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SOME METHODS FOR QUALITY TESTING OF CYANOBACTERIAL BIOFERTIZERS

Arvind Kumar*[1] and R. Chandra[2]

[1]Department of Ag. Chemistry and Soil Science, R.K. (P.G.) College, Shamli - 247 776 (U.P.).
[2]Depatmnent of Soil Scince, College of Agriculture, G.B. Pant Univ. of Agricul. and Tech., Pantnagar - 263 145 (U.K.).

ABSTRACT

Cyanobacteria are a large group of Gram- negative Prokaryotes that perform oxygenic photosynthesis. These Prokaryotes have ability to fix atmospheric nitogen symbiotically as well as free living. These Prokaryotes uses sunlight as the source of energy for the fixation of nitrogen and therefore have potential as bio fertilizer. Availability of a good quality inoculants is a major constraint in popularization of cyanobacteria Taxonomic Characters of Cyanobacteria changes so drastically that reliable identification of species becomes difficult.

Key words : Cyanobacteria, Inoculants, Biofertilizers, Nitrogen Fixation, Taxonomy.

INTRODUCTION

Cyanobacteria (blue green algae or BGA) are unique in possessing the capacity of oxygenic photosysthesis. These prokaryotes have ability to fix atmospheric nitrogen symbiotically as well as free living. Nitrogen fixing cyanobacteria uses sunlight as the source of energy for the fixation of carbon and nitrogen and therefore have potential as biofertilizer. The composite inoculants consisting of cyanobacterial cultures viz. Nostoc. Anabaena, Calothrix, Tolypothrix, Oscillatoria and Scytonema have been used for inoculation in rice (Kannaiyan, 1993). The biofertilizer techniques of cyanobacteria are limited mainly due to the non-availability of good quality inoculants. Traditionally cyanobacteria have been distinguished on the basis of phynotypic properties, structure and physiology. Taxonomic characters change so drastically that reliable identification of species becomes difficult (Thajuddin *et al*., 2002; Rajkumar, 2004 and Chillappa *et al*., 2004). More importantly, the morphology of cyanobacteria in laboratory cultures is often considerably altered from the original morphology of environmental isolates and the diversity of strain within a culture may be reduced because of selective culturing condition. Many techniques have been used to study the phylogenetic perspectives, which are more reliable in cyanobacterial strain identification. These techniques could be effectively utilized in checking genetic purity of the strains.

Various methods for quality testing are tried but few rapid methods to ensure quality of inoculants are

MATERIALS AND METHODS

Visible and Planktonic samples were collected from various Ponds and Canals in and around Shamli Distt. M. Nagar (U.P.) using net and khurpi along with algal mat water samples were also collected and were analysed for Physicochemical and Biological parameters such as

*Corresponding author : Arvind Kumar

disolved oxygen, nitrate, total and inorganic phosphorus using standard methods (APHA, 1975).

The collected cyanobacterial samples were transferred into 250 ml conical flasks and were tested for quality. The following rapid methods were used to ensure the quality of inoculants.

Molecular methods

The difference in genome size and DNA base composition has been used in cyanobacterial taxonomy, which can demonstrate genetic differences between different taxa. The GC content is a promising criterion to classify cyanobacteria at genetic level, as this usually does not show large differences within a genus. One area in which a significant application has been developed recently is in the phylogenetic characterization of natural microbial communities by 16S rRNA sequence analysis, as the rRNAs are ancient molecules and extremely conserved in overall structure. Hence, homologous rRNAs are readily identifiable by their sizes. Nelissen *et al.* (1996) developed a cyanobacteria specific oligonucleotide probe with 16SrRNA genes. DNA/RNA hybridization is based on the feature that denatured single stranded DNA will renature under suitable conditions. The extent of renaturation and the stability of renatured hybrid molecule are proportional to the complementarity of the DNA strands involved. The relative binding and the differences in thermal stability can be used to assess the taxonomic status among organisms. Apte and Haselkorn (1990) demonstrated the potential of the hybridization method to identify differentially expressed genes in cyanobacteria. RFLP markers have proven to be a reliable and highly informative tool for characterizing genetic diversity. Planktonic filaments cyanobacterial strain belonging to the general Anabaena, Nadularia, Nostoc and Aphanizomenon were characterized by SDS-PAGE of whole cell proteins and PCR/RFLP of the 16S rRNA gene (Lyra *et al*., 1997).

By RAPD, polymorphisms can be easily analyzed by small amount of template DNA. The strategy is based on amplification of characteristic DNA fragment by a thermostable DNA polymerase, directed by a single oligonucleotide primer of arbitrary sequence in a thermocycling reaction. DNA amplication fingerprinting has been very useful for distinguishing among closely related genotypes and it is a novel method for classification. This technique is sensitive and specific because the entire genome of an organism is used as the basis for generating a DNA profile. Neilan (1995) reported the use of RAPD-PCR to generate uniquences in phylogenetic analysis of toxic cyanobacteria. Kumar *et al*. (2002) analyzed the DNA profile of seventeen different cyanobacterial cultures including free-living and symbiotic form using RAPD technique. The dendrogram, constructed based on the RAPD profile of cyanobacterial cultures, revealed 3 major clusters. There was similarly of 60-90 percent within Westiellopsis cultures. Nostoc cultures shared 50-80 percent similarity with Westiellopsis cultures. Anabaena analysis clearly revealed that free-living cyanobacterial cultures are closely related with each other and are diverse from the symbiotic forms.

Immunological methods

In the immunometric assay, the antisera developed in white leghorn chicken against specific antigen of different cyanobacterial strains reacted only with their respective antigen and there was no cross reaction. The high specificity of the antisera developed against the cyanobacterial proteins proved the utility as an effective serological marker to identify the cyanobacterial strains. The serological techniques, such as fluorescent antibody, immunoprecipitation and ELISA are highly specific for identifying microorganisms.

Thus the development of an integrated system consisting of various biochemical, immunological and molecular biomarkers for various cyanobacterial strain identification is the need of the hour. Instead of depending on a single biomarkers, this integrated system certainly facilitates to employ various biomarkers in combination, which is the objective of polyphasic taxonomy that provides reliability and reproductivity to the process of cyanobacterial strain identification.

Taxonomic methods

In taxonomic methods the fatty acid composition is one of the tools available for the classification of cyanobacterial strains in culture. However, the fact that physiological conditions that alter the fatty acid composition restricts the use of this character in taxonomic analysis. 2002 analysed the fatty acid profile of filamentous cyanobacterial strains of the composite culture inoculums used for algal biofertilization of rice crops using GC-MS. They found that the filamentous cyanobacterial cultures tested were highly diverse with respect to fatty acid composition. In several morphologically very similar species, more or less stable modifications in the pigment content were found. Proteins and isozyme pattern as taxonomic probes have been well documented. Electrophoretically detectable isozyme profiles have been used for identifying strains especially soil inoculation studies. Sample preparation methods that maximize zymogram consistency of Anabaena strains

have been developed.

RESULTS AND DISCUSSION

The G C Content is a promising criterion to classify cyanobacterio at generic level not showing large differences within a genus. The relative binding and the thermal stability difference can also be used to assess the taxonomic status among organisms. RFLP markers are a reliable and highly informative tool for characterizing genetic diversity. Specific antisera against the cyanobacterial proteins proved as an effective marker to identify the strain of cyanobacteria these result are also supported by Lakshamanan (2003). Besides this serological techniques, are highly specific for strain identification. A combination of various biochemical, molecular and immunological biomarkers is the need of the hour for cyanobacterial strain identification. As far as taxonomic methods are concerned electrophoretically detectable isozyme profiles could be used for identifying strains.

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