Effect of a Common Medium on the Growth of Nitrogen Fixer
*Rhizobium* and Phosphate Solubilizer *Bacillus megaterium*

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Mass culturing of two beneficial organisms used as biofertilizers for crops would reduce the risks in production and minimize the capital involved and this demands appropriate media that supports both organism and also selection of organisms that are not antagonistic to each other. A study was initiated to culture a nitrogen fixer (*Rhizobium*) and phosphate solubilizer (*Bacillus megaterium*) in a single medium and to study their growth patterns and shelf life in carrier. The growth of *Rhizobium* and *Bacillus megaterium* was assessed in different media and a slight modification in the traditional yeast extract mannitol media promoted the growth of both the organisms. The growth of the individual organisms in the modified medium was assessed by estimating the population at regular intervals and compared to their original medium. Maximum population of *Rhizobium* and phosphobacteria was at 60 hr when the phosphobacteria inoculation of later was after 48 hr of *Rhizobium* inoculation. The shelf life of the individual inoculants in the inoculant containing both the organism in a sterile carrier base revealed no significant differences compared to individual organisms inoculated in a sterilized carrier. The population of both organisms in carrier based mixed inoculant remained at 10³ cells till 90 days.

**Key words:** *Rhizobium* sp., *Bacillus megaterium*, Dual inoculation, N-fixer, Phosphate solubilizer

**Introduction**

The vast majority of plant associated soil organisms inhabit the rhizosphere, where there is a continuous interaction between and within the microbes and the plant roots. The plant microbe microbe-interaction that is of recent focus has an important influence on plant growth. Soil microorganisms such as *Rhizobium*, *Azospirillium*, etc., play an important role in improving soil fertility and crop productivity due to their capability to fix atmospheric nitrogen which inturn increases nitrogen supply to crop. Bacterial genera such as *Pseudomonas* and *Bacillus sp.* help to convert insoluble phosphate into soluble phosphate and thus improve phosphate availability to plants (Frank, 2001). The biofertilizers (containing these microorganisms) applied for the nutrients availability also play a commendable role in amelioration of biophysical properties of soil, constituting an important role in crop productivity (Pal, 1998).

It is estimated that approximately 139 million Mg of nitrogen is fixed biologically out of which legumes contribute 25.2%. *Rhizobium* is the most widely used biofertilizer, which colonize the root of legumes and form nodules, which act as factories of nitrogen fixation. The *Rhizobium*-legume association can fix 100-300 kg N ha⁻¹ in a season and leave substantial quantity of nitrogen for the succeeding crop (Balasubramanian, 1996). The largest contribution of Biological Nitrogen Fixation (BNF) to agriculture is derived from *Rhizobium* legume symbiotic association and inoculation with efficient *Rhizobium* strain specific to each crop is very essential for the N gains and better crop yield. Tippannavar et al. (2001) reported a yield increase upto 54.8 and 50% reduction in nitrogen dose recommended through the application of *Rhizobium* strains in pigeon pea.

Efficient and economic use of phosphate fertilizers could be achieved by using phosphate solubilizing microorganisms (PSMs) in legumes, cereals and other useful crops. Application of PSMs causes a replacement of 25% of phosphate fertilizers (Gupta et al., 1998). Ramesh and Sabale (2001) recorded pod yield significantly higher under P₂O₅ application along with phosphate solubilizer in
groundnut. They also reported higher protein percentage and oil content due to P solubilizer. Seed inoculation with PSB significantly enhanced seed, stover and oil yield and uptake of N, P and S over no inoculation in mustard (Baldev and Pareek, 2000).

Biological nitrogen fixation depends appreciably on the available form of phosphorus. So the combined inoculation of nitrogen fixers and PSMs may benefit the plant better than either group of organisms alone. Dual inoculation of plants with a nitrogen fixer and PSM showed increased nodulation, growth, yield, and P and N uptake (Tiwari et al., 1988).

Though the nitrogen fixers and phosphate solubilizing bacteria are recommended to farmers, presently they are being produced and supplied in individual packets involving a high production cost. So the present study aimed at culturing a nitrogen fixer (Rhizobium) and a phosphate solubilizer (Bacillus megaterium) together in a common medium and its feasibility to use as a carrier based inoculant by assessing their shelf life in the carrier. The term phosphobacteria is used to denote the phosphate solubilizing bacteria Bacillus megaterium and they are used interchangeably.

Materials and Methods

Bacteria and culture conditions Authentication cultures of Rhizobium strain BMBS P47 and phosphobacterial strain Bacillus megaterium var phosphaticum Pb1 obtained from the culture collection centre of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore were used in the studies.

A simple Streak method was carried out to assess the growth of Rhizobium and phosphobacteria in different media. The yeast extract mannitol (YEMA) medium slightly modified by reducing the concentration of mannitol to 0.5% and adding glycerol at 5 mL L\(^{-1}\) is here referred to as modified YEMA (MYEM). The media that showed better growth of the two cultures was further selected and used for broth culture and dual inoculation studies.

Broth culture studies One mL of log phase culture of Rhizobium strain BMBS P47 grown in YEMA medium was used to inoculate 100 mL of YEMA broth and MYEM broth individually. Similarly phosphobacteria was inoculated in nutrient broth and MYEM broth and the flasks were incubated at 28 ± 1°C in a shaker. The growth was measured at regular intervals viz., 12 hr for Rhizobium (BMBS P47) unto 144 hr and 6 hr for phosphobacteria (Pb1) unto 72 hr, by both turbidometric and plate counts simultaneously.

To a cuvette, 5 mL of the culture sample was transferred aseptically and the absorbance read at 660 nm in a Spectronic 20 photocolorimeter. Simultaneously, one mL of the broth culture was taken, serially diluted and plated for the viable cells estimation. A quantity of 10\(^{4}\) to each dilution of 10\(^{4}\), 10\(^{3}\), 10\(^{2}\) and 10\(^{1}\) was used for plating in the respective media.

Dual inoculation studies The effect of the time of inoculation of the two cultures on the growth of individual organisms was assayed by a dual inoculation study. The Rhizobium strain BMBS P47 was inoculated in a series of 250 mL conical flasks containing 100 mL of sterilized modified yeast extract mannitol broth. One mL of phosphobacteria was inoculated to the Rhizobium inoculated flasks individually at different periods of time and population of both Rhizobium and phosphobacteria was estimated by serial dilution and plating as mentioned above. The treatment details are mentioned in Table 2.

Studies on the survival of the mixed inoculant in carrier based formulation The shade dried and sieved (106 µm IS sieve) lignite obtained from Neyveli Lignite Corporation adjusted to a neutral pH using commercial grade calcium carbonate was packed in 50 g lots in opaque low density grade polythene bags of 75 µm thickness and sterilized for one hour. Broth culture of Rhizobium/phosphobacteria/mixed inoculant having a cell load of 10\(^{10}\) cells mL\(^{-1}\) in late log phase was inoculated in the carrier bags using a sterile, plastic syringe fitted with hypodermic needle until 40-45% moisture content was obtained. The bags were thoroughly kneaded to ensure absorption of the liquid culture in to the carrier. The inoculated packets were stored at room temperature and survival of the cultures, pH and moisture content were estimated.

Analysis of pH, moisture content and population in the carrier One g of inoculated carrier was transferred under aseptic conditions and serially diluted. Plating in YEMA medium and nutrient agar medium respectively assessed the population of Rhizobium and phosphobacteria in the inoculant. The plates were incubated at 28 ± 1°C and the population was estimated