COPPER TOLERANT STRAIN OF AZOTOBACTER CHROOCOCCUM

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A B S T R A C T

Copper is one of the few metallic elements to occur in nature and an importance lies in the participation of number of biochemical reactions in plants life. Copper is used in agricultural processes. Microorganisms develop more resistance to heavy metals and adapt particular condition containing toxic metal in their media. Among metals copper and zinc are considered to be essential for the growth and yield of crop plants. Each of the metal has one or more specific functions and growth is determined by the presence of an optimum concentration of these metals in the growth media. Bacteria are most dominant group of microorganisms in soil and probably equal one half of the microbial bio-mass in soil. In present study we developed the copper strain of Azotobacter chroococcum in Jensen's medium. An inhibitory level of organism to Cu was determined by selecting the range of 0.01 - 10 mgl^{-1}. The metal resistant strain of Azotobacter was obtained by repeated subculturing. The inhibitory level 0.08 mgl^{-1} of copper metal for Azotobacter. The tolerance index (TIC) of bacteria increased to 0.1 to 0.5 mgl^{-1} in about 35 generations. TIC of tolerant strain was obtained at Cu-t 0.36 for Azotobacter chroococcum.

Keywords: Azotobacter chroococcum, Copper, metal

1 Introduction

The fixation of atmospheric nitrogen by free living microorganisms as distinguished by fixation in association with another host system is known as non-symbiotic nitrogen fixation. The aerobic bacteria capable of fixing nitrogen come under genera *Azotobacter*, *Azomonas*, *Beijerinckia*, *Derxia*, *Mycobacterium* and *Azospirillum* (Becking, 1981). Among these bacteria, many are heterotrophic and depend on energy derived from the degradation of plant residues. The lack of organic matter in soil is a limiting factor in the proliferation of *Azotobacter in soil.* Soil microbiological problems related to the rhizosphere attracted the attention of several workers in India. The occurrence of *Azotobacter* in soil types of India. Many workers believe that increased respiration by *Azotobacter* excludes oxygen from nitrogens which may serve as natural tool to scavenge oxygen from the site of nitrogen fixation (Dalton and Postgate, 1969 and Postgate, 1971, 1974)

2 Materials and methods

2.1 *Collection and isolation of Azotobacter from soil*

The *Azotobacter* collected from rhizosphere of plants from 10 cm - 30 cm depth of soil of the experimental field of Nagpur District. Isolation was done by serial dilution technique in Jensen's medium. Plates were incubated for 3-4 days.

2.2 *Estimation of growth*

The growth of both bacteria was monitored in measuring optical density at 660 nm for bacteria by using UV spectrophotometer, model CL-54D.

2.3 *Preparation of stock solutions for heavy metals and determination of inhibitory level*

The stock solutions of metals were prepared by adding 3.928 g of $CuSO_4$. $5H_20$, in 100 ml distilled water separately. The metal solution was diluted to various concentrations in the range of 0.01 - 10 mgl^{-1} in determining the inhibitory level of each metal. An inhibitory level of organism to Cu was determined by selecting the range of 0.01 - 10 mgl^{-1}. Bacterial growth was observed under microscope after 4 days and inhibitory level of organism.

2.4 *Determination of tolerance index concentration (TIC) for azotobacter chroococcum and selection of new strain*

The metal resistant strain of *Azotobacter* species was obtained by repeated sub-culturing. The inhibitory level was found to be 0.08 mgl^{-1}Cu for the growth of *Azotobacter*. In the present investigation influence of various environmental factors have been studied on wild (Laboratory) strain and Cu-t 0.36 *Azotobacter chroococcum*

3 Results and Discussion

3.1 Heavy metal toxicity to microorganisms

The pollution of natural environment by heavy metal is potential menace to the health. Most of these occur in small or trace amounts in aquatic bodies. Sixty eight of these metals have a density five time that to water and known as heavy metals. The presence of one heavy metal influences the uptake of the others (Silverberg et al, 1976).

3.2 Influence of Copper on microorganisms

Copper is one of the few metallic element to occur in nature and an importance lies in the participation of number of biochemical reactions in plants life (Malstrom & Neilands, 1964). The increase in number and dry weight of nodules and grain yields of lentil when seeds were soaked in 0.35 percent copper sulphate prior to *Rhizobium* inoculation (Khurane et al, 1976). The heavy metals absorbed to bacterial wall of *Bacillus subtilis* by copper upto 100% (Urrutia & Beveridge, 1993)

4 Conclusion

In the present study it has been shown that *Azotobacter chroococcum* can adapt more heavy metal copper after 35 generation in respective metal. The copper and zinc are micronutrients required for crops. Thus the application of these nutrients through the organism can have improved the soil fertility, integrated plant nutrient supply system (IPNS) and yield of crops. Use of wild and metallic strains of biofertilisers culture improved growth yield and quality of crop along with improves soil fertility and could save 25% of chemical fertilizers needed for the crop plants. Use of wild and metallic strains of biofertilisers culture improved growth yield and quality of crop along with improves soil fertility and could save 25% of chemical fertilizers needed for the crop plants.

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