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ORIGINAL ARTICLE

Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculant

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Abstract *Azotobacter chroococcum* (A101) was examined for some biological activities such as nitrogenase, phosphatase, potassium solubilization, and production of some plant hormones such as indole acetic acid, gibberellic acid, and cytokinin. Six different formulations were prepared, using different carrier materials namely; peat moss, mixture of peat moss plus vermiculite 1:2 (w/w), wheat bran, rice husk, clay, and sodium alginate. Each carrier material was packed using polyethylene pages, and then divided into three groups. The first group was sterilized by autoclaving at 121 °C for 20 min, and the second one was sterilized by gamma irradiation at a dose rate of 4.0 kGy for 1 h. However, the third group was left without sterilization. Half of the inoculated polyethylene bags, containing the tested formulations either sterilized by autoclaving or gamma irradiation, were incubated at 8 °C and the other bags were incubated at 30 °C for 6 months. The non-sterilized bags were incubated under the same condition but only for 3 months. For testing the survival of *Az. chroococcum* (A101), the prepared formulation samples were taken every month during the storage period. Nitrogenase activity was evaluated in the prepared formulations which exerted survival cells equal to or more than 10^8 CFU/ml after 6 months of storage period. Results revealed that non-sterile formulations exerted high numbers of total fungi and bacteria along the storage period; however, *Az. chroococcum* (A101) numbers were decreased over incubation time. No contam-

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inants have been detected in all sterilized carriers. *Az. chroococcum* (A101) inoculated on wheat bran exhibited the highest densities among the tested carriers. Encapsulated formulation of alginate exerted the high stability in *Az. chroococcum* (A101) densities up to the end of the incubation period (6 months) at both 30 °C and 8 °C, being 11.905 log 10 CFU/g.

Introduction

The biofertilizers have the ability to convert nutritionally important elements from unavailable to available form through biological processes (Vessey, 2003). Some bacteria are capable to fix the atmospheric nitrogen. The free-living non-symbiotic nitrogen-fixing bacteria those belonging to genus *Azotobacter* sp. which is a heterotrophic, aerobic microorganism being broadly dispersed in different environments, such as soil, water, and sediments (Palleroni, 1984).

Different investigations stated that inoculation with *Azotobacter* sp. has beneficial effects on plant yields (Idris, 2003), due to the increase in fixed nitrogen content in soil (Mrkovac et al., 1996) and to the microbial secretion of stimulating hormones, like gibberellins, auxins, and cytokinins (Salmeron et al., 1990; Gonzales Lopez et al., 1991). Also, Kumar and Singh (2001) established the ability of *Azotobacter* sp. to solubilizing phosphates.

Variety of materials used as carriers has been shown to improve the survival and biological effectiveness of inoculants by protecting bacteria from biotic and abiotic stresses (van Veen et al., 1997). Suitable carrier should be cheap, easily used, mixable, packageable, and available. Also, the carrier must permit gas exchange, particularly oxygen, and has high organic matter content and high water holding capacity as well (Bashan,1998; Ben Rebah et al., 2002). According to Somasegaran and Hoben (1994), the good carrier material must be non-toxic either to the bacterial inoculants or to the plant itself. Furthermore, Stephens and Rask (2000) and Ferreira and Castro (2005) stated that the carriers should have near neutral or readily adjustable pH, be abundant locally at a reasonable cost and able to sterilize. These properties only indicate the potential for a good carrier, while final selection of carrier must be based on microbial multiplication and survival during storage, the general method of planting, equipment used for planting, and acceptable cost.

Among carriers that can sustain high levels of microbial load, the peat is considered the most widely used carrier (Burton, 1967; Peterson and Loynachan, 1981) but is not universally available (Tilak and Subba Rao, 1978). Alternatively, different materials such as industry by-products, organic wastes, mineral soils, plant by-products, coal, perlite, and agro-industrial wastes have been tested as culture media for the microbial growth (Brockwell and Bottomley, 1995; Stephens and Rask, 2000).

Alginate is dry, synthetic, simple to use, uniform, biodegradable by soil microorganisms, and non-toxic in nature. It contains a large uniform bacterial population and provides slow release of the bacteria for long periods (Bashan, 1998). It causes no ecological pollution and can be produced on large scale by the proper industry. The beads can be stored for long periods in a relatively small volume without any apparent effect on the size of the bacterial population (Bashan and Gonzalez, 1999).

It is of importance to support high number of bacterial inoculants on carrier during the storage period before use and to prevent undesirable spreading of pathogenic bacteria to agricultural field (Somasegaran and Halliday 1982). Different methods were used for the sterilization of carrier materials to obtain the most suitable one without any effect on their quality. Strijdom and Deschodt (1976) reported that steam sterilization by autoclaving is the most commonly used and has the superiority among all employed methods due to low cost and its ability to allow absolutely pure culture of inocula to be prepared. However, Strijdom and van Rensburg (1981) reported that gamma-irradiation is the most suitable way for carriers sterilization, because this process makes almost no changes in physical and chemical properties of the material and the final materials are more quality.

The present work was designed to study the effect of different products and wastes as carrier materials for preparation of *Azotobacter chroococcum* formulation and evaluating the survival and biological efficiency of tested strain in these formulations at the end of storage period using two different methods of sterilization and stored at different temperatures.

Materials and methods

Source of strain

Az. chroococcum (A101) was kindly provided from Unit of Biofertilizer, Fac. Agric., Ain Shams Univ., Cairo, Egypt.

Materials used for formulation of solid state inoculants

Peat moss and peat moss and vermiculite 1:2 (W/W) were obtained from "Shama Company for Agricultural Requirements", wheat bran was obtained from Mills & Bakeries, north of Cairo, rice husk was obtained from El-Sharkia Governorate, Egypt, clay was obtained from the farm of Faculty of Agriculture, Ain shams University, Cairo, Egypt. Alginate was obtained from El Nasr Pharmaceutical Chemicals Co., Egypt.

Assessment of some physiological activities of Az. chroococcum (A101)

Az. chroococcum (A101) was activated in Modified Ashby's broth medium (Abd El-Malek and Ishac, 1968). After 7 days at 30 °C, cell densities were adjusted to be 35×10^7 cfu/ml and examined for the following metabolic activities.

a. Nitrogenase activity according to the method described by Hardy et al. (1973).
b. Phosphatase activity and potassium solubilization according to the method described by Jackson (1973).