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# Combined inoculation effect of pink pigmented facultative *Methylobacterium* (PPFM) and other bioinoculants on cotton

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**A field study was conducted to evaluate the individual and combined inoculation effect of PPFM and other bioinoculants on cotton. Initially a detailed survey was conduced in different agro climatic zones of Tamilnadu to isolate an efficient strain of *Methylobacterium.* Isolated PPFM strains were analyzed through polymerase chain reaction (PCR) for the presence of *mdh* gene using *mdh* gene specific probes. Only the isolates having the expected size of *mdh* gene product were chosen for further studies. Selected isolates were screened for plant growth promoting efficiency through vigor index studies. In the field trial conducted with cotton crop, PPFM was inoculated with a diazotroph and a phosphate solubilizing organism (PSB) as individual and combined inoculant treatments. PPFM inoculation has resulted in increased seedling vigor, drymatter production and yield. Inoculation of all the three inoculants together has resulted in increased rhizosphere population of the inoculants, drymatter production and yield.**

**Key words :** Cotton, PPFM, Azospirillum, Phosphate solubilizing bacteria, *Mdh* gene, Vigor index, Field studies

## INTRODUCTION

WORLD Cotton production for 2005 was estimated around 23000 metric tons, while the over all world cotton use has increased 2.5 % than 2004. With ever increasing cotton demand and dramatic increase in prices of chemical fertilizers, the widening gap between supply and demand and concern over environmental hazards caused by chemical fertilizers, now the focus has turned towards sustainable agriculture. Knowing the deleterious effects of using only the chemical fertilizers, use of soil microorganisms which can either fix atmospheric nitrogen or solubilize phosphorous or stimulate plant growth through synthesis of growth promoting substances will be environmentally benign approach for nutrient management and ecosystem function (Tilak and Annapurna, 1993).

The genus *Methylobacterium* commonly known as Pink Pigmented Facultative Methylotrophic (PPFM) bacteria are of ubiquitous in nature. These bacteria are widely found on seeds, plant phyllosphere and in plant rhizosphere (Ivanova *et al.,* 2001b). Scientific investigations have identified the close association of aerobic methylobacteria with plants through which the methanol synthesized and excreted by plants is used by *Methylobacteria* as a source of carbon and energy (Fall, 1996). In turn these bacteria secrete variety of auxins (Ivanova *et al.,* 2001a) and cytokinins (Long et al, 1997) which can be utilized by the plants leading to an increased plant growth and yield. The genus *Methylobacterium* includes a group of pink-pigmented facultatively methylotrophic bacteria with the ability to grow on one-carbon compounds such as formate, formaldehyde and methanol as sole carbon and energy sources, as well as on a wide range of multi-carbon growth substrates (Green, 1992). At present, this genus comprises 15 different species (Jourand et al., 2004 and Doronina et al., 2002). Utilizing PPFM for crop growth promotion is gaining imporance and exploiting the potential of the bacterium can lead to improved plant growth and yield in a sustainable way (Sundaram *et al.*, 2002).

*Azospirillum*, an associative symbiotic $N_2$-fixing bacterium, occurring abundantly in tropical conditions, can positively influence plant growth, crop yields and N-content; these properties may be attributed to its ability to fix nitrogen and synthesize plant growth regulators (PGR) such as auxins, gibberellins, cytokinins and ethylene (Strzelczyk *et al*., 1994).

Phosphorus is one of the major essential macronutrients for biological growth and development. About 98 per cent of Indian soils have inadequate supply of available phosphorus. It is present at levels of 400-1200 mg/kg of soil. Its cycle in the biosphere can be described as open or sedentary because there is no interchange with the atmosphere. Thus even if the total P is high and P fertilizers are applied regularly, the P is rapidly fixed to unavailable forms and accounts for low P use efficiency. Insoluble phosphate, which is not readily available for plant, is 95-99 per cent of the total soil phosphate (Walker, 1975). *Bacillus megaterium* is an efficient phosphate solubilizing bacterium (Rajarathinam *et al*., 1995) and significant increase in crop yield can be obtained through the inoculation of *Bacillus megaterium* (Thakuria *et al.*, 2004).

Counting the benefits of all the three bioinoculants together, the present experiment was aimed to evaluate individual and combined inoculation effect of PPFM and other inoculants on cotton crop through field trial study and biometric observations.

## MATERIALS AND METHODS

### *Isolation of PPFM strains*

A detailed survey was conducted in different agroclimatic zones of Tamilnadu (Aruppukottai, Kovilpatti, Paiyur and Coimbatore) for isolation of an efficient strain of PPFM. About 25 different PPFM isolates from the rhizosphere and phyllosphere of cotton and cotton based cropping systems were obtained. AMS medium (Ammonia Mineral Salts medium) which contains methanol as sole carbon source was used for the isolation of *Methylobacterium* strains (Whittenbery *et al*., 1970).

### *Authentication of PPFM isolates*

Identification of methanol dehydrogenase (mxaF) gene by PCR
The presence of *mxaF* gene is unique to *Methylobacterium* and detected by PCR amplification of *mxaF* gene using specific probes. The total genomic DNA from the PPFM isolates was extracted according to the protocol given by Ivanova *et al.,* (2000) with slight modifications. The primers *mxa* f1003 5'GCG GCA CCA ACT GGG GCT GGT 3' and *mxa* r1561 5'GGG CAG CAT GAA GGG CTC CC3'(Mc Donald and Murrell, 1997) which target the highly conserved region of *mxaF* gene in *Methylobacterium* genera was used in the PCR reactions.

### *Vigor index studies*

These PPFM isolates were screened for growth promoting efficiency of cotton crop through vigor index studies as described by ISTA (1993). The most efficient strain of PPFM isolate with

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