Proc. Indian natn. Sci. Acad. **B59** Nos. 3 & 4 pp. 295-302 (1993)

Algal Biofertilizer for Vital Soil and Free Nitrogen

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The diazotrophic cyanobacteria contribute substantially to the fertility of the soil especially under tropical rice field conditions. In addition to contributing upto 30 kg N ha^{-1} $season^{-1}$, these algae benefit the crop plants through excreting part of the biologically fixed nitrogen and secreting growth promoting substances like vitamins and amino acids, add appreciable amount of organic matter into the soil, solubilize insoluble phosphates and improve the physical and chemical nature of the soil. Algalization increases the fertilizer use efficiency of the crop plants, reduces the loss of fertilizer nitrogen and above all, provides these benefits in a recurring manner.

Key Words: Biofertilizer, Cyanobacteria, Diazotrophy, Growth promoting substances, Soil fertility

Introduction

Soil is a dynamic system in which the physical, chemical and biotic components are in a state of equilibrium. Application of chemical fertilizers without taking care of the other soil constituents, disturbs this equilibrium which adversely affects the productivity of the soil. Biofertilizers are the microorganisms which help in better crop nutrient management and maintenance of the soil health while working in perfect harmony with the nature.

Under waterlogged conditions of rice fields, the diazotrophic cyanobacteria play a vital role in maintaining the soil fertility and sustaining the crop yield even in the absence of any added nitrogenous fertilizer. They are able to withstand extremes of temperature and drought and show remarkable variation in growth, nitrogen fixation and stress compatibility. Recently, algalization has been recognised as an important input in rice cultivation as it forms a perpetually renewable source of nutrients and improves the soil health (De 1939, Singh 1961, Venkataraman 1981, Roger & Kulasooriya 1981 and Goyal 1987).

Nitrogen Fixation by Blue-green Algae

The term algal biofertilizer was coined in early seventies to embody such blue-green algae which are capable of growing or molecular nitrogen. These algae, with the help of the enzyme nitrogenase, bring about the reduction of molecular nitrogen to ammonia at ambient conditions. This enzyme is inactivated by the presence of oxygen because of the oxidation of the labile sulphide groups and reduced synthesis of both the components. In cyanobacteria, under aerobic conditions, nitrogen fixation is carried out in specialized cells known as heterocysts, which provide necessary anaerobic conditions for the activity of nitrogenase. Heterocysts have thick walls which physically prevent the entry of oxygen; have high rate of respiration which scavenges the diffused oxygen and lack photosystem II

(Thomas 1970), due to which there is no endogenous evolution of oxygen during photosynthesis. Till the findings of Wyatt and Silvey (1969), only the heterocystous cyanobacteria were considered to be capable of fixing nitrogen. Since then many non-heterocystous blue-green algal genera have been added to the list. Out of these, only *Gloeocapsa* is an aerobic nitrogen fixer while the rest need anaerobic or microaerophilic conditions (Stewart et al. 1979). Stewart and Lex (1970) had opined that all the vegetative cells of the trichome of diazotrophic blue-green algae contain nitrogenase, but in the vegetative cells, it gets inactivated due to the presence of oxygen.

Like Haber-Bosch process, conversion o elemental nitrogen to ammonia is an energy intensive process. The necessary energy for the process in the cyanobacteria is supplied through oxidative phosphorylation and photophosphorylation. When placed in an environment of combined nitrogen, the nitrogen fixing cyanobacteria show accelerated growth (Kratz & Myers 1955) and progressively reduce their dependence on molecular nitrogen. Thus it seems that the cyanobacteria resort to nitrogen fixation only under combined nitrogen deficient conditions. Deficiency of combined nitrogen has been shown to cause degeneration of photosystem II in *Anabaena variabilis* and *Mastigocladus laminosus*, which leads to the activation of nitrogenase because of the creation of anaerobic conditions. In presence of combined nitrogen the enzyme nitrogenase remains repressed and this effect is more pronounced in the presence of ammonium nitrogen.

Similar to oxygen inhibition, chemical nitrogen inhibition of nitrogenase in blue-green algae is reversible. According to Stewart and Gallon (1980), this difference from the bacterial nitrogenase may be because in cyanobacteria the repression of nitrogenase is not regulated by glutamine synthetase (GS), instead presence of ammonia increases ADP/ATP ratio and carbamyl phosphatase activity which in turn cumulatively and independently inhibit the nitrogenase activity. Removal of ammonium nitrogen stress reduces the ADP/ATP ratio to less than 0.5 and brings down the carbamyl phosphatase activity which derepresses the nitrogenase. The revival of nitrogenase activity takes place within about 24 hr of the removal of the stress.

Higher concentrations of combined nitrogen have been shown to inhibit both growth and nitrogen fixation. However, the tolerance limit for growth is much higher than for nitrogen fixation. Ammonium nitrogen is the putative inhibitor of nitrogen fixation and proves toxic for algal growth at 75 ppm (Stewart 1964, Goyal & Marwaha 1985). Nitrate nitrogen is preferred by the cyanobacteria and even at 100 ppm, there is no detrimental effect on growth and only a marginal reduction is observed in nitrogen fixation. Stewart (1964) reported that in certain cyanobacteria, the nitrogen fixation continues even at 50 ppm ammonium nitrogen.

Nitrogen Contribution by Blue-green Algae

Tropical conditions ensure increased incidence of cyanobacteria in the rice field soils and high humidity and temperature and shade provided by the crop canopy favour the luxuriant growth of cyanobacteria (Roger & Reynaud 1979). Direct evidences showing enrichment of the soil nitrogen due to algal application have been deduced using ^{15}N and ARA technique. *In situ* estimations using ARA technique have shown an addition of 18-45 kg N ha^{-1} due to the activity of diazotrophic cyanobacteria (Watanabe & Cholitkul 1979). Availability of nitrogen fixed by the blue-green algae to the rice plant was shown with the help of ^{15}N studies conducted by Reynault et al. (1975). Cyanobacteria have also been shown to liberate variety of amino acids like aspartic acid, glutamic acid and alanine and their liberation has been found to be maximum during the stationary phase. Being soluble in water, they form readily available sources of nitrogen for the crop plants.

Growth and yield of the rice crop provide an indirect estimate of the algal contribution. Series of multilocational trials conducted with different rice varieties under varying agroclimatic conditions have shown that algal inoculation can result in an addition of upto 30 kg N ha^{-1} $season^{-1}$ (Venkataraman 1979). This however, depends upon the agroecological conditions which regulate the establishment and activity of the introduced algae.

In addition to providing biologically fixed nitrogen, algal biofertilizer increases the availability of fertilizer nitrogen. The typical rice field conditions favour denitrification resulting in rapid loss of the fertilizer nitrogen. This compounded with the nitrogen loss through leaching and run-off, reduces the nitrogen uptake by the crop to about 35% of the total fertilizer nitrogen applied. Colonization of the rice field soils by the blue-green algae appreciably reduces this loss through metabolization of the combined nitrogen. The nitrogen fixing machinery of cyanobacteria is endowed with the unique property of temporarily shutting off the process of utilization of the molecular nitrogen when placed in an environment containing combined nitrogen. Under field conditions in presence of higher levels of fertilizer nitrogen, the cyanobacteria grow faster and may fix comparatively much less nitrogen. But they revert back to nitrogen fixing state when the combined nitrogen stress is removed due to progressive utilization and natural loss. The increased algal biomass is now expected to contribute much more nitrogen than what it would have contributed in the absence of chemical nitrogen. The metabolised combined nitrogen and the biologically fixed nitrogen subsequently becomes available in a gradual manner through exudation and decomposition. Thus, the cyanobacteria prevent a part of the applied fertilizer nitrogen from being lost and make it available to the crop plants at a later stage.

Stress Compatibility in Cyanobacteria

Since the blue-green algae have to function from outside the crop plant, they are always exposed to the vagaries of the environment which can be natural or man made. The situation may be more difficult if the introduced algae happen to be aliens.

Heavy soils with more organic matter and water holding capacity, harbour rich algal flora in contrast to the sandy soils. While quite a few cyanobacteria can grow at extremes of temperature, majority of them have their growth optima at 30-40°C. Temperate soils harbour more green algae while tropical soils are generally dominated by the blue-green algae. Soil *p*H also has a selective effect on the distribution and predominance of algae. Although, *p*H specific cyanobacteria growing at *p*H as low as 3.8 and as high as 9.5 are not rare, most of them have a wide *p*H range showing good growth at 6.5-8.5.

The soil algal flora and its activity is greatly influenced by the type, quantity and mode of application of the nitrogenous fertilizers. In addition, the fertilizer nitrogen has differential effect on growth and nitrogen fixation by cyanobacteria (Goyal & Marwaha 1985, Goyal 1989). Under field conditions, the cyanobacteria are generally exposed to ammonium nitrogen as it is the common form of fertilizer nitrogen used in rice cultivation. Stewart (1964) and Venkataraman (1979) did not observe any reduction in nitrogen fixation in presence of ammonium nitrogen upto 50 ppm. Goyal and Marwaha (1985) reported a concentration dependent increase in growth and absorption of ammonium nitrogen by

Anabaena fertilissima upto 50 ppm. At concentrations higher than 50 ppm, reduction in nitrogen fixation was more drastic than in growth. These studies show that for diazotrophic cyanobacteria, the tolerance limit for combined nitrogen with respect to growth is much higher than for nitrogen fixation (Goyal 1989). Nitrate nitrogen appears to be more compatible than ammonium nitrogen for the cyanobacteria. Comparatively higher levels of nitrate nitrogen have been shown to appreciably benefit the growth without affecting the process of nitrogen fixation by blue-green algae. These attributes of cyanobacteria contribute to their increased supplementation effect at lower levels of fertilizer nitrogen.

The cyclic availability is another constraint on maximum utilization of the fertilizer nitrogen by the crop plants. Presence of considerably large amount of nitrogen at the time of fertilizer application makes it prone to increased loss leading to uneven availability of nitrogen to the crop. Contrary to this, algal biofertilizer provides the biologically fixed nitrogen in a linear fashion maintaining a steady flow throughout the crop growth cycle.

Variety of pesticides are used in rice cultivation which are expected to influence the algal activity. The effect, however, depends upon physical and chemical properties of the soil and nature and concentration of the pesticide. At the recommended doses, herbicides like 2,4-D, ordram, stam, F-34, trifluralin, butachlor, benthiocarb, pandimethalin and oxadiazon; insecticides like gamma BHC, phorate, carbofuran, endosulphan, malathion and parathion and fungicides like MBC, diflotan and captan have been found to be completely harmless to the diazotrophic cyanobacteria (Venkataraman & Rajalakshmi 1971, 1972, Sardeshpande & Goyal 1982, Goyal 1986, 1989). The beneficial effect of some of the pesticides on algal growth under field conditions may be because of the possible utilization of the pesticide or its degraded products, reduced competition from other susceptible microbial denizens of the soil and control of the population of algae grazers.

The paramount significance of algal biofertilizer lies in the fact that unlike the chemical fertilizers, they themselves are not utilized by the crop plants. Since only the products of the biological activities of blue-green algae are taken up by the crop, there is always a possibility of building up of the cyanobacterial population in the soil. During a growing season, the algae grow, multiply, add organic matter and metabolize elemental and combined nitrogen. With the approach of dry season, they form perennating bodies which become active with the onset of favourable season. Thus, superimposed inoculations for 3 to 4 consecutive seasons, generally lead to the building up of the algal population in the soil, which in the subsequent seasons may sustain the crop yield equivalent to the application of 25-30 kg N^{-1}.

This is substantiated by the observation that the post harvest soil samples from an algalized field do not show any increase in the organic matter as well as residual nitrogen (Singh et al. 1990) Thus, the cumulative effect of superimposed algal application can only be attributed to the gradual build up of the algal population in the soil leading to increased ecological benefit.

Algalization and Crop Yield

Algal nitrogen fixation under no circumstances can completely substitute the nitrogenous fertilizers in rice cultivation. However, in areas where no fertilizers are used for various reasons, use of algae can result in a benefit of upto 30 kg N ha^{-1}. Algal biofertilizer is recommended only as a supplement to nitrogenous fertilizers. The supplementation effect remains perceptible even in the presence of high levels of fertilizer nitrogen (Venkataraman & Goyal 1969). Present evidences show that algalization in presence of higher levels of nitrogen results in a saving of 10-20% nitrogen which increases to 20-30 kg N ha^{-1} at lower levels. Pronounced additive effect of algal application at lower levels (Goyal 1982, 1989), becomes important in extensive agriculture which envisages use of less fertilizer nitrogen on larger areas for reducing the loss of fertilizer nitrogen and ensuring maximum utilization of the natural process of nutrient recuperation in the soil.

Increase in the crop yield above the level which can be attributed to the contribution of biologically fixed nitrogen by the cyanobacteria is the consequence of liberation of growth promoting substances and prevention of the loss of part of the applied fertilizer nitrogen. The growth promoting substances have been shown to accelerate root growth (Venkataraman & Neelakantan 1967), which in turn enables the crop plant to take up more nitrogen from the soil (Sundara Rao et al. 1963).

Future Strategy

Information gathered on various aspects of algal biofertilizer technology during the last over three decades has shown that diazotrophic cyanobacteria form an important input in rice cultivation especially for small and marginal farmers. The present day need is to produce quality inoculum and put it within easy reach of the farmers.

Since in our country, rice is grown under varied agroecological conditions ranging from tropical to temperate climate, sandy loam to clay loam soil, lowland to rainfed conditions and highly acidic to highly alkaline soil reaction, it is essential to develop region specific, stress compatible cyanobacteria which will have better ability of establishing and proliferating in their own niche.

Systematic survey of the autochthonous cyanobacterial flora of the rice field soils will help in developing such strains in addition to the preparation of a distribution map of the country as well as in the identification of the algae deficient areas. A beginning in this direction has been made at the National Facility for Blue-green Algal Collections of Indian Agricultural Research Institute, New Delhi, which harbours a germplasm collection of region specific strains of blue-green algae of known attributes.

In view of the existence of natural variation in growth, nitrogen fixation, moisture requirement and compatibility with the fertilizer nitrogen and pesticides, perceptible even at intraspecies level, exploration of the natural cyanobacterial population can be used as an easy approach to select region specific strains performing the desired functions. The most important outcome of the recent efforts made in this direction is the identification of cyanobacteria like *Aulosira, Calothrix, Scytonema* and *Tolypothrix,* which grow luxuriantly on moist and shaded soil surface and fix more nitrogen than the most widely distributed genera like *Anabaena* and *Nostoc.* Such saphophytic forms hold promise for upland and rainfed paddies, which form a major part of the area under rice cultivation in our country.

Cyanobacteria which readily form spores will have an added advantage in better establishment of the inoculum as these perennating bodies will not only help the cyanobacteria in withstanding the unfavourable conditions in the post harvest period but will also increase their population through the formation of increased number of germlings.

Extracellular substances liberated by the cyanobacteria have been shown to increase the uptake of fertilizer nitrogen. We do not have any information on the quality and quantity of these substances being liberated by different cyanobacteria. We are also not aware of the extent to which their liberation is regulated by the environmental factors.

Presence of combined nitrogen and consequent reduction in the amount of nitrogen fixed is bound to influence the type and amount of these extrametabolites. Identification of the factors inducing and accelerating the process of liberation of these substances will help in increasing the supplementation effect of algalization.

Cyanobacteria capable of absorbing and growing at higher levels of fertilizer nitrogen will be more useful in preventing the loss of fertilizer nitrogen on one hand and increasing the availability of biologically fixed nitrogen on the other. Strains with derepressed nitrogenase, capable of fixing nitrogen in presence of comparatively higher levels of combined nitrogen, will be of added advantage under these conditions.

Cyanobacteria partially defective in glutamine synthetase activity will liberate substantial amount of the fixed nitrogen as ammonia. Such strains will increase the linear availability of nitrogen to the rice crop. Strains efficient in photosynthesis with a capacity to build a large intracellular pool of photosynthates will be able to continue to fix nitrogen for a considerable period in the absence of light.

Most of the pesticides do not affect the biological activity of the diazotrophic cyanobacteria at the field application doses. In some cases, these chemicals even accelerate the growth and nitrogen fixation. It is, therefore, necessary to develop pesticide compatible cyanobacterial inoculants which not only are able to function but show enhanced activity in their presence.

The open air tank culture method for producing the soil based algal culture is simple, easily adaptable and economical. However, it is prone to aerial and soil borne contamination with unwanted algae which leads to deterioration of the quality of the inoculum. In addition, since the multiplication is done under natural conditions, the production from the open air ponds is not assured. Bulky nature of the soil based inoculum poses transportation problems. The suggested decentralised production of the algal inoculum at farmers' level introduces another factor contributing to the low quality of the inoculum. It is, therefore, necessary to develop a method which can be operated under completely controlled conditions to ensure continuous production of quality inoculum.

Open circulation system in an enclosed space using artificial illumination seems to be quite promising as the reduction in handling costs due to reduced volume of the inoculum will partly offset the energy input cost. Suitable carrier material alternative to the soil, capable of harbouring a higher population load in viable state for a longer period, will help in reducing contamination with the unwanted algae and cut on the space and transport cost.

Conclusion

Algal biofertilizer can substantially contribute in making the rice cultivation sustainable on long term basis. It helps the small and marginal farmers by providing a part of the much needed nitrogen. It enables the crop to utilize more of the applied fertilizer nitrogen and contributes to the vitality of the soil. The sustained additive effect due to population build up is an added attribute of this input. All that is needed is to identify and develop promising region specific stress compatible strains and place quality inoculum within easy reach of farmers.

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