Engineering cyanobacteria nitrogen bio-fertilizer for rice cultivation in stressful environment

Shree Kumar Apte

Bhabha Atomic Research Centre, India

Keyword: Bio-fertilizer, Biotechnology, Biofuel, Bioengineering, Nitrogen fixation

Abstract:

As a naturally abundant, photosynthetic, nitrogen-fixing microbe, the cyanobacterium Anabaena contributes significantly to the nitrogen and carbon economy of tropical soils, especially in cultivation of rice paddy. However, its nitrogen bio-fertilizer potential is adversely affected by common abiotic stresses. Engineering enhanced nitrogen fixation and stress tolerance capabilities in this microbe through genetic manipulation is seriously limited due to the unavailability of appropriate tools and techniques and knowledge of suitable candidate genes. In recent years, our laboratory has devised an electroporation protocol for genetic transformation that achieves high frequency gene transfer and overcomes problems associated with the current practice of triparental conjugation between E. coli strains and Anabaena. We have also constructed (a) a suitable vector for new gene discoveries, and (b) a novel integrative expression vector pFPN, placing desired genes at a defined locus in Anabaena genome and facilitates their high level expression from an eco-friendly light-inducible promoter. Using these tools we have identified several genes responsible for enhanced heterocyst formation and nitrogen fixaton (hetR), chaperones (groESL, cpn60) for protein folding and homeostasis, and several oxidative stress tolerance genes (superoxide dismutases, catalases and peroxiredoxins) which confer superior stress tolerance to Anabaena. The approach has proved very useful for constructing recombinant Anabaena strains capable of nitrogen fixation in stressful environments.

Introduction:

A bio-fertilizer also known as bio-fertilizer is a substance which contains living microorganisms when applied to seeds, plant surfaces or soil surfaces colonize the rhizosphere or the interior of the plant & promotes growth by increasing the supply / availability of primary nutrients to the host plant population. Biofertilizers supply nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus and stimulating plant growth through the synthesis of growth-promoting substances required for their development. The microbes in bio-fertilizers restore the soil's natural nutrient cycle & build organic matter in soil. Through the use of bio-fertilizers healthy plants can be grown, while enhancing the sustainability and the soil health and soil properties. Bio-fertilizers can be expected to reduce the use of synthetic fertilizers and pesticides, but they are not yet able to replace their use. Since they play several roles, a preferred scientific term for such beneficial bacteria is PGPR- "plant-growth promoting rhizobacteria".

Bio-fertilizers provide organic and "eco-friendly" inputs required for agriculture. Bio-fertilizers such as Rhizobium, Azotobacter, Azospirillum and blue green algae - BGA have been in use for a long time. Rhizobium inoculant is used for leguminous crop plants. Azotobacter can be used with crops like wheat, maize, mustard, cotton, potato and also for other vegetable crops. Azospirillum inoculations are recommended mainly for crops like sorghum, millets, maize, sugarcane and wheat. Blue green algae belonging to a general cyanobacteria genus, Nostoc or Anabaena or Tolypothrix or Aulosira, fix atmospheric nitrogen and are used as inoculations for paddy crop grown both under upland & low-land conditions. Anabaena in association with water fern Azolla contributes nitrogen up to 60 kg/ha/season and also enriches soils with organic matter. Seaweeds are rich in various types of mineral elements (potassium, phosphorus, trac elements etc) hence they are extensively used as manure by people of coastal districts. Seaweed - manure also helps in breaking down clays. Fucus is used by Irish people as manure on a large scale. In tropical countries, bottom mud of dried up ponds which contain abundant blue green algae is regularly used as manure in fields. The mixture of seaweeds and blue green algae may serve as ideal fertilizer.

Bio-fertilizers are means of fixing various nutrient availability in the soil for plant growth and development mainly Nitrogen deficiencies. Since a bio-fertilizer is technically living, it can be Symbiotically associated with hot plant roots. Involved microorganisms could readily & safely convert complex

organic material which is not absorbed by the plant into simple compounds, so that they are easily taken up by the plants to promote growth. The soil Microbes function is in long duration, causing improvement in the soil fertility. It maintains the natural habitat of the soil without any harm. It increases crop yield nearly by 20-30%, replaces chemical nitrogen & phosphorus by about 30% and also helps in stimulating plant growth. It can also provide protection against drought and also protects from some soil-borne diseases. Researches has also been shown that to produce a larger quantity of crop produce, bio-fertilizers with the ability of nitrogen fixation and phosphorus solubilizing will result in the greatest possible effect.

They advance shoot & root growth of various crops versus control groups. This can be important when implementing new seed developments. Bio-fertilizers also promote soil health, leading to greater sustainability. Important types of bio-fertilizers includes: Symbiotic Nitrogen Fixing Bacteria(Rhizobium),Loose Association of Nitrogen-Fixing Bacteria(Azospirillum),Symbiotic Nitrogen-Fixing Cyanobacteria(Blue-Green algae or Cyanobacteria, Azolla),Free-Living Nitrogen-Fixing Bacteria(Clostridium, Beijerinckii, Azotobacter and Bacillus polymyxin). Usage of biofertilizers is important for many reasons including improvement in soil texture and yield of crop plants.some beneficial microbes do not allow pathogens to flourish or colonize in the field. They are not harmful & eco-friendly also cost-effective, Bio-fertilizers protects the environment from pollutants since they avoid chemical components as they are natural fertilizers. They are capable of destroying many harmful substances present in the soil that can cause plant diseases; they are proved to be effective even under climatic conditions like semi-arid regions.

Conclusion:

Our research says that Bio-fertilizers can improve the status of soil fertility by providing all the nutrients require for the plant growth and development in every stage and also in adverse condition like stress etc.., here some types of bio-fertilizers that enhance the crop production in rice Azospirillum is a symbiotic bacteria and also an important bio-fertilizers used in paddy cultivation. Azospirillum treatment is recommended for seed, seedlings and in the field. Azospirillum bacteria thrive in root zones of paddy and are capable of fixing more atmospheric nitrogen into the form which is absorbed by the plants. It also solubilizes phosphorus & silicon to some extent required for rice crop plants. It helps in drought tolerance, when irrigation or rainfall is delayed or not available. Blue Green Algae -BGA is another type of bio-fertilizer used in an algal form. Important specie includes: Cyanobacteria, Anabaena, Nostoc & Tolypothrix. The nitrogen fixed by these Blue Green Algae is about 15 kg/ha over the complete season. Blue Green Algae elaborates vitamin Biz & growth factors that makes the plant grow vigorously and healthy. It oxygenates the water impounded in the rice field (in puddling condition). It excretes organic acids that help phosphorus solubilisation which provides phosphorus in available form for the plants. Azolla is a water fern is an algal symbiont Anabaena azolla that can fix atmospheric nitrogen. Azolla excretes nitrogen (organic) in water during its growth and also immediately upon trampling it in the field. It also absorbs traces of potassium from irrigated water. Azolla provides nitrogen, potassium organic carbon etc. It prevents weed growth in the rice field.