RESEARCH PAPER

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Standardization of media for co-culturing of *Azospirillum*, phosphobacteria and *Methylobacterium* (Azophosmet)

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Nitrogen fixers, phosphate solubilizers and a plant growth hormone synthesizer were cocultured in a suitable single medium The compatibility nature of *Azospirillum brasilense* Sp7, *Bacillus megaterium* var phosphaticum PB1 and *Methylobacterium extorquens* CO 47 in a common growth medium was investigated to coculture the bioinoculants (Azophosmet). All the three had better growth in YEMA. During coculturing the survival of *Azospirillum* Sp7, PSB PB1 and PPFM CO 47 was maximum in YEMB supplemented with 0.5% methanol. Biochemical characters of YEMB culture filtrates (0.5% methanol) recorded the highest amount of ammonia secretion, cell protein and polysaccharide content when compared to nutrient broth and glycerol peptone broth. The shelf life studies of Azophosmet in lignite carrier based formulation assessed for four months indicated that *Azospirillum* Sp7, PSB PB1 and PPFM CO 47 had cell load of 10^8 cfu g^{-1}. The cotton seeds treated with the Azophosmet revealed the surviving ability of *Azospirillum* Sp7, PSB PB1 and PPFM CO 47 noticed up to 24 hr on the seeds.

Key words : *Azospirillum*, *Bacillus*, Methylobacterium *and coculturing*.

INTRODUCTION

There is world wide consensus now that sole dependence on chemical inputs based agriculture is not sustainable in the long run and only integrated nutrient system involving a combination of fertilizers, organic acid or green manures and biofertilizer are essential to sustain crop production, preserve soil health and soil biodiversity. The high cost of chemical fertilizer, the widening gap between supply and demand and their adverse effect on environmental has led agricultural scientists to look for new strategies. Biofertilizers are the alternative sources to meet the nutrient requirement of crops and to bridge the future gaps in chemical fertilizers production. But the key constraint in successful commercialization of biofertilizer is overcoming difficulties in formulating a viable, cost effective and user friendly final product. The development of new microbial formulations is a challenging task and requires greater efforts (Jones and Burges, 1998). The inoculation of consortium containing more than one microbial inoculants is more advantageous than single inoculants application, since the combined inoculation brings in the benefits of two or more inoculants together. The present study was aimed to develop a new formulation of the bioinoculants as a consortium of microbial inoculants with a view to improve the efficiency of bioinoculants, reduce cost of production and to facilitate easy application. *Azospirillum brasilense* as associative nitrogen fixing bacterium, *Bacillus megaterium* var phosphaticum a phosphate solubilizing bacteria and plant hormone synthesizer Pink pigmented facultative methylotrophs (PPFMs) belonging to the genus Methylobacterium are persistently present in the rhizosphere and phyllosphere regions of plants and even on the surface of the seeds of various plants. They were revealed on leaves of almost all plants (Corpe and Rheem, 1989). The three inoculants were selected to form a consortium of inoculants that can promote plant growth efficiently.

MATERIALS AND METHODS

Culture collection:

Azospirillum brasilense Sp7 (*Azospirillum* Sp7), *Bacillus megaterium* var phosphaticum PB1 (PSB PB1) were obtained from the culture collection center of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore.

Invitro growth assay of Azospirillum brasilense, Bacillus megaterium and Methylobacterium extorquens on liquid and solid media:

The bioinoculants strains *Azospirillum, Bacillus megaterium* and *Methylobacterium extorquens* were tested in individual and coculture media. The *Azospirillum* Sp7 was tested in nitrogen free malic acid broth, Phosphobacteria PB1 in Pikovskya's broth and PPFM CO 47 in ammonium mineral salt broth. All the bioinoculants strains were inoculated simultaneously