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Azotobacter chroococcum: Utilization and potential use for agricultural crop production: An overview

Sellamuthu Gothandapani[1,2], Soundarapandian Sekar[2] and Jasdeep C Padaria[1]*

[1]Biotechnology and Climate Change Laboratory, National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi, India

[2]Department of Industrial Biotechnology, School of Biotechnology Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

*Corresponding author: *jasdeep_kaur64@yahoo.co.in*

Abstract

It is important to develop integrated crop production strategies that enhance the productive ability of crops in a sustainable manner for the developing nations worldwide. Intensive farming practice of high yielding crop production is mainly dependent on chemical inputs as fertilizer, pesticide and weedicides. However, excessive use of chemical inputs has negative impact on environment in the form of soil infertility, ground water contamination, imbalance of biological ecosystem and cost inputs. Soil health is an important aspect of productive sustainable agriculture and in recent years maintaining soil quality can reduce the problems of land degradation, decreasing soil fertility through use of advanced technologies in the field of biofertilizers. Biofertilizers can produce mineral nutrients through natural processes of biological nitrogen fixation, phosphate solubilization and also stimulation plant growth through the synthesis of plant growth promoting substances. Thus, in recent years biofertilizers have become an important component of integrated management for enhanced crop production. This paper reviews the facts and observations regarding an important biofertilizer microorganism *Azotobacter chroococcum* and its potential for crop production based.

Keywords: *Azotobacter*, Biofertilizer, Crop improvement, Sustainable agriculture.

Introduction

In recent years salinization, soil erosion and ground water contaminations are the most important ecological concerns which affect the agricultural land causing it to become unsuitable for crop production. Biofertilizer plays key role in productivity and sustainability of soil health management in terms of solubilizing plant nutrients; both micro and macro nutrients, and stimulating plant growth. Use of biofertilizer in agriculture protect the environment as they are eco-friendly and economical for the farmers (Khosro and Yousef, 2012). Increasing and extending the application of biofertilizers can reduce the negative impact of chemical fertilizers and decrease the adverse environmental effects of agrochemicals. Biofertilizers or bio inoculants are minute organisms which are beneficial to the growth of plants and responsible for enhanced crop yield. The various naturally available free living bacteria which are very beneficial for the overall plant growth are collectively known as plant growth promoting rhizobacteria (PGPR) (Kloepper, 1994). These PGPRs are mainly involved in metabolic process related to nitrogen fixation, phosphate solubilization and overall plant growth promotion. *Azotobacter* sp, *Azospirillum* sp, blue green algae, *Azolla*, *Rhizobium* sp are few of the commonly used phosphate solubilizing microorganisms which are also used as biofertilizers. *Sinorhizobium mycorrhizae* is one of the most studied microbe with phosphate solubilizing and other PGPR properties (Selvakumar et al, 2009).

Since the advent of green revolution and high input agriculture practices, chemical fertilizer have become the major source of nitrogen for crop plants (Peoples et al., 1995). Due to public awareness of environmental safety, use of biofertilizes have increased tremendously in recent years. Biological nitrogen fixation plays an important role in maintaining of nitrogen status of the soil. Biofertilizer are able to fix atmospheric nitrogen as single microbial inoculants or as consortia. Microbes that are able to fix atmospheric nitrogen are grouped in to three broad categories as: 1) non-symbiotic bacteria, 2) blue green algae and 3) symbionts (Rajendra et al., 1998). The genus *Azotobacter*, belonging to family *Azotobacteriaceae*, represents the main group of heterotrophic non-symbiotic nitrogen-fixing bacteria principally inhabiting neutral or alkaline soils (Sartaj et al., 2013). The non-symbiotic free living *Azotobacter* is largely associated with nitrogen fixation in plant rhizosphere (Lakshminarayana 2000). It is also involved in various other metabolic pathways of plant growth promotion (Gonzalez-Lopez et al. 1986), antagonistic activity against plant pathogens in soil (Verma et al. 2001) and activation of potential rhizospheric bio-inoculants for enhancement of plant yield (Lakshminarayana et al. 2000). Vessey (2003) reported *Azotobacter* sp. and *Azospirillum* sp. are the most commonly used biofertilizers in different agricultural crops either as combined or as single inoculation. The estimated contribution of these free-living nitrogen fixing bacteria to the nitrogen input of soil ranges from 0–60 kg/ha per year. Studies have shown that inoculation of maize crop with *Azotobacter* significantly increased its plant height, grain weight and grain yield. Inoculation of *Azotobacter* increased grain yield in maize upto 35% over the non inoculated treatment (Bandhu and Parbati, 2013). Therefore, in the development of sustainable agricultural use of *Azotobacter* as biofertilizer has great importance in improved of nutrient profile of plant and soil and increased crop yield accompanied by protection of environmental pollution and soil contamination (Saini et al., 2004; Namvar et al., 2012; Rana et al., 2012).

Need of Bio-Fertilizers

In recent year's intensive farming practices have led to high crop yield which has been achieved by extensive use of chemical fertilizers and these chemical fertilizers are environmentally and economically inhibitory. Continuous use of chemical fertilizers has led to the environmental pollution and contamination of the soil, pollution of water basins, and disruption of flora-fauna of ecosystem and ultimately reduced soil fertility (Mishra., et al., 2013). Dependence on available and required chemical fertilizers for the future agricultural growth will result in further reduction in soil quality, acidification, ground water contamination and hence loss of ecological balance. The field of eco-friendly biofertilizers is creating advancement in growing level of concern towards environmental safety sustainable agricultural practice (Debojyoti et al., 2014). Nitrogen plays important role in increasing the crop yield, crop nutrients for agricultural crop productivity. The long term use of biofertilizers is not only economical and eco-friendly but is also found to be increasing crop productivity and improving soil health over chemical fertilizers. Easy accessibility of biofertilizer is an added advantage especially for small and marginal farmers for whom the cost of chemical fertilizer is becoming unaffordable (Mishra et al., 2013).

Characteristic features of *Azotobacter chroococcum*

The genus *Azotobacter*, belonging to the family *Azotobacteriaceae* is an aerobic, heterotrophic, non-symbiotic biological nitrogen fixing microbe. It has been found that some *Azotobacter* species exist in association with some crops especially cereals (Martyniuk et al., 2003). *Azotobacter* sp. are generally present in natural and alkaline soil with its most commonly occurring species found in arable soils. The genus *Azotobacter* comprises of different species: *A.chroococcum, A. vinelandii, A. beijerinckii, A. paspali, A. armeniacus, A.nigricans* and *A. salinestri*. The free-living, gram-negative, motile and mesophilic *Azotobacter* spp. are capable of fixing on an average 20 kg N/ha/per year (Rawia et al., 2009). *Azotobacter* spp. was commonly found as small, medium or large rod shaped cells. The colonies developed on Jensen's agar medium were raised, spherical flat and with irregular margins. The colony size varied from ≥2 mm to ≥5 mm in 7days. The colony characters such as colony-margin, size, colour and consistency also differed among the different species of *Azotobacter* spp. (Tejera, et al. 2005;

Ahmad, et al. 2008). The agronomic importance of *Azotobacter* spps is due to its the capability of biological nitrogen fixation, synthesis of antibiotics, plant growth hormones (Pandey et al., 1998), vitamins, exopolysaccharides and pigment (Jimenez et al., 2011) and also its antifungal activity (Sudhir et al., 1983). *A.chrococcum* is a common nitrogen fixing microbe found in the rhizosphere of agricultural crops. The first representative of the genus *A. chroococcum*, was discovered and described in 1901 by the Dutch microbiologist and botanist Martinus Beijerinck (Martyniuk and Martyniuk, 2003).

Nitrogen fixation process is highly sensitive to O_2, but *Azotobacter* sp.have special mechanism of oxidases and catalases to reduce the concentration of O_2 in the cells (Shank et al., 2005). *Azotobacter* species have two types of nitrogenases viz., molybdenum – iron nitrogenase, vanadium– iron nitrogenase (Neeru et al., 2000).

Development of Biofertilizer

To develop any effective biofertilizer we need to aptimize a several factors related to growth of microbe under field conditions. The microbial property such as microbes growth pattern, optimum conditions for microbes growth, interactive behaviour with other microbes need to be screened before selecting a microbe for developing it as biofertilizer, . Important characteristics like pH, shelf life of the microbe, its rate of multiplication, level of microbial contamination, viable count under field conditions and carrier size (Table-1) are very important aspects that need to be worked out before proceeding with biofertilizer development. For high effectiveness of biofertilizers the carrier material also plays a major role. The carrier material is selected should be easy to handle, highly diffusible in soil, long-term storage. A good carrier material must also be non-toxic to inoculant strain and to plant. It should have good pH buffering capacity, easy to process and could be sterilized by either autoclaving or gamma radiation. Sterilization of carrier material is essential to keep high number of inoculants bacteria on carrier for long storage period and to avoid other bacterial contamination. Gamma irradiation or autoclaving can be used as method for sterilization to avoid other microbial contaminants. If using seed as carrier for microbes, it should have good moisture absorption capacity and good adhesion to seeds property (Khosro and Yousef ., 2012).

Table-1: Specification of biofertilizers *Azotobacter* (Yadav and Chandra., 2014)

S.No	Specification	Recommended range
1	Base	Carrier based* in form of moist/dry powder or granules, or cyst based (liquid) and seed based
2	Viable cell count	CFU minimum $5 \times 10^7 - 1 \times 10^9$ cell/g of carrier material or $1 \times 10^9 - 1 \times 10^{12}$ cell/ml of liquid.
3	Contamination level	No contamination at 10^{-5} dilution
4	pH	6.5–7.5
5	Particle size in case of carrier based materials	All materials should pass through 0.15–0.212 mm IS sieve
6	Moisture percent by weight maximum in case of carrier based	30–40%
7	Storage temperature	below 30^{o}C
8	Efficiency character	The strain should be capable of fixing at least 10 mg of nitrogen per g of sucrose consumed

*Type of carrier: The carrier materials such as peat, lignite, peat soil and humus, wood charcoal or similar material favoring growth of organism.

Production of plant growth hormone and their Subsequent Effects

Plant growth promoting substances, or plant growth promoting hormones, are natural substances that are produced by microorganisms, which affect certain physiological-biochemical processes in plants and thereby promote their growth.*Azotobacter* sps. which exerted a beneficial effect upon plant growth was observed to produce indol-3-acetic acid (IAA) when tryptophan was added to the medium. Such microbes therefore may be used as biofertilizers for agriculture (Broughton et al., 2003).

Root colonizing bacteria like, *Azospirillum* and *Pseudomonas* species are known to produce plant growth hormones which often leads to increase root and shoot growth (Debojyoti et al., 2014). It has been observed that treating roots of tomato seedlings with *A. vinelandii* and *A. beijerinckii* cultures accelerated the plant growth and increased fruits yield. Studies postulated that these effects may be caused by the activity of the auxin, gibberellins and cytokinin plant growth hormones from the cultures of *A. vinelandii* and *A. beijerinckii* (Azcon and Barea 1975). The treatment of seedlings of tomato and lettuce with *A. paspali* changed the growth and development of the seedlings with significant increase in the weight of leaves and roots. It was observed that *A. paspali* culture supernatant contained indole-3-acetic acid, gibberellins and cytokinins which may be responsible for significant improvement in growth of tomato and lettuce. There was distinct difference in fruit size of young plants which were treated with *A. paspali* indicating the influence of *A. paspali* plant differentiation and development (Barea and Brows.,1974).

Azotobacter has the ability to produce vitamins like thiamine and riboflavin and plant hormones viz., indole acetic acid, gibberellins, siderophores and cytokinins (Fig:1) (Abd El-Fattah et al., 2013). These plant growth promoting substance are exogenously released by *Azotobacter* and thus the growth and productivity of the plant is improved. Strains of *Azotobacter* are known to also improve uptake and utilization of essential nutrients by the associated plants (Pandey and Kumar 1989). Barik and Goswami (2003), reported that seed inoculation with *A. chroococcum* strains significantly influenced the growth and yield of wheat.

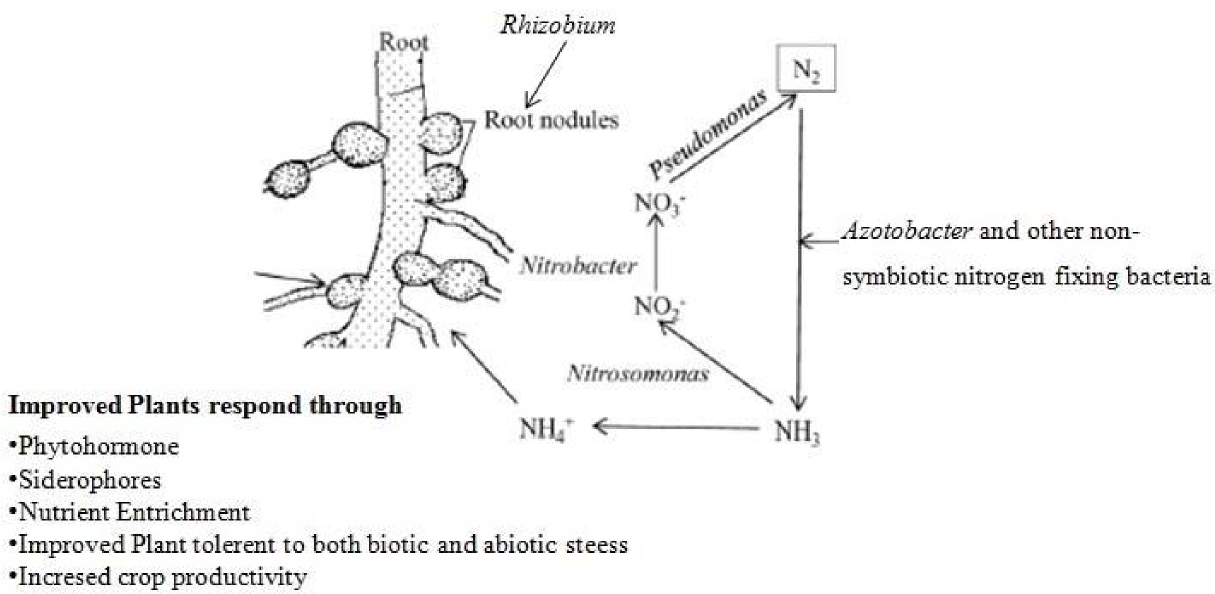

Figure:1. Potential use of *Azotobacter* in sustainable crop production. (Source: Bhardwaj et al., 2014. Modified)

Effect of biofertilizer on the plant growth and yield

The macro and micro plant nutrients play an important role in the productivity of agricultural crops as well as for the environment. Macro nutrients such as nitrogen, phosphate and potassium are required by crops to develop a natural ability to tolerant different biotic and abiotic stress (Tsai et al., 2007). Biofertilizer will influence the soil ecosystem and environment and produce supplementary element for the plants. It also includes organic fertilizers, which are rendered in an available form due to the interaction of micro-organisms and plants through plant microbe interaction (Sujanya and Chandra, 2011).

Mehrotra and Lehri (1971) observed the effect of *Azotobacter* inoculation on growth and yield of vegetable crops. They found that inoculation of seeds or roots with *Azotobacter* cultures seems to influence the fertility of the soil and distinctly increased the yield of the plant. *Azotobacter* inoculation on tomato seeds resultedlated in increase in fruit yield vigorous growth in terms of plant spread, number of leves, as compared to control. An increase in total plant protein content and chlorophyll content were absorbed in tomato plants/seeds inoculated with *Azotobacter* culture as compared to control. This was because of the vigorous growth in terms of plant spread, number of leaves in the plants treated with *Azotobacter*. (Ramakrishnan and Selvakumar G.,2012). Singh (2001) observed the same response of significantly increased tuber yield in *Azotobacter* treated potato grown under north-eastern hill conditions. Combined application of microorganisms along with 150 kg N/ha resulted in highest yield of tuber productions which was significantly higher than the control. In a study on cabbage with various treatments including application of poultry manure and biofertilizers, reported that application of *A. chroococcum* saved 25 per cent nitrogen in cabbage cultivation (Devi et al. 2003). Kumar et al. (2008) studied the response of knolkhol (Brassica oleracea L. var.) to various integrated nutrient management practices and found that the application of *Azotobacter* as a earliness seedling treatment was best for getting higher yield and earliness is a prerequisite for high intensity cropping system.

In form of consortia

Recent studies show promising trends in the field of microbial consortia development (combinations of microorganisms) for enhanced crop productivity and sustainable agriculture. Microbial inoculants have been in use for traditional farming for enhanced crop productivity without realizing their exact role. The most important characteristic common to most of biofertilizers is unpredictability of their efficiency. In order to known the benefits of biofertilizers in agriculture, the consistency of their performance must be improved (Wani and Lee, 2002). Plant studies have shown that the beneficial effects of non-symbiotic microbes on plants can be enhanced by co-inoculation with other microorganisms as compared to single inoculation.

Wheat growth and grain yield was increased in plants wherein seeds were treated with *Azotobacter* sp. and *Azospirillum* sp. as compared to non-inoculated plants (Kandil et al. 2011). Study in pearl millet suggested that when the biofertilizers were inoculated with combined treatment of *Azotobacter* and *Azospirillum, it* resulted in significantly higher growth and grain yields as compared to independent treatment of *Azotobacter* (Tilak, 1995). Similar results were also obtained in case of black pepper (Bopaiah and Khadeer, 1989) and tomato plants (Ramakrishnan and Selvakumar, 2012). In *Moringa oleifera* using combination of different biofertilizers such as *A. chroococcum, A. brazilense, Bacillus megatherium, B. circulans, Pseudomonas fluorescens* and *Saccharomyces cerevisiae* resulted in improvement of growth and nutritional quality (Zayed, 2012).

Significance of biofertilizer in Agriculture

Biofertilizer could be used as a source of nutrient for agricultural crops for improved crop yield and quality and low availability at a production costs (Caalcante et al., 2012). Nitrogen fixing microorganisms plays an important role in increasing yield by making atmospheric nitrogen available to the plant in soluble ammonia from either symbiotic or non-symbiotic microbes. The symbiotic bacteria *Rhizobia* are associated with legumes and nitrogen fixation occurs within the root where the bacterium resides (Saikia and Jain, 2007). The non-symbiotic bacteria as *Azotobacter* and *Azospirillum* are most important N-fixing bacteria in non-leguminous crops. Under appropriate conditions, *Azotobacter* and *Azospirillum* can enhance plant growth development and yield of several agricultural important crops in different agro climatic soils (Okon and Labendera-Gonzalez, 1994). *Azotobacter* along with other biofertilizers has important function in soil as they can fix 15-20 kg nitrogen/ha/crop and give about 10-15per cent increase in yield as compared to singly treated *Azotobacter* (Singh and Singh 2007).

Conclusion

Evolution of modern agriculture has lead to extensive use of agricultural chemicals which have degraded the soil fertility, soil profile and rendered the soil unsuitable for plant growth. It has further ultimately led to deleterious effects on human health, environment, soil health, ground water etc. Bio-fertilizers are being recognized as an essential component for increasing the sustainable agricultural productivity and maintaining long term soil fertility in an eco-friendly manner. At present research focus on identification of microorganism with efficient plant growth promoting activity needs to be targeted. Further, roduction of protocol for enhanced biofertilizer inoculam needs to standardized so as to providing fundamentals to farmers for increasing crop productivity. In this review the role of non-symbiotic microbes as biofertilizer in agriculture has been discussed. Proper use non-symbiotic microbes as biofertilizers showed increase in growth and yield. The application of inorganic chemical fertilizers was thus significantly reduced to 40-50%. It helps through environmental friendly and sustainable agriculture.

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Conflict of Interest

The authors declare that there is no conflict of interest

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