Internat. J. Plant Sci. Vol.2 No.2 July 2007 : 174-179

Influence of micronutrients on growth, survival and *in vitro* nitrogen fixing efficiency of *Azospirillum brasilense* strains

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(Accepted :May, 2007)

SUMMARY
A laboratory experiment was conducted to study the effect different concentrations of micronutrients (Zinc, iron and molybdenum) on the survival, growth and *in vitro* nitrogen fixation efficiency of *Azospirillum brasilense* strains (ACD-15, ACD-20 and ACD-L_4). Among the different concentration of micronutrients, 100 ppm was found to have a positive effect on the growth of *Azospirillum* strains, whereas 250 ppm concentration of of micronutrients has an adverse effect on the growth of *Azospirillum*. Significant differences were observed between additions of micronutrients individually and in combination. In case of addition of micronutrients individually, zinc has supported maximum growth in case of ACD-15 and ACD-20 strains whereas addition of iron to the medium has supported maximum growth in case of ACD L_4 strain. When all the strains were subjected for *in vitro* nitrogen fixation in N free semi solid malate medium, significantly higher nitrogen fixation was observed in all the treatments over control (without any micronutrients). Among different treatment combinations, addition of 100 ppm of Zn, Fe and Mo into the N free semi solid malate medium gave higher nitrogen fixation in ACD-15 (26.93 mg N/g of malate), ACD-20 (27.17 mg N/g of malate) and ACD L_4 (27.53 mg N/g of malate) when compared to control (19.90 to 21.15 mg N/g of malate used). Thus it can be concluded that, enrichment of growth medium with different micronutrients had significant influence on the growth as well as nitrogen fixing efficiency of *Azospirillum* strains.

Key words: *Azospirillum*, Micronutrients, Assoc. symbiotic N fixers etc.

Azospirillum is gram negative, vibroid and 1-1.5 mm in diameter, possessing peritrichous flagella for swarming and apolar flagellum for swimming. It belongs to a group of rhizosphere bacteria often referred to as plant growth promoting rhizobacteria (PGPR) contains poly b hydroxy butyrate (PHB) granules (Kloepper *et al.*, 1989). *Azospirillum* is essentially an aerobic microorganism but able to grow under micro-aerophilic condition and under such condition only it is capable of fixing the nitrogen and not otherwise (Vande-Broek and Vanderleyden, 1995). Micronutrients like zinc and iron are known to play an important role on the growth of microorganisms, as they are the constituents of many enzyme systems. Molybdenum is also known to be a key element required by the microorganisms for nitrogen fixation. It is a constituent of nitrogenase enzyme and nitrate reductase enzymes.

The nitrate reductase is essential in the assimilation of nitrates since it catalyses the first step of the reduction of NO_3 to NH_3. The other major molyboprotein of plants include nitrogenase, which fixes atmospheric nitrogen to NH_3, which is assimilated by plants. It was, therefore, thought necessary to develop biofertilizer enriched with micronutrients particularly for the cereals. As a preliminary study, the present investigation was undertaken to know the effect of different levels of micronutrients on the growth as well as their nitrogen fixing efficiency of *Azospirillum brasilense* strains (ACD-15, ACD-20 and ACD-L_4).

MATERIALS AND METHODS

Laboratory experiments were conducted to study the effect of different concentration of micronutrients (Zn, Fe and Mo) as alone and in combination on the growth and *in vitro* nitrogen fixing capacity of *Azospirillum* strains in the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad. The details of the material used and methods followed are listed below.

Three strains of *Azospirillum brasilense* viz., ACD-15, ACD-20 and ACD-L_4 were obtained from the culture collections of the Department of Agricultural Microbiology. These strains were identified and maintained on nutrient agar slants at 4°C. Among the different micronutrients, sulphate form of iron and zinc, and molybdenum as sodium molybdate were selected for the present study.

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