

But there still are many uncertainties about the methodological aspects of blue-green algae inoculation and the nature of inocula to be used. Most algalization trials have been conducted using inocula developed from a mixture of laboratory cultures, but almost none of the published inoculation experiments have paid attention to the establishment of inoculated strains. There is increasing evidence that most of the blooms obtained with various treatments combined with inoculation are from indigenous strains (Bisoyi and Singh, 1988; Grant et al., 1985, Reddy and Roger, 1988; Reynaud and Metting, 1988). When the inoculum is produced on the spot in small plots or trays, it is probable that strains present in the added local soil may outcompete the intended isolates, even before the inoculum is added to the field.

Available data are not sufficient to draw definite conclusions, but they clearly suggest that use of an inoculum produced from the soil to be inoculated should be tested whenever experiments are conducted.

Biotechnology and Improved Strains of Blue-Green Algae

The prokaryotic nature of blue-green algae has permitted use of molecular methods developed for other bacteria (Brusslan and Haselkorn, 1988). Significant progress toward genetic manipulation of blue-green algae has been made, particularly in the areas of herbicide resistance and N_2 fixation (see Chap. 16). One can speculate on the possibility of selecting or designing efficient strains for inoculation. Several authors have screened for high N_2-fixing activity, but their findings may not be useful, because there is little reason *a priori* why high N_2-fixing activity should correlate well with in situ colonization potential. In fact, most fast-growing strains (doubling time less than 12 hr) belong to the genus *Anabaena,* have short filaments and, therefore, are susceptible to grazing. A study of 12 strains showed that those with high N_2-fixing activity in vitro did not become established in situ (Antari-kanonda and Lorenzen, 1982; Huang, 1983).

The selection of nitrogenase-derepressed mutants, which excrete NH_4^+ into the medium, is another attractive approach. Such an *Anabaena variabilis* mutant was found to provide N to rice in a N-free gnotobiotic culture more efficiently than the parent strain (Latorre et al., 1986). However, preliminary studies at IRRI showed that the strain is not competitive and did not multiply when inoculated in soil microplots (Roger et al., unpublished data).

Biological engineering of blue-green algae is currently limited to unicellular strains that are morphologically, physiologically, and ecologically very different from the N_2-fixing strains considered for inoculating rice fields. Probably "super N_2-fixing blue-green algae" can be selected or designed and grown in test tubes, but the characteristics that will enable them to survive, develop, and fix N_2 in rice fields are still largely unknown. The immediate need is for a better understanding of cyanobacterial ecology, but genetic engineering may also contribute to their agronomic use in the long term.

V. MANAGEMENT OF THE RHIZOSPHERE MICROFLORA

A. Inoculation with Nitrogen-Fixing Bacteria

Nitrogen-Fixing Bacteria and Associative Biological Nitrogen-Fixation in the Rice Rhizosphere

Genera of N_2-fixing bacteria isolated from the rice rhizosphere include *Agromonas, Alcaligenes, Aquaspirillum, Azospirillum, Beijerinckia, Citrobacter, Enterobacter, Flavobacterium, Klebsiella,* and *Pseudomonas* (Roger and Watanabe, 1986). The N_2-fixing bacteria most frequently isolated from rice using root exudates of rice seedlings as the C source were Enterobacteriaceae, *Azospirillum* spp., and *P. paucimobilis* (Bally et al., 1983; Omar et al., 1989; Thomas-Bauzon et al., 1982)).

Estimates of associative biological N_2 fixation summarized by Roger and Watanabe (1986) show that ARA associated with rice is usually highest at or near heading stage and ranges from 0.3 μmol/hr of C_2H_4 per plant in temperate regions to 2 μmol/hr of C_2H_4 per plant in the tropics. If we assume (1) that ARA measured

at heading lasts for 50 days, (2) an ethylene/N_2 rate of 4:1, and (3) a plant density of $25/m^2$, the estimated N_2-fixing rate would be 0.8–6 kg/ha of N per crop cycle. The N_2-fixing rate extrapolated from ^{15}N incorporation experiments is 1.3–7.2 kg/ha per crop, suggesting that the potential for exploiting associative biological N_2 fixation is the lowest among the N_2-fixing agents discussed in this review.

General Effects of Bacterial Inoculation

Table 2 presents a summary of the analysis of data from 23 articles reporting 210 trials on bacteria inoculation of rice. Most of those studies considered grain yield or grain and straw yield only. Therefore, as with blue-green algae, it is difficult to interpret the results because experimental data on inoculum establishment and N_2-fixing activity in inoculated plants are scarce.

Table 2 Analytical Summary of 210 Sets of Data on the Effect of Bacterial Inoculation on Rice Yield: Bibliographic Study

	Grain yield			Straw yield difference (%)	Harvest index (grain/straw)		N efficiency (kg grain/kg N)	
	control (t/ha)	difference (t/ha)	difference (%)		Control	Inoc.	Control	Inoc.
All data (123 field experiments, 87 pot experiments)								
Mean	4.0	0.5	19.8	15.9	0.66	0.69	18.7	19.1
SD	1.6	0.5	25.0	16.2	0.20	0.22	17.1	13.7
Maxi	11.1	2.4	125.0	66.7	1.12	1.20	78.0	54.7
Mini	1.0	−1.5	−32.6	−20.9	0.10	0.10	±20.0	−12.0
No. of data	121	121	210	130	130	130	60	59
Field experiments (123)								
Mean	3.99	0.52	14.35	15.06	0.57	0.57	18.68	19.10
SD	1.64	0.52	14.14	14.88	0.17	0.17	17.15	13.68
Maxi	11.09	2.35	59.63	64.84	0.86	0.88	78.00	54.67
Mini	1.00	−1.50	−25.00	−7.49	0.25	0.25	−20.00	−12.00
Count	121	121	123	51	51	51	60	59
Inoculation with *Azotobacter* (40 field experiments, 18 pot experiments)								
Mean	3.9	0.4	16.6	13.3	0.52	0.54	18.5	17.0
SD	1.6	0.5	20.4	15.5	0.20	0.21	18.9	12.3
No. of data	40	40	58	28	28	28	26	23
Inoculation with *Azospirillum* (83 field experiments, 11 pot experiments)								
Mean	4.1	0.6	15.2	16.0	0.62	0.62	18.8	21.4
SD	1.7	0.5	18.3	13.3	0.21	0.21	16.0	14.8
No. of data	81	81	94	45	45	45	34	33
Inoculation with other bacteria (58 pot experiments, no field experiments)								
Mean			30.6	17.1	0.75	0.81		
SD			34.2	18.5	0.15	0.15		
No. of data	0	0	58	57	57	57	0	0

Source: Data from Balasubramanian and Kumar, 1987; Balasundaram and Sen, 1971; Charyulu et al., 1985; Gopalaswami and Vidhyasekaran, 1987a,b; Jalapati Rao et al., 1977; Jeyaraman and Purushothaman, 1988; Kannaiyan et al., 1980; Kavimandan, 1986; Kundu and Gaur, 1984; Maskey, 1976; Nayak et al., 1986; Omar et al., 1989; Patil et al., 1976; Prasad N. N., 1986; Prasad J., 1987; Raman Rai, 1985; Rajagopalan and Rangaswamy, 1988; Rajaramamohan, 1983; Subba Rao et al., 1979; Watanabe and Lin, 1984.

When all data are considered, the average effect of inoculation is a 19.8% increase in grain yield, but the responses are highly variable, ranging from −33 to +125% (see Table 2). As with blue-green algae (Roger and Kulasooriya, 1980), pot experiments seem to overestimate the effects of inoculation on grain yield. Average increase is 27.6% in pot experiments (87 datum points) and 14.4% in the field (123 datum points).

Field experiments show an average increase in yield (+14.4%) that is close to the minimum detectable difference (14.5%) that can be expected from the experimental design most commonly used in field experiments (16-m^2 plots, with four replicates) (Gomez, 1972). This indicates that experiments in which no statistical analysis was conducted should be interpreted with caution. In field experiments, the relative differences in grain yield between inoculated and noninoculated plots is also quite variable, ranging from −25 to +69%, which corresponds to a coefficient of variation (cv) of about 100% (see Table 2). The distribution of the values (Fig. 7) is very asymmetric, and the median (11%) might be a better index of the average effect of inoculation than the mean. The general shape of the histogram, which exhibits an abrupt raise of the first class of positive values, suggests a bias in the data and the possibility that unsuccessful field trials were not always reported.

Inoculation did not invariably increase yield. Different investigators found (1) no effect (Rajagopalan and Rangaswami, 1988), (2) inconsistent effects among successive trials in time or various simultaneous treatments (Maskey, 1976; Rajaramamohan Rao et al., 1983), and (3) negative effects of inoculation. Kavimandan (1986) reported no effect of *Azotobacter* inoculation on grain and straw yield and erratic effects of *Rhizobium,* including significant increases and decreases in grain and straw yield. Jeyaraman and Purushothaman (1988) reported positive effects of *Azospirillum* inoculation in the absence of fertilizer and when N was applied at a rate of 50 kg/ha, but also a statistically significant decrease in yield by 25% in plots for which N was applied at a rate of 75 kg/ha. Similarly, in a field experiment with two rice varieties, *Azospirillum* inocuation significantly increased yield by 19 and 43% without N fertilizer, but significantly decreased yield by 33 and 31% with 92 kg/ha of N (Charyulu et al., 1985). This was attributed to enhanced denitrification by the added bacteria.
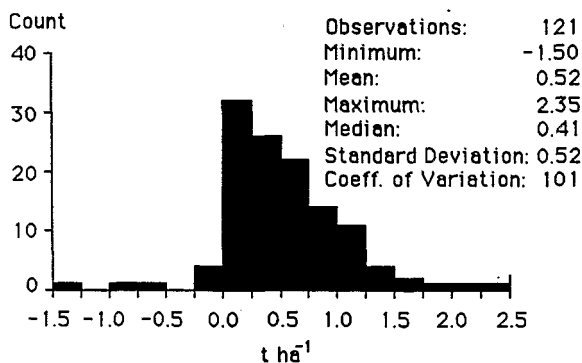

Figure 7 Histogram of the difference in yield between noninoculated plots and plots inoculated with various strains of bacteria, calculated from 121 sets of data reported by various authors listed in Table 2.

Effect of Nitrogen

Associative biological N_2 fixation seems to be less sensitive to N fertilizer application than other N_2-fixing systems (Roger and Watanabe, 1986). This might explain why almost any kind of trend in the response of rice to inoculation at various levels of N fertilizer has been reported. Patil and associates (1976) found no clear relation between N fertilizer level and effect of inoculation with *Azotobacter*. Some reports indicate a better effect of inoculation with *Azotobacter* and *Azospirillum* without N fertilizer (Dewan and Subba Rao, 1979), or at low N levels (Balasubramanian and Kumar, 1987). Jalapathi Rao et al. (1977) observed a negative correlation between N level and effect of inoculation with *Azotobacter*. Several reports indicate that the effect of inoculation was lower without N fertilizer or at high levels of N fertilizer than at low or at medium levels (Balasubramanian and Kumar, 1987; Balasundaram and Sen, 1971; Rajaramamohan Rao et al., 1983). There are also reports of significant effects of inoculation with *Azospirillum* at high N levels (Charyulu et al., 1985; Omar et al., 1989).

Nitrogen fertilizer has also been reported to affect efficiency of N_2 fixation. A *Spirillum* sp. isolated from the roots of rice plants grown with 20–40 kg/ha of N had a higher N_2-fixing efficiency (3.5–4.0 mg N fixed per gram malate in 72 hr) than a *Spirillum* sp. isolated from plants grown with 60–100 kg/ha of N (2.5 mg N fixed per gram malate in 72 hr). The *Spirillum* sp. isolated from the roots of rice plants grown without N had the lowest efficiency (2 mg N fixed per gram malate in 72 hr) (Nayak and Rajaramamohan Rao, 1977).

Inoculation Methods

Inoculation of rice has been performed by (1) dipping seeds or coating them with various carriers, (2) dippling seedlings in bacterial cultures, (3) nursery soil inoculation, (4) field inoculation, and (5) foliar application. Little attention has been paid, however, to the relative efficiency of these methods. In a comparison of seven combinations of seed, seedling, and soil inoculation, a significant increase in yield was obtained only when seedlings and soil were inoculated (Gopalaswamy and Vidhyasekaran, 1987a,b). Foliar application of *Azotobacter* increased yield by 7.5% without N ferilizer and by 3.7% with 50 kg/ha of N (Kannaiyan et al., 1980).

No cultural practice has been shown to consistently enhance the associative biological N_2 fixation process and to favor rice response to inoculation.

Strain Selection

The nature of the strain might influence the inoculation effect. However, the overall effects of inoculations with *Azotobacter* and *Azospirillum* (see Table 2) do not statistically differ, as shown by relative increases in grain yield by 16.6% with *Azotobacter* and 15.2% with *Azospirillum*. The higher increase observed with other bacteria (see Table 2) is, most probably, because these experiments were conducted only in pots.

If the positive effect of inoculation on plant growth is at least partly because of enhanced biological N_2 fixation, the selection or improvement of N_2-fixing strains is one possibile way to increase efficiency. In selecting the most efficient combination of a N_2-fixing bacterial strain and a specific rice cultivar, Heulin et al. (1982, 1989) used a two-step process in which bacterial strains were isolated from the rhizosphere of actively N_2-fixing rice plants and were then tested with rice cultivars by a gnotobiotic

system known as the spermosphere model in which an axenic rice seedling is grown in darkness in a Pankurst tube on a medium without C or N (Thomas-Bauzon et al., 1982). This system provides a means to test bacterial strains with rice exudates as the sole C source. The atmosphere of the tubes contains 1% acetylene and each "strain × cultivar" combination is characterized by its maximum ethylene production using 15–20 replicates. This approach was used by Charyulu et al. (1985) to screen seven strains of bacteria and seven rice varieties. The association considered most efficient (*Azospirillum lipoferum* 4B × rice Cesario M7) was tested in the field, but differences in yield were erratic in three independent field experiments (+27% with no N, −28% with 92 kg/ha of N, +43% with 120 kg/ha of N, and −4% with 150 kg/ha of N). Omar et al. (1989) used a similar approach and reported significant yield increases, ranging from 6 to 21% in field experiments in Egypt. Higher increases were obtained at higher N levels (76–96 kg/ha of N). However, yields of 6 and 9 tons/ha obtained in the control plots without N fertilizer indicate that the experiment was conducted under conditions not frequently observed in rice culture. The approach to improve the effects of inoculation used by Charyulu et al. (1985) and Omar et al. (1989) considers both the soil to be inoculated and the rice variety to be grown for selecting the most efficient strain. The potential for practical utilization of this method strongly depends on the degree of specificity required to select an efficient bacterium for given agroecological conditions.

Another aspect to consider is inoculation with a mixture of strains. A better response to inoculation was observed when *Azotobacter* was combined with P-solubilizing bacteria (Kundu and Gaur, 1984). Dewan and Subba Rao (1979), however, observed that dual inoculation with *Azospirillum brasilense* or *Azotobacter chroococcum* gave lower (26%) increase in yield than single inoculations (49 and 62%, respectively).

Establishment and Persistence of Inoculated Strains

Little information is available on the establishment of inoculated strains. Studies on *Azotobacter* reported better establishment in the rhizosphere of plants grown in sterile than in nonsterile soil, with no effect on soil populations (Gopalakrishnamurthy et al., 1967; Neelekantan and Rangaswami, 1965; Purushothaman et al., 1976). No statistical analysis of the data was made, but the range of variations in the number of microorganisms per gram of rhizosphere soil observed were most often lower than one to five and, considering the low accuracy of dilution techniques, differences were probably not significant in many cases. Watanabe and Lin (1984) found higher counts of N_2-fixing bacteria at maximum tillering, early flowering, and late flowering in the roots of rice inoculated with *Azospirillum* or *Pseudomonas*, but the difference was considered significant only at early flowering when N_2-fixing populations were more than ten times higher in inoculated plants than in the control.

With use of a marker strain of *Azospirillum lipoferum* resistant to streptomycin and rifampin, Nayak et al. (1986) found survival but no establishment of the strain. In one experiment the strain disappeared within 1 month, whereas in a second experiment it survived in the soil, the rhizosphere, and on the stems for 50–70 days without an increase in numbers. The greatest density of inoculated *Azospirillum* (5.3×10^4/g dry weight of root) was recorded in the rhizosphere 40 days after transplanting, whereas putative indigenous populations of *Azospirillum* were about

500 times larger (2.8×10^6/g dry weight of root). Despite the low level of inoculated *Azospirillum,* inoculation increased the dry weight and total N of the plant. The only information on long-term effects of inoculation is a report by Balasundaram and Sen (1971) showing no residual effect of rice inoculation on a succeeding wheat crop.

Few of the mechanisms involved in the colonization of rice root by inoculated bacteria have been studied. Balandreau and Knowles (1978) and Diem et al. (1978) reported a significant role of mucigel in rice root colonization by *Beijerinckia.* Murty and Ladha (1987) observed that root colonization by *A. lipoferum* was unaffected by root mucigel and suggested that, instead, colonization was related to the nature of the root surface and not the presence or absence of mucigel. Tabary and colleagues (1984) isolated and purified a lectin from rice embryos that interacted in vitro with different bacteria isolated from the rice rhizosphere. The most efficient binding was observed with a strain of *Beijerinckia.*

Little is known about the competition between indigenous and inoculated strains. Rai (1985) observed that mutants of *Azospirillum* resistant to the herbicide machete increased yield more efficiently in a machete-treated soil (+44%) than the parent strain in a nontreated soil (+5%).

Mode of Action on Rice

Field inoculation experiments show a relative increase in grain yield (14.3%, 123 datum points) similar to that of straw yield (15.1%, 51 datum points) (see Table 2). Similar harvest index values (ratio between grain and straw yields) observed in the controls and inoculated plots (0.66 and 0.69) indicated that the effect of inoculation probably takes place early in the crop cycle during the vegetative phase.

The beneficial effect of bacterial inoculation can be attributed to a combination of (1) increased associative biological N_2 fixation in rhizosphere, (2) production of PGRs that favor rice growth and nutrient utilization, (3) increased nutrient availability through solubilization of immobilized nutrients by inoculated bacteria, and (4) competition of inoculated strains with pathogens or detrimental bacteria in the rhizosphere. The relative importance of these four components has not yet been determined.

Current estimates of biological N_2 fixation in rice rhizosphere are not sufficient to explain the increases in yield reported in the literature. If one assumes that all N fixed is absorbed by the plant, the average increase in yield of about 0.5 tons/ha reported in field experiments would require at least an increase of biological N_2 fixation by 10 kg/ha per crop. But no available data demonstrate a marked and durable increase of biological N_2 fixation in inoculated rice. Rao and Rajamamohan Rao (1983) studied the ARA of rice rhizosphere soil for 72–108 days after sowing in plots inoculated with *Azospirillum.* The relative increase in ARA was 65% without N, 80% with 30 kg/ha of N, and 38% with 60 kg/ha of N. Average increase in ARA for the three N levels was 60% (median 30%). In 10 of the 18 measurements, the increase was not significant. If we assume that the average difference in ARA observed between 72 and 108 days persisted through the complete crop cycle and that there is about 250 g dry weight of rhizosphere soil per rice hill, extrapolation of their data gives an increase in N fixed of about 4 kg/ha per crop in inoculated plots. Similarly, Watanabe and Lin (1984) recorded a significant increase in ARA at flowering stage in rice inoculated with *Pseudomonas* or *Azospirillum,* but it was

equivalent to an increase of about 6.5 mg N per plant over 100 days. Thus, ^{15}N content of inoculated and control plants did not differ. Nayak and associates (1986) found that inoculating with *A. lipoferum* did not increase associative biological N_2 fixation (estimated by ARA, ^{15}N and N balance studies), but did increase tiller number and early reproductive growth.

Another hypothesis to explain the beneficial effect of inoculation is PGR production. Omar et al. (1989) consider that inoculation of *Azospirillum* should not be regarded as a substitute for N fertilizers and a technology for low-input rice cultivation, but rather, as a means to extend N fertilizer use even at high levels. However, the hypothesis that PGR production by inoculated bacteria increases nutrient absorption by rice does not agree with the absence of significant differences in N fertilizer efficiencies between control plots (18.7 kg grain per kilogram of N applied) and inoculated plots (19.1 kg) in field experiments (see Table 2).

B. Rice Varietal Differences in Promoting Associative Biological Nitrogen Fixation

There are several reports on the existence of varietal differences in the ability to support associative biological N_2 fixation. Methodologies used have included N balance studies (App. et al., 1986), ARA measurements (Tirol-Padre et al., 1988), and N isotope ratios (Watanabe et al., 1987). Differences were genetically analyzed by Iyama et al. (1983). The plant traits associated ($p < 0.01$) with associative biological N_2 fixation determined by Ladha et al. (1988) were, by decreasing importance, dry weight of roots and submerged portions of the plant at heading, dry weight of shoots at heading, and N uptake at heading, and N uptake at maturity. With use of plant traits and a short-term ARA assay, Ladha et al. (1988) established a ranking for biological N_2-fixation and N utilization of 21 rice genotypes that was fairly reproducible in two consecutive dry season trials. Nothing is known, however, about the physiological basis of the apparent varietal differences. The idea of breeding varieties with higher N_2-fixing potential is attractive because it would enhance biological N_2 fixation without additional cultural practices. However, a prerequisite is the availability of a rapid screening technique. Even short-term ARA assays are time-consuming and do not allow the screening of a large number of genotypes (Tirol-Padre et al., 1988). An ^{15}N dilution assay could be used for screening and genetic studies, but reference varieties with low biological N_2 fixation stimulation ability must first be identified.

C. Phosphate-Solubilizing Bacteria

Phosphate-solubilizing microorganisms are present in the rice rhizosphere and in rice soils (Raghu and Macrae, 1966), yet few experiments indicate a potential for increasing rice yield by the inoculation with such organisms. Datta and associates (1982) tested the effects of a strain of *Bacillus firmus,* known to produce indoleacetic acid and to have a high ability to solubilize P. They compared superphosphate and insoluble rock phosphate and two rice varieties at seven levels of P. Inoculation increased yield by 10–70% (median 28%). The highest yield increase was observed in plots without P fertilizer. The P content of the grain increased in inoculated plots. Because the yields in controls receiving superphosphate and rock phosphate did not

differ, the relative importance of possible PGR effects and a P-solubilizing effect of the inoculated strain could not be quantified. Kundu and Gaur (1984) tested the effect of *Azotobacter* and two P-solubilizing bacteria on yield and P absorption by rice. Inoculation with *Azotobacter* had less effect on yields (+12%) than inoculation with P-solubilizing bacteria alone (+25%) or inoculation with two (+34%) and three (+52%) strains. Replacing superphosphate with rock phosphate and microbial inoculation did not affect rice response to P fertilizer.

VI. USE OF MICROBIAL INHIBITORS TO REDUCE NITROGEN LOSSES

A. Losses by Nitrification and Denitrification

Mechanism and Quantification of Losses

The establishment of nitrification–denitrification processes requires an aerobic–anaerobic interface. First, ammonium is nitrified in the aerobic zone. Its consumption creates a gradient that causes NH_4^+ present in the anaerobic zone to diffuse into the aerobic zone. Concurrently, the nitrate accumulating in the aerobic part diffuses in the anaerobic part of the interface, where it is denitrified into N_2O and N_2, which escape into the atmosphere.

In continuously flooded rice fields, environments conducive to nitrification–denitrification are the oxidized soil–reduced soil interface and the rhizosphere. Denitrification has also been observed to be associated with green algal clumps in floodwater (Kimura, 1986). The development of anaerobic microsites in algal clumps or mats may explain Kimura's observation (Paerl et al., 1988).

In rice fields subjected to alternating desiccation and submergence, an interface results from the temporal succession of aerobic and anaerobic conditions, with the bulk of soil successively hosting nitrifying or denitrifying activities. Under such conditions, a large soil volume is available for nitrification, and losses by denitrification can be high.

Although denitrification has long been considered an important mechanism of N losses in wetland soils and has been the subject of many studies, quantification of the losses has been hindered by the lack of a reliable methodology for direct measurement (Watanabe and Mitsui, 1979). The recent review by Buresh and DeData (1989) on denitrification losses from puddled rice soils in the tropics summarizes methodological problems for assessing N losses by denitrification as follows:

1. Techniques utilizing the inhibition of the reductions of N_2O to N_2 by C_2H_2 are not suitable for assessing denitrification in wetland rice fields because of slow dispersion and instability of C_2H_2 in flooded soil.
2. Indirect determination from the difference between total N loss (estimated by ^{15}N balance) and NH_3 loss (estimated by micrometeorological techniques) is prone to high variability and may overestimate losses.
3. The direct measurement of $^{15}N_2 + {}^{15}N_2O$ from ^{15}N-enriched urea may strongly underestimate losses.

Denitrification losses estimated at 13 locations using the most accurate method for measuring NH_3 volatilization ranged from zero to 39% of applied N (average

17%), representing zero to 82% of gaseous losses (De Datta and Buresh, 1989). Losses estimated while using a simplified measurement of NH_3 volatilization ranged from 3 to 70% of applied urea. However, as pointed out by De Datta and Buresh (1989), such denitrification rates would require nitrification rates as high as 2.6 kg/ ha of N per day, whereas the highest reported daily N value is but 1.2 kg/ha per day and the average N value during a 48-day study was about 0.5 kg/ha per day (Watanabe et al., 1981). In 12 experiments, recoveries of $^{15}N_2$ + $^{15}N_2O$ between 10 and 20 days were <0.1 to 2.2% of N applied, whereas total losses were 10–56% of N applied (Buresh and De Datta, 1989).

If one considers that (1) denitrification is primarily limited by NH_4^+ diffusion and nitrification (Reddy and Patrick, 1986), (2) most N losses from fertilizer take place during the first 40 days (IRRI, 1988), and (3) nitrification rates measured over 48 days totaled 28 kg/ha of N in fertilized plots and 19 kg/ha of N in the control (Watanabe et al., 1981), it could be hypothesized that N losses from fertilizer by denitrification might be moderate, but that N losses from soil N should also be considered when dealing with overall N fertility of rice fields.

Nitrification Inhibitors

A possible approach to reduce denitrification is to reduce nitrate production. The preference of rice for ammonium N over nitrate N suggests the usefulness of nitrification inhibitor application with ammonium fertilizer.

Many compounds have been found to inhibit the growth or activity of nitrifying mircoorganisms by creating unfavorable microenvironments or by interfering with metabolic activities common to autotrophic microorganisms (Hauck, 1980). The ideal nitrification inhibitor for use in agriculture should (1) block ammonia oxidation (= inhibit *Nitrosomonas*), but not nitrate oxidation (*Nitrobacter*); (2) not adversely affect other soil organisms and plants; (3) be nontoxic for humans and animals at the level of utilization; (4) be active for several weeks; and (5) be economical. Hauck (1984) lists seven chemicals produced commercially worldwide for use as nitrification inhibitors.

In laboratory studies, several inhibitors, inluding nitrapyrin (2-chloro-6-trichlor-omethylpyridine), AM (2-amino-4-chloro-6-methylpyrimidine), dicyan-diamide, thi-ourea, ST (2-sulfanil-amidiothiazole), and potassium azide have effectively retarded denitrification, but their effectiveness in the field has been less clear (De Datta, 1981). Experiments on nitrapyrin utilization on rice in the southeastern United States, summarized by Touchton and Boswell (1980), showed inconsistent response. When a significant effect was observed, yield increases were moderate averaging 0.1 and 0.4 ton/ha for nitrapyrin rates of 1.12 and 1.24 kg/ha, respectively. They concluded (1) that although nitrapyrin application with ammonium fertilizer may increase yield, it will not work well on clay soils commonly used for rice production where effective incorporation and mixing is not feasible; and (2) the volatile nature of nitrapyrin, which does not allow its granulation with solid N fertilizer, will probably discourage its use. These were the reasons for a renewed interest in thiourea and dicyan-diamide, the commerical formulation (DCD) of which was designed mostly for the use with solid N fertilizer and direct seeded rice (Hauck, 1984). However, IRRI trials did not show any yield benefit with DCD (De Datta, 1985).

Neem (*Azadirachta indica* Juss.) cake, known for its insecticidal properties, was also reported to be a nitrification inhibitor when coated on urea (Prasad and

De Datta, 1979; Reddy and Prasad, 1975). A 2-year experiment by John et al. (1989) showed a significant yield increase of 0.4 tons/ha the first year when applied urea was coated with 20% neem cake, despite no noticeable effect on N loss estimated from ^{15}N balance studies. Neem might increase yield by several other mechanisms, such as pest control and promoting photodependent biological N_2 fixation (Grant et al., 1985). The mechanisms involved in yield increase need further study.

The use of nitrification inhibitors with ammonia fertilizer broadcast on flooded soil may decrease denitrification losses, but may also increase losses by NH_3 volatilization by maintaining high NH_4^+ concentration in the floodwater. Nitrification inhibitors might be more valuable in direct-seeded rice where nitrate formed during the preflood period is lost by denitrification after flooding. No formulation has yet been shown to be consistently effective in rice.

B.   Nitrogen Losses By Ammonia Volatilization

Mechanism and Quantification of Losses

Ammonia is continuously formed in the soil and water of rice fields. When floodwater pH increases, ionized NH_4^+ increasingly converts to nonionized NH_3, which may escape from the water as a gas. Many factors affect NH_3 loss by volatilization, the most important of which are pH and NH_3 concentration and wind speed at the floodwater surface (De Datta, 1981). Because pH is a major factor in determining the rate and extent of loss (up to pH 9, NH_3 concentration increases by a factor of 10 per unit increase in pH), aquatic photosynthetic organisms, especially microalgae, have a key role in NH_3 volatilization. They deplete CO_2 in floodwater during the day and partly replenish it at night through respiration. This causes diurnal changes in floodwater pH, which may reach values as high as 10 by midday and decrease by 2–3 units at night (Mikkelsen et al., 1978).

Fillery and co-workers (1986b) estimated the photosynthetic biomass in fields where N losses from fertilizer applied 18 days after transplanting were evaluated. One week after fertilizer application, a limited and uneven growth of algae (100–300 kg fresh weight per hectare) was observed in N-treated plots where pH at noontime ranged from 7.8 where there was no visible algal growth, to 10.5 near algal colonies. Despite the low algal biomass, significant N losses (40%) occurred, suggesting that large populations of algae are not required to increase floodwater pH to levels that support rapid N losses.

Losses by NH_3 volatilization range from 2 to 60% of N applied (Fillery et al., 1986b). Most losses occur at the beginning of the crop cycle, when there is almost no canopy and the resulting high light availability permits microalgae to develop a photosynthetic activity sufficient to induce a significant pH increase in the floodwater, whereas their biomass is not large enough to limit losses through immobilization. Losses from application around panicle initiation are generally lower (10–15% of the N applied) because (1) the larger canopy reduces wind speed at the water surface; (2) the canopy also reduces photosynthetic activity in the floodwater and, therefore, the maximum value of the floodwater pH; and (3) N uptake by the crop is more rapid.

Algicides

Practices that decrease algal growth, such as applying $CuSO_4$ (Mikkelsen et al, 1978) and other algicides (Bowmer and Muirhead, 1987), and deep placement of N fertilizer (Cao et al., 1984), decrease pH diurnal variations and maxima and also N losses by NH_3 volatilization.

Various compounds have been tried for control of algal mats that can cause mechanical damage to young rice seedlings, but many were found to be toxic to rice (Dunningan et al., 1976). Few reports describe the potential use of algicides to decrease N losses and improve fertilizer efficiency. Algicides currently being tested for their effect on NH_3 volatilization are $CuSO_4$, simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine], diuron [3-(3,4-dichlorophenyl)-1,1 dimethylurea], and terbutryne [2-(*tert*-butylamino)-4-(ethylamino)-6-(methylthio)-*s*-triazine].

Copper is registered as an algicide in many countries, but it has several disadvantages in rice fields: (1) many species of algae are not controlled, (2) Cu is expensive, (3) it is toxic to fish, and (4) it is rapidly inactivated by precipitation and absorption on colloids. Although it is effective at low concentrations in large bodies of water, it is often ineffective in rice fields at rates up to 20 kg/ha (Dunnigan, 1974). Copper applied at 1.5 kg/ha with various levels of urea had little effect on algal growth and no significant effect on pH, O_2 content, and ammoniacal N content of the water (Muirhead et al., 1990).

Simazine applied with urea (Vlek et al., 1980), diuron applied with ammonium sulfate (IRRI, 1977), and terbutryne applied with urea (Bowmer and Muirhead, 1987), reduced pH and increased ammoniacal N concentration in the floodwater, compared with the controls. Terbutryne applied at panicle initiation in a temperate rice field decreased water pH for 6 days and N losses by 43%, but the resulting 5% increase in yield was not significant (Bowmer and Muirhead, 1987). Terbutryne applied with urea 10 days after transplanting and at panicle initiation in a tropical rice field reduced the maximum floodwater pH by 0.9 units (dry season) and 0.5 units (wet season) for more than 1 week. The estimated N savings averaged 4.7 kg/ha when 90 kg/ha of N was applied and 9.6 kg N when 150 kg/ha of N was applied (Muirhead et al., 1990).

Available data suggest that fertilizer savings from algicide use is low. More data are needed for definite conclusions, but deep placement of fertilizers, which does not favor the growth of non–N_2-fixing algae and prevents high concentration of ammonia in floodwater, seems a more efficient method to decrease N losses and increase fertilizer efficiency. Deep placement is also less inhibitory to photodependent biological N_2 fixation than broadcasting (Roger et al., 1980; Roger, 1990b).

Finally, Bowmer and Muirhead (1987) pointed out that by reducing photosynthetic O_2 production at the soil, algicides might also reduce nitrification and, thereby, the potential for losses by denitrification (see next section).

Urease Inhibitors

Another possible method for preventing the buildup of ammonia in the floodwater following fertilizer application is the inhibition of urease, which hydrolyzes urea to ammonium bicarbonate (Vlek et al., 1980). Urease inhibitors might offer the attractive possibility of reducing N loss through a modified fertilizer formulation, without requiring a change in the farmer's application practices (De Datta and Buresh,

1989). However, very few of the chemicals tested met the requirements of an effective urease inhibitor for use with urea fertilizer (Hauck, 1984).

Phenylphosphorodiamidate (PPD) and N (N-butyl)thiophosphoric triamide (NBPT) were tested for rice. The PPD decreased NH_3 evolution from some soils in the laboratory, but the results of early yield trials were negative, which was attributed to a fast degradation of PPD by soil microorganisms. Hauck (1984) concluded that no substance patented or under study could now be recommended for commercial production. In further studies (Fillery and De Datta, 1986), PPD decreased N losses by volatilization, but did not consistently increase yield (Fillery et al., 1986a). Recent studies by Buresh and co-workers (1988a,b) compared PPD with NBPT. Both inhibitors retarded the disappearance of urea from floodwater; but whereas PPD only delayed the buildup of ammonia in the floodwater, NBPT prevented it, reducing partial pressure of NH_3 by an average 80–98% during the 10 days following urea application. A significant increase in yield (average 8%) was observed at only one of two sites.

In a 2-year ^{15}N balance study of multiple urea rates in the Philippines, Buresh et al. (1988c) estimated the maximum potential of urease inhibitors. In the absence of leaching and runoff they considered unaccounted ^{15}N as the potential savings when urea N is used with an ideal inhibitor that would completely eliminate gaseous losses without detrimental effect on rice. They concluded that an ideal urease inhibitor could increase yield by a maximum of 6–8%.

Urease inhibitors, although they reduce the buildup of ammoniacal N after urea application, tend also to delay the disappearance of urea from floodwater, which results in a higher total (urea + NH_4) concentration (De Datta and Buresh, 1989). This may increase loss by runoff where water flows out of the fields soon after fertilizer application.

VII. SUMMARY

Nitrogen is usually the limiting factor to high yields in rice fields. Therefore, the use of biological N_2 fixation as an alternative or supplementary source of N for rice has been the major approach in microbiological management of wetland rice. Whereas N_2-fixing green manures have been used for centuries in some rice-growing areas, research on N_2-fixing algal and bacterial inoculants for wetland rice is relatively recent, being initiated in the early 1950s for free-living blue-green algae and in the 1960s for rhizosphere bacteria.

Azolla has proves useful as an N biofertilizer in some rice-growing countries. Like legumes it has a high N potential, but is easier to incorporate and grows well with rice under flooded conditions. Environmental, technological, and economic factors limit use of *Azolla*. Recent progress in strain hybridization and recombination has opened new ways to alleviate some environmental and nutritional limitations. Socioeconomic limitations are important and are probably increasing, as shown by the setback of *Azolla* in China and Vietnam where it was traditionally used. However, recent studies have shown that *Azolla* has a potential not only as a green manure, but as a multipurpose biofertilizer that can also decrease N losses by NH_3 volatilization. It can also be a weed suppressor, a K source through its ability to concentrate this element, an animal feed, a primary producer in rice–fish–*Azolla*

culture (IRRI, 1987; Liu, 1987; Watanabe, 1987). The major limiting factors—including space, water, nutrition, available labor, agronomic practices, and economic feasibility—and the potential of *Azolla* as a multipurpose crop, which may revive interest in its use, will decide the extent of its future utilization.

Biomass estimates, ARA measurements, and inoculation experiments indicate that free-living blue-green algae, as an additional source of N for rice, have a moderate potential of about 30 kg/ha of N per crop cycle which may translate to a yield increase of 300–450 kg/ha. However, the technology for blue-green algae inoculation is still at the experimental stage of large-scale field testing. This is mostly due to insufficient knowledge of conditions that favor blue-green algae establishment in the field. Cultural practices to enhance the growth of indigenous or inoculated blue-green algae are known, but their efficiency and economic viability have to be determined. As long as blue-green algae inoculation is applied on a trial-and-error basis, it will have little chance of success in many rice-growing areas. Recent developments indicate that the whole principle of blue-green algae inoculation should be reconsidered, and more attention should be paid to promoting indigenous strains. In-depth agroecological research is required before blue-green algae technology can be substantially improved.

Information on the effects of bacterial inoculation of rice is found in fewer than 40 papers. Most of the quantitative data given are only yield values. Effects are inconsistent with some reports of positive effects, some of no effect, and some reporting statistically significant yield decreases. Currently, most strains tested for inoculation have been N_2-fixing forms, but ARA, ^{15}N, and N balance studies have not provided clear evidence that the promotion of growth and N uptake was due to increased biological N_2 fixation. Therefore, several authors refer to the production of PGRs to explain the beneficial effect of bacterial inoculation. No experiment has yet supported this hypothesis. However, one of the very few non–N_2-fixing bacteria used for rice inoculation, a strain of *Bacillus firmus* known for its ability to produce indoleacetic acid, increased yield very effectively (Datta et al., 1982). If it is verified that the ability of inoculated strains to produce PGRs is more important than their N_2-fixing ability, it is clear that screening of bacterial strains for inoculation should not be limited to N_2-fixing strains. The few data available on strain establishment showed that, in most cases, inoculated strains disappeared or established themselves for various periods, but did not multiply. That would limit the effect of inoculation to the earlier growth stage of the plant, a hypothesis that agrees with the absence of an inoculation effect on harvest index. Given the current status of knowledge on bacterial inoculation of rice, no definite conclusion for the potential of this technology can be drawn.

Experiments on algal and bacterial inoculation have two features in common that call for caution when interpreting reports for which no (or improper) statistical analysis was done: (1) field experiments that show an average increase in yield that is close to or below the minimum detectable difference (14.5%) for the experimental design most frequently used in field experiments (16-m² plots with four replicates); (2) most experiments have paid no attention to the establishment of the inoculum. The ideal inoculation experiment with N_2-fixing organisms should present not only yield data with a valid statistical analysis, but also environmental and agronomic parameters, information on the inoculum composition and method of

application, quantitative or at least qualitative data on its establishment, and biological N_2 fixation measurement. Very few of the several hundred of reports on inoculation (mostly with blue-green algae) satisfy these criteria.

If we consider the rapid progress in genetic engineering, one can speculate on the possibilities to select or design efficient N_2-fixing strains of cyanobacteria and eubacteria for inoculation. This includes, inter alia, screenings for various properties, the enhancement of N_2-fixing ability of the strains by recombinant DNA techniques, or the development of derepressed strains in which biological N_2 fixation is not suppressed by NH_4^+. In the long-term, genetic engineering may contribute to the microbiological management of wetland rice fields, but it is not yet known if and how engineered strains can establish or compete with the indigenous microflora.

The selection and breeding of rice according to the variety's ability to stimulate an associative microflora that promotes biological N_2 fixation and soil N utilization is still limited by the absence of an efficient screening method. The relatively low N_2 fixation potential of associative biological N_2 fixation is not a hindrance to this approach, the major advantage of which is that the N potential is inherent to the plant and thus requires no additional cultural practice by the farmer.

Biological N_2 fixation in rice fields has been the most effective system for sustaining production in low-input traditional cultivation. The general impression when considering the microbiological management of N_2-fixing organisms in rice fields is that 40 years after the first inoculation experiments, the agronomic potential of biological N_2 fixation is still largely underutilized, and benefits from its intentional use are far less than contributions by indigenous microorganisms. Considering that rice obtains most of its N from the soil, regardless of the amount of chemical N fertilizer applied, concerns in recent high-input, intensive rice cultivation are sustainability of high yields and the possible environmental influences of intensive management on soil fertility. Knowledge on this aspect is still limited, but the key roles of the rhizosphere, the photosynthetic aquatic biomass, and their N_2-fixing components in maintaining the fertility of rice soils under intensive cultivation have been recognized and need further study (Watanabe et al., 1988).

A relatively recent approach in microbiological management of wetland rice is the utilization of inhibitors to decrease N losses. To some extent, algicides and urease inhibitors, which both aim at decreasing N losses by NH_3 volatilization, share a similar potential for saving N fertilizer and increasing grain yield. Estimates by Muirhead et al. (1990) for algicides and by Buresh et al. (1988c) for urease show a moderate potential of a few kilograms of N per hectare. Similarly, increases in yield attributable to nitrification inhibitors are moderate and also erratic. The greatest potential for benefit is when inhibitors are used with N fertilizer rates at or below optimum. When N application rates are greater than required for optimum yield, yield benefits are less likely. However, for the relatively low cost of N fertilizer and the moderate potential of inhibitors to reduce losses, as compared with other practices such as timing of application, water management, and various methods of incorporation, more efficient inhibitors are needed before cost-competitive formulations can be designed (De Datta and Buresh, 1989). Environmental concerns, such as release of N oxides and nitrate pollution of water table are a new incentive for designing fertilizer formulations less susceptible to losses.

ACKNOWLEDGMENTS

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