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EARTHERN POT CULTURE METHOD TO CHECK THE STABILITY OF MARINE *AZOTOBACTER* IN THE SOILS BY ASSESSING GROWTH OF GREEN GRAM

A. Karthick and V. S. Jayashree
Department of Microbiology, Dr. G. R. Damodaran College of Science, Coimbatore-641 014, T.N.

ABSTRACT

Azotobacter sp. is a gram-negative soil dwelling organism with a wide variety of metabolic capabilities which include the ability to fix atmospheric nitrogen by converting it to ammonia. In this study, 45 samples were positive out of 50 marine samples, collected from different locations of Rameshwaram marine region at the depth of 1-2m. The nitrogen fixing ability of the isolated *Azotobacter* sp. from marine source is compared with soil *Azotobacter* sp. and standard *Azotobacter* sp. by pot cultivation method by assessing the growth of green gram. Seed germination, shoot length, root length and chlorophyll content were measured. Therefore, irrespective of the environment, marine *Azotobacter* sp. is capable of fixing atmospheric nitrogen in a terrestrial environment at a higher frequency rate. Since it is a non-symbiont, it can be a suitable for all kinds of crop plants as a biofertilizer.

INTRODUCTION

During 1980s, the use of chemical fertilizers in agriculture fields in Colombia reflect an average of 20% of the total production investments (Ortega 1992), increasing the acquisition costs of agricultural products. Therefore, scientists and biotechnologists in particular, while trying to cut on these costs, have used different soil-isolated bacterial genera, in the form of bio-fertilizers, which may eventually reduce chemical fertilizers overuse, as well as all derived serious problems in soils where they are applied. Therefore, this survey intends to evaluate an inoculum to be used as a bio-fertilizer, which is made up of phosphate-solubilizing bacteria (PSB) and *Azotobacter chroococcum,* and that may be able to solubilize sources of insoluble phosphate and improve the use of nitrogen. In this way, use of chemical fertilizers could be diminished and productivity could be optimized, thus providing enhanced benefits to growers who are directly dependent on this crop for their survival .

Nitrogen fixation which is catalysed by nitrogenase, is the reduction of N_2 (atmospheric nitrogen) into NH_3 (ammonia). Nitrogen fixation can be an important source of nitrogen for biological productivity in the marine environment. The estimated contribution of free-living N-fixing prokaryotes to the N input of soil ranges from 0-60 kg/ha/year (Burgmann et al. 2003). Dinitrogen (N_2)-fixing microorganisms (diazotrophs) play important roles in ocean biogeochemistry and plankton productivity (Church et al. 2005). Interest in nitrogen fixation in the sea has usually been focused on rates of nitrogen fixation, but information on the types of species present with the capability for nitrogen fixation can be important for predicting nitrogen fixation rates *in situ*. Understanding how fixed N regulates nitrogenase availability is necessary for devising strategies to increase the amount of ammonium synthesized by nitrogen fixing bacteria with the potential to be used in agriculture (Kennedy et al. 2004).

When *Azotobacter* is applied to seeds, seed germination is improved to a considerable extent, so also it controls plant diseases due to above substances produced by *Azotobacter* (Kader et al. 2002.)

There are different strains of *Azotobacter*, each of which has varied chemical, biological and other characters. However, some strains have higher nitrogen fixing ability than others (Burgmann et al. 2003). Besides nitrogen fixation, *Azotobacter* also produces, thiamine, riboflavin, indole acetic acid and gibberellins. There is firm evidence that indole-3-acetic acid, gibberellins and cytokinins, all produced by plants and essential to their growth and development, are produced also by various bacteria which live in association with plants (Ahmand et al. 2004). There is also evidence that the growth hormones produced by the bacteria can in some instances increase growth rates and improve yields of the host plants (Brown et al. 1976).

MATERIALS AND METHODS

Sample collection: Samples were collected in different locations of Rameshwaram marine region at the depth of 1-5 m. The randomly collected samples were kept in an ice-cold box and transported safely to the lab for further analysis within 12 hrs.

Isolation of *Azotobacter* from water and sediment samples (Mary et al. 1985)**:** Media used for the isolation of nitrogen fixing organism (*Azotobacter*) from marine sources were Jensen's agar medium, *Azotobacter* agar medium, Burk's medium and marine agar medium. *Azotobacter* strains used for this study were maintained and cultured in Burk's medium. As the isolates are of marine origin, the media were prepared with the 3.5% sodium chloride (NaCl) solution.

Culture characteristics (Bagwell et al. 1988): Gram-staining characteristics and cell morphologies were determined by standard methods (Gerhardt et al. 1981). Motility was observed in wet mount using phase contrast microscope. Preliminary physiological characterization such as catalase test and starch hydrolysis test were also carried out.

Pot culture method: The broth containing active culture of *Azotobacter* sp. was selected. Three marine strains (400, 408, 409), 3 soil strains, (1, 2, 6) and standard cultures such as *Azotobacter* sp. (2632), *Azotobacter chroococcum* (2452), and *Azotobacter lactinogens* (2633) procured from MTCC, Chandigarh, were used. Forty healthy seeds of green gram were mixed with 3mL of *Azotobacter* inoculum and 3mL of cooled rice porridge. Approximately (10×10^7 CFU/pot) broth inoculum was introduced in all the 18 pots (original and duplicate) except the control pot. The pots were watered regularly at an interval of 5 days and the length of the root and shoot were observed and recorded. After 15 days interval, leaves were collected for estimating the chlorophyll content. Chlorophyll was extracted in 80% acetone and the absorption at 663nm and 645nm were read in a spectrophotometer.

RESULTS AND DISCUSSION

Totally 50 samples were collected in marine region of both water and sediments at the interval of approximately 20 days. These samples were processed for isolation of *Azotobacter* spp. followed by culture characteristics for identification of free-living diazotrophic *Azotobacter*. The results of these studies show that *Azotobacter* is Gram negative rod shaped with motile cells which shows positive result for starch and catalase tests. The morphology of *Azotobacter* colonies shows that they were clear, large, mucoid and watery drops like initially i.e. from the marine source. All the isolated *Azotobacter* strains were numbered for the easy identification and convenience.

The main objective of the pot culture study was to examine the influence of *Azotobacter* on green gram. Five days after sowing various characteristics of growth such as percentage of germina-

Table 1: Percentage of seed germination, shoot and root length afer 15 days of sowing of green gram.

Pot culture isolate	% of germination	Shoot length	Root length
400 M	91.25	25.0	12.5
408 M	78.75	24.2	14.5
409 M	80.00	19.6	13.0
1 S	92.50	21.8	14.0
2 S	86.25	22.6	11.4
6 S	82.50	24.0	11.2
2452 R	88.75	22.0	10.8
2632 R	90.00	22.0	11.0
2633 R	91.25	21.5	10.4
Control	82.50	19.0	11.2

M = marine strain, S = soil strain, R = reference strain

tion, shoot and root length were measured. Results for percentage of seed germination, shoot and root length for 15th day are shown in Table 1. The estimation of total chlorophyll content by spectrophotometric method shows that the values range from 0.257mg/g to 0.353mg/g. The plants inoculated with *Azotobacter* were taller than that of control pot. Marine strain 400 and soil strain 6 show remarkable effect on shoot length of the plant, while marine strain 408 and soil strain 1 show remarkable effect on the root length of the plant on 15th day.

Beside seed inoculations, the banana's sucker as well as the soil were inoculated with *Azotobacter* and maximum plant height and leaf size was obtained using one-half the recommended nitrogen fertilizer (Tiwary et al. 1998). Plants inoculated with marine *Azotobacter* show values of total chlorophyll content nearer to that of standard strains by biometric analysis. Generally, plants inoculated with *Azotobacter* show higher amount of total chlorophyll content than the plant devoid of *Azotobacter* strains (control). From this experiment it was found that there is pronounced effect of marine *Azotobacter* as nitrogen fertilizer on the growth of green gram. The hypotheses from this study were that the plants inoculated with *Azotobacter* will exhibit more growth than plants not inoculated, and plants fertilized with nitrogen will exhibit more growth than plants not fertilized.

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