Azobacter: A Plant Growth-Promoting Rhizobacteria Used as Biofertilizer

Santosh Kumar Sethi¹ • Siba Prasad Adhikary²

ABSTRACT

Nitrogen fixation is mainly responsible for improvement of crop yield. In this regard, diazotrophs like Rhizobium, Azotobacter and Azospirillum are important as they enrich nitrogen nutrition in N-deficient soils. Of these, Azotobacter promotes plant growth as well as nitrogen fixation. Thus technology has been developed for making use of Azotobacter biofertilizer for nitrogen and non-nitrogen fixing plants and popularized by educating about their benefits in agriculture to users for practicing integrated nitrogen management.

Keywords: Azotobacter, biofertilizer, growth and yield, nitrogen fixation, organic farming, PGPR

INTRODUCTION

In agriculture, one of the limiting factors is providing plant nutrients, particularly nitrogen (N) and phosphorous (P), to crops. So the improvement of crop yield by inoculation with diazotrophs like Azotobacter, Rhizobium and Azospirillum has been suggested as an eco-friendly technology (Choudhary and Kennedy 2004). These microorganisms colonize the rhizosphere of plants and remain in close association with roots and influence their growth. Of these, Azotobacter is one of the most extensively studied plant growth-promoting microorganisms because its inoculation benefits a wide variety of crops. These are polymorphic, possess peritrichous flagella and produce polysaccharides; they are sensitive to acidic pH, high salts and temperature above 35°C and can grow on a N-free medium thus utilize atmospheric nitrogen (N₂) for cell protein synthesis. Cell proteins are mineralized in soil after death of Azotobacter and contribute to N availability to crop plants. Several types of azotobacteria have been found in the soil and in the rhizosphere: A. chroococcum (Beijerinck 1901), A. nigricans (Krassilnikov 1949), A. puspi (Débereiner 1966), A. armenicus (Thompson and Skenman 1981), A. salinestris (Page and Shvivprasad 1991) and A. vinelandii (Lipman 1940). A. chroococcum is the species most commonly found in Indian soils. Plant growth promotion by Azotobacter may also be attributed to other mechanisms such as ammonia excretion (Narula et al. 1981). Plant growth promotion by Azotobacter also contributes to other mechanisms such as ammonia excretion (Narula et al. 1981). Besides N₂ fixation, they also produce siderophores and antifungal substances (Suneja et al. 1994) and plant growth regulators (PGRs) such as hormones and vitamins (Shende et al. 1977; Verma et al. 2001). PGRs such as auxin and cytokinin produced by Azotobacter sp. have also been reported (Pilet et al. 1979; Harmann et al. 1983; Horemans et al. 1986; Falik and Okon 1989; Nieto and Frankenberger 1989; Taller and Wong 1989; Barbiri and Galli 1993; Patten and Glick 1996; Verma et al. 2001; Patten and Glick 2002).

AZOTOBACTER AND NITROGEN FIXATION

Azotobacter belongs to the Azotobacteriaceae family. These are Gram-negative, non-symbiotic, aerobic diazotrophs. The young rod-shaped cells vary from 2.0-7.0 to 1.0-2.5 μm and occasionally an adult cell may increase up to 10-12 μm, and be oval, spherical or rod-shaped cells. Azotobacter can grow well on simple N-free nutrient medium containing phosphate, magnesium, calcium, molybdenum, iron and carbon sources. Its catabolic versatility in utilizing several aromatic compounds such as protocatechuic acid, 2,4-D (2,4-dichlorophenoxyacetic acid), 2-chlorophenol, 4-chlorophenol, 2,4,6-trichlorophenol, aniline, lindane, tolune, p-hydroxy benzoate, benzoate and benzene is well documented (Hardisson et al. 1969; Balajee and Mahadevan 1990; Gahlot and Narula 1996; Moreno et al. 1999; Revillas 2000; Thakur 2007). Azotobacter contributes significant amounts of fixed N₂ in, on, or near a plant. The energy requirement for the process of N₂ fixation is met by a very high rate of aerobic metabolism which contributes to high oxygen demand for the maintenance of minimal intracellular oxygen tension, a requirement of the oxygen-sensitive nitrogenase to accomplish N₂ fixation (Robson and Postgate 1980).
Diazotrophic bacteria in the rhizosphere of plants utilize the products of N₂ fixation for their own growth and release little while they are alive (Imam and Badaway 1978; van Berkum and Bohllool 1980; Apte and Shende 1981; Balajee and Mahadevan 1990; Das et al. 1992; Jana and Mishra 1994; Verma et al. 2001; Johnson et al. 2006; Damir et al. 2011; Sayeda et al. 2011). When bacteria die, only a small quantity of fixed N₂ is assimilated by the plant. N₂ fixation by the diazotrophic bacteria in the environment. The N₂ fixation process in root-associated bacteria can fix N₂ gas in the presence of repressive levels of combined N₂ and export a major portion of the Nitrogenase-produced ammonia or organic N₂ by-product from their cells into the rhizosphere and/or roots. Thus, plants which form associations with desired bacteria have an additional source of combined N₂ available for growth. This microbe possesses three genetically distinct nitrogenase varieties with vanadium, molybdenum and ammonium as activators. Nitrogenase-II is expressed only when vanadium is present in the culture medium (Bishop et al. 1988; Bishop and Joerger 1990; Harvey et al. 1974). Ammonia is responsible for the repression of synthesis of all three Nases. Energy requirement for N₂ fixation is obtained from EMP and TCA cycle (Jackson and Dawes 1976). Acetate is utilized via the glyoxylate pathway. Both GS (Glutamine synthase) and GOGAT (Glutamate Oxoglutarate Amino Transferase) accomplish NH₄⁺ assimilation through the GS-GOGAT pathway (Kleiner and Kleinschmidt 1976).

Polysaccharide or gum production is one of the characteristic features of Azotobacter (Moulder and Brontonegoro 1974). Some of the species produce polysaccharides in more quantities forming a capsule around the cell. These EPS (extracellular polysaccharides) have a composition similar to alginic acid and contain rhamnose, mannose and galactose with trace amounts of glucose (Haran et al. 1983; Cote and Krull 1988). The role of EPS has not been clearly established but it has been suggested that they protect against desiccation, mechanical stress, phagocytosis and phage attack, participate in the uptake of metal ions as adhesive agents, ATP sinks or are involved in interactions between plants and bacteria (Fyfe and Govan 1983; Hamad 1998). Pigments are also an important characteristic and are produced by all Azotobacter species. A. chroococcum produces black, brownish-black water-insoluble mela- nin like pigment in old cultures (Zimovyeva 1962; James 1970). A yellow-green, fluorescent pigment is excreted by A. vinelandii and A. paspali. A red-violet or brownish-black pigment is seen in the extracellular product of A. nigricans and A. armeniacus. These organisms produce a cyst which is a living dormant cell with two coats namely exocytory and two layers of exine. Cyst is found to be rich in poly-β-hydroxy butyric acid. With the onset of favorable conditions, the cyst gives rise to vegetative cells. Calcium is essential for cyst formation (Page and Sadoff 1975; Gimmestad et al. 2009).

USE OF AZOTOBACTER AS BIOINOUCULTANTS/ BIOFERTILIZERS

Azotobacter, a Gram-negative, free living and plant growth-promoting rhizobacteria, was first reported by Kloeper and Schrot (1978). Its use as a biofertilizer was first advocated by Gerlach and Voel (1902) with the purpose of supplemen-
ting soil-N with biologically fixed N₂ due to the activity of this microbe. Since then they have been reported to play a multifaceted role in stimulating the growth of plants not only by fixing atmospheric N₂ under free-living conditions but also possess other plant growth-promoting activities like phosphate solubilization, production of PGIs like auxins, gibberellins, cytokinins, vitamins and amino acids (Barea and Brown 1974; Tien et al. 1979; Apte and Shende 1981; Burlingham 1968; Nieto and Frankenberger 1989). Azotobacter produces IAA (indole-3-acetic acid) when tryptophan is added to the medium (Brakel and Hilger 1965) as trypto-
phan is the precursor of IAA and is converted to IAA through a primary Trp-aminotransferase reaction. Inocula-
tion of Azotobacter improved seed germination rate and enhanced the vegetative growth of the inoculated plants (Apte and Shende 1981). Three gibberelin-like substances and five cytokinins were found in A. chroococcum (Brown and Burlingham 1968; Nieto and Frankenberger 1989). Azoto-
bacter also improved plant growth indirectly by suppressing phytopathogens or reducing their deleterious effects (Pande and Kumar 1990) and reduced the incidence of fungal, bacterial and viral diseases of several crops (Mersham 1984; Pandey and Kumar 1990). A. chroococcum reduced nematode infection by up to 48% followed by Pseudomo-
nas (11%) and Azospirillum (4%) (Chahal and Chahal 1988). Competition of iron is one of the well known mechanisms of biocontrol under iron-limiting conditions. The bacterium produces a range of iron chelating compounds or sidero-
phores which have a high affinity to ferric ion. These side-
rophores bind most of the iron (Fe³⁺) available in the rhi-
zosphere and thereby making unavailable to pathogens pre-
 sent in soil. Pathogens may not have the ferri-siderophore receptor for uptake of the iron-siderophore complex. So they do not proliferate immediately due to a lack of iron in the soil (O’Sullivan and O’Gara 1992; Tindale et al. 2000; Duhme et al. 1998; Kraepiel et al. 2009; Yoneyama et al. 2011).

AZOTOBACTER INOCULATION AND CROP YIELD

Improvement in crop production due to Azotobacter inoculation has been reported in a number of crops. Artificial inoculation of wheat (Triticum aestivum) seeds with A. chroococcum increased the dry matter 42% more than the control (Gerlach and Vogel 1902). Similarly, a 10-18% increase in yield of bean, corn and potato (Phaseolus vulgaris, Zea mays, Solanum tuberosum) (Skeloumova 1935), 10-23% in wheat (Triticum aestivum), 13-19% in oat (Avena sativa), 14-27% in clover (Trifolium repens) and 10-20% more than the control due to Azotobacter inoculation has been reported (Krasilnikov 1945; Barea and Brown 1974; Tien et al. 1979; Barbieri et al. 1986; Kermi and Gupta 1986; Das et al. 1992; Abbas and Okon 1993; Yadav et al. 1996; Paul and Verma 1999; Paul et al. 2002; Nagamanda 2010; Damir et al. 2011). Azotobacter inoculation also significantly increased the weight of plant, grain yield and N₂ content of plant in wheat (Triticum aestivum), mustard (Brassica alba, Sinapis hirsutum) crops (Apte and Shende 1981). A foliar spray of Azotobacter significantly increased the grain and straw yield of rice (Oryza sativa) (Kanniyan et al. 1980). The use of Azotobacter inoculation has great potential in oilseeds and also a diverse array of crops in terms of crop yield. An increase in yield of mustard (Brassica oleracea) (Gerlach and Vogel 1902; Schmidt 1960), sunflower (Helianthus annuus) (Badve et al. 1977; Yadav et al. 1986), sugarcane (Saccharum officinarum) (Agrawal et al. 1987),
Table 1 Summary of the work on use of Azotobacter and other growth promoting rhizobacteria as biofertilizer for different crops.

<table>
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<tr>
<th>Organism</th>
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<th>Isolated from</th>
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<td><em>Azotobacter</em> sp.</td>
<td></td>
<td>Vegetable gardens, grasslands and cultivated fields</td>
<td>All cultures were grown at 0°C on Winogradsky nitrogen-free agar medium and washed off the agar surface for preparation of antiserum or antigens for immuno electrophoresis.</td>
<td>All members of the <em>Azotobacteraceae</em> have some taxonomic relationship, since they all share some antigens with the reference strains. It is apparent that members of <em>A. paspali</em> and <em>A. vinelandii</em> are immunologically homogeneous within species, as the immunological distances from the corresponding reference strains ranged from 0 to 0.2 for <em>A. paspali</em> and from 0 to 0.08 for <em>A. vinelandii</em>. <em>A. chroococcum</em> strains may be considerably less homogeneous, as their immunological distances from the reference strain ranged from 0.5 to 0.3.</td>
<td>Tehan et al. 1983</td>
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<td><em>Azotobacter</em> and <em>Azospirillum</em></td>
<td></td>
<td>Obtained from University of Agricultural Science, GKVK, Bangalore and Research Institute, Madurai, Tamil Nadu</td>
<td></td>
<td>Application of <em>Azotobacter</em> and <em>Azospirillum</em> biofertilizer in irrigated mulberry under graded level of nitrogen was studied. Better response to <em>Azotobacter</em> than <em>Azospirillum</em> under low nitrogen with 150 kg N/ha was observed. Leaf nitrogen and crude protein were also significantly higher in <em>Azotobacter</em> 150 kg N/ha/year inoculation.</td>
<td>Das et al. 1992</td>
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<td><em>Azotobacter</em></td>
<td>Ale-3</td>
<td>HAU, Hissar</td>
<td></td>
<td>Field trials were conducted in rabi seasons of 1987-88 and 1988-89 at research farm, HAU, Hissar. Growth and yield were significantly enhanced with the application of <em>Azotobacter</em> with and with out nitrogen. Higher plant height and yield attributes of wheat as compared to other treatments or over control was observed due to <em>Azotobacter</em>.</td>
<td>Hooada and Dahiya 1992</td>
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<td><em>Azotobacter</em> and <em>PSB</em></td>
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<td>Obtained from Microbiology Division, IARI</td>
<td>Jansen and Pikovaskyas tricalcium phosphate broth</td>
<td>Effect of cotton seed (var. ‘SRT-1’) inoculation with <em>A. chroococcum</em> and <em>Pseudomonas</em> in combination at graded doses of nitrogen and phosphorous on the uptake of N and P. Plant height, dry matter weight and yield were studied. A combination of fertilizer and <em>Azotobacter</em> inoculation saved half on the N and P fertilizers.</td>
<td>Pottukhe et al. 1992</td>
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<td><em>A. chroococcum</em></td>
<td>BI2</td>
<td>Soil of West Bengal</td>
<td>N-free Burk’s medium containing 1% (w/v) glucose as the carbon source</td>
<td><em>A. chroococcum</em> BI2 showed higher nitrogen fixation at pH 7.0 with a low concentration of potassium nitrate (25 mgN/L) and ammonium sulfate (100 mgN/ml) at 28°C. This strain showed tolerance to NaCl (0.12%). A pesticide (Rogor) inhibited growth as well as acetylene reduction at very low concentrations.</td>
<td>Jana and Mishra 1994</td>
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<td><em>A. chroococcum</em></td>
<td>MKU 201; B-8005; BKMB-1030; A-41</td>
<td>Tropical soil</td>
<td>Jansen agar medium</td>
<td>The temperature optima for high survival and efficiency in nitrogen fixation varied among the strains of <em>A. chroococcum</em>. It ranged between 20-35°C. Performance of temperate strains 8005 and BKMB-1030 was better at low temperature (20°C) and that of tropical strains B and MKU 109 was appreciable even at high temperature (40°C). Strain A-41 exhibited tolerance over a wide range of temperatures; however, extreme temperatures reduced its growth and efficiency of nitrogen fixation.</td>
<td>Rajkumar and Lakshmanan 1995</td>
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<td><em>A. chroococcum</em></td>
<td>RH-30; WH-147; MAC-27; E-12</td>
<td>Department of Plant Breeding CCS HAU, Hisar</td>
<td>Jensen medium containing trace element, sodium glutamate as ‘N’ source and EDA-HCl (EDA, 0.05-1%) incubated at 30°C for 48-72 h</td>
<td>Ethylene diamine (EDA)-resistant mutants (MAC-27) fixed nitrogen in the presence of high concentration of NH₄⁺ and also excreted NH₃⁺ E-12, which exhibited low glutamine synthetase (GS) activity, reduced NH₃⁺ uptake in the mutant due to GS-induced deficiency in ammonia assimilation. Yield and dry matter in mustard and grain yield of wheat were greater with E-12 inoculation than with parent MAC-27 under greenhouse conditions.</td>
<td>Narula et al. 1999</td>
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<td><em>Azotobacter</em> and <em>Rhizobium</em></td>
<td><em>Azotobacter</em> strains: W-5; CBD-15 and C-11; <em>Rhizobium</em> strains: BG-256 (chickpea)</td>
<td>Collected from Division of Microbiology, IARI, New Delhi</td>
<td></td>
<td>The effect of inoculation of chickpea seeds with three strains of <em>A. chroococcum</em> in combination with <em>Rhizobium</em> in a non-sterile soil was studied. Inoculation with either <em>A. chroococcum</em> or <em>Rhizobium</em> alone increased nodule number, weight and yield of chickpea.</td>
<td>Paul and Verma 1999</td>
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<td><em>A. chroococcum</em> and <em>Trichoderma viride</em></td>
<td>W-5; ITCC 1433; 1662; 2185; 3235; 3255</td>
<td>Obtained from Division of Microbiology, IARI</td>
<td></td>
<td>Strains of <em>T. viride</em> were used for solid state fermentation (SSF) of sorghum straw after adjusting the C: N ratio to 35:1 to study the effect of the fermented residues alone and in combination with <em>A. chroococcum</em> W5 as biofertilizer for wheat. Inoculation with W5 alone increased the biomass and yield by 25% over that in control. Fermented residue of <em>T. viride</em> ITCC 1433 applied in combination with <em>A. chroococcum</em> decreased the yield.</td>
<td>Nain et al. 2000</td>
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<td><em>A. chroococum</em></td>
<td>Mala-11 and HT54</td>
<td>Collected from Department of Microbiology, CCS, HAU, Hisar</td>
<td>Jensen’s N-free medium at 30°C for 18 days</td>
<td>Plant growth regulators (PGRs) like gibberellin, kinetin and indole-3-acetic acid were produced by <em>Azotobacter</em>. Out of 20 <em>Azotobacter</em> isolates, 4 produced all three PGRs, 14 produced GA₃ and 10 produced kinetin. All the isolates except for Mala-11 and HT-54 produced one of the three PGRs.</td>
<td>Verma et al. 2001</td>
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<td><em>Azotobacter</em> sp.</td>
<td>Soil</td>
<td>Microbiology Laboratory, GB. Pant, University of Agriculture and Technology, Pant Nagar</td>
<td>Jensen’s broth 28 ± 2°C for 7 days</td>
<td><em>Azotobacter</em> culture differed greatly in intrinsic resistance to streptomycin, tetracycline, trimethoprin, nalidixic acid and rifampicin. 14 cultures inhibited growth of <em>Fusarium oxysporum</em>. In modified JAM-PDA medium none of the <em>Azotobacter</em> strains inhibited the growth of <em>Microphoma phaseolina</em> and <em>Sclerotium rolfsii</em>. No relationships could be observed between the fungal inhibition and the antibiotic resistance of the diazotrophs.</td>
<td>Agrawal and Singh 2002</td>
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<td><em>B. japonicum</em> and <em>A. chroococum</em></td>
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<td>The effect of inoculation of <em>Bradyrhizobium japonicum</em> and <em>A. chroococum</em> on soybean [<em>Glycine max</em> (L) Merril var. Ransom] was studied. Dual inoculation most enhanced plant growth parameters. Inoculation with <em>Azotobacter</em> alone was better than uninoculated control. 16 isolates of <em>A. chroococum</em> were studied for azide resistance. Azide-sensitive mutants were developed which was widely prevalent among the isolates. Azide resistance showed no significant correlation with rate of respiration, ATP concentration, activity of cytochrome-o-oxidase and nitrogen fixation.</td>
<td>Vasudeva et al. 2003</td>
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<td><em>A. chroococum</em></td>
<td></td>
<td>Collected from Department of Microbiology, CCS, HAU, Hisar</td>
<td>Grown in Jensen N-free medium for 72 h at 30°C</td>
<td>Sixteen isolates of <em>A. chroococum</em> were studied for azide resistance. Azide-sensitive mutants were developed which was widely prevalent among the isolates. Azide resistance showed no significant correlation with rate of respiration, ATP concentration, activity of cytochrome-o-oxidase and nitrogen fixation.</td>
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<td><em>Azotobacter</em> and <em>Azospirillum</em></td>
<td>BG-13 and BG-33</td>
<td>Rice and wheat fields, vegetable gardens, grasslands</td>
<td>Jensen’s ‘N’ free medium with sucrose (0.25%) and with 2500 ppm 2,4-D</td>
<td>Four <em>A. chroococum</em> strains from soils enriched with 2,4-D were studied for metabolism of the compound. All 4 strains degraded 2,4-D to chlorocatechol even at 2500 ppm in the presence of sucrose as the C source and with out any additional C source in soil. Chlorocatechol formation was observed even at stationary phase of cells indicating co-metabolism of 2,4-D. Nitrogenase activity in these strains remained unaffected up to 50 ppm of 2,4-D. Accumulation of chlorocatechol with less cell density indicates that some strains may not have metabolized the intermediary product.</td>
<td>Gahlot and Narula 2004</td>
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<td><em>Azotobacter</em></td>
<td></td>
<td>Rice and wheat fields, vegetable gardens, grasslands</td>
<td>Bacteria isolated by serial dilution and plating technique in nitrogen-free medium and general purpose medium (glucose-yeast extract agar)</td>
<td>Total <em>Azotobacter</em> population decreased with increasing soil moisture content. Maximum <em>Azotobacter</em> population was recorded in March. When the soil moisture content was &lt;15% during May-June declined sharply. Grassland field had highest <em>Azotobacter</em> population compared to other fields. Similar trend was also observed in rhizospheric soils collected from vegetable garden and grasslands which were not waterlogged.</td>
<td>Sharma and Bhattacharjee 2004</td>
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<td><em>Azotobacter vinelandii</em></td>
<td>ATCC 9046</td>
<td>Rhizospheric soil of <em>Trigonella</em> plant</td>
<td>Strain grown in modified Burk’s medium and incubated during 24 h at room temperature.</td>
<td>Experimental results showed that sucrose catabolic regulon was developed in <em>A. vinelandii</em> by using genomic fusions. <em>IscS</em>, <em>IscU</em>, <em>HscBA</em>, and <em>Fdx</em> genes are essential in <em>A. vinelandii</em> for the functional analysis, whose products are involved in the maturation of [Fe-S] proteins; their depletion causes deficiency in the maturation of aconitase, an enzyme that requires a 4Fe-cluster for its catalytic activity. Depletion of <em>IscS</em> results in a null growth phenotype only when cells are cultured under conditions of elevated oxygen.</td>
<td>Johnson et al. 2006</td>
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<td><em>Azotobacter</em></td>
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<td>Bacteria isolated by serial dilution till 10⁷ and 0.1 ml plated on Ashby’s agar medium and incubated at room temperature for 4-7 days.</td>
<td>The root length, shoot length, fresh weight, protein, carbohydrate and chlorophyll content of <em>Trigonella</em> plantlets was maximum after 15 days of growth in vitro; 100% seed germination was observed when seeds were treated with <em>Azotobacter</em> cell at 2.3 × 10⁵ cells ml⁻¹.</td>
<td>Nagananda et al. 2010</td>
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<td>Azotobacter vinelandii</td>
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<td>N-deficient combined carbon sources medium at 32°C in a rotary shaker for 5 days</td>
<td>In the two dissolved oxygen conditions evaluated, strictly controlled by gas blending at 0.5 and 5% DOT (dissolved oxygen tension), an increase in the agitation rate (from 300 to 700 rpm) caused a significant increase in the OTRmax (oxygen transfer rate) from 17 to 100 mmol L^-1 h^-1 for 5% DOT and from 6 to 70 mmol L^-1 h^-1 for 0.5% DOT. This increase in the OTRmax improved alginate production, as well as the specific alginate production rate (SAPR), reaching a maximal alginate concentration of 3.1 g L^-1. Using ecofriendly biofertilizers instead of chemical ones in fish aquacultures the impact of inoculation of two strains of Azospirillum brasilense and Azotobacter chroococcum was studied. Chemical characteristics of water, specific growth rate, aspartate amino transferase, alanine amino transferase and histopathological changes were analysed in Oroschromis niloticus aquaculture. Dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, NPP, NO3N and O-PO4 levels were significantly increased by treatment with Azotobacter while Azospirillum gave lower levels. In other hand single or mixed bacterial treatment increased fish specific growth rate especially treated with Azotobacter (34.62% increase in growth). Study induces single inoculation of Azotobacter bacteria biofertilizer as a suitable probiotics in aquacultures.</td>
<td>Lozano et al. 2011</td>
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<td>Azotobacter chroococcum</td>
<td>AZt</td>
<td>DSM 2286 Isolated from Hordeum vulgare</td>
<td>Optimization the production of bacterial biomass cultivation of A. chroococcum was done by using different media and cultivation techniques (batch, fed batch and repeated batch). Chemically defined and complex media with 20 g/l of sugar were selected as the most appropriate media for batch cultivation in stirred tank bioreactor. Higher fed batch and repeated batch techniques increased the bioprocess efficiency parameters (yield coefficient and productivity). Repeated batch technique appeared to be the most suitable for the bacterial biomass production at industrial scale.</td>
<td>Sayeda et al. 2011</td>
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fruit trees (Kerni and Gupta 1986; Pandey et al. 1986), pearl millet (Wani et al. 1988), sorghum (Jadav et al. 1991), jute (Poi and Kabi 1979), cotton (Gossypium hirsutum) (Apé and Shende 1981; Paul et al. 2002) has been reported. Several vegetable crop like tomato (Lycopersicon esculentum), brinjal (Solanum melongena), cabbage (Brassica chinensis), onion (Allium cepa), potato (Solanum tuberosum), radish (Raphanus sativum acanthiformis), chillies (Capsicum annuum) and sweet potato (Ipomoea batatas) responded positively to azotobacterisation (Joi and Shinde 1976; Imam and Badaway 1978; Khuller et al. 1978; Sethi and Adhikary 2009). Synergistic effect of co-inoculation of Azotobacter with Rhizobium in pea (Pisum sativum) (Paul and Verma 1999), chickpea (Cicer arietinum) (Verma et al. 2000) and groundnut (Arachis hypogeae) (Rashid et al. 1999) was also observed. Detailed summary of the work carried out on use of Azotobacter and other PGPRs as biofertilizers for different crops is provided in Table 1.

MASS PRODUCTION PROTOCOLS OF AZOTOBACTER BIOFERTILIZER AND FIELD APPLICATION METHODS

Efficient strains of Azotobacter can be obtained from established culture collection centres for mass production. Alternatively, region-specific and environmental stress-compatible strains of the bacterium can be isolated and used. For isolation, soil samples adhering to uprooted plants should be collected and cultured through serial dilution and plating techniques using Azotobacter isolation media containing (g/l): Sucrose - 20.0, K2HPO4 - 1.0, MgSO4·7H2O - 0.5, Na2MoO4 - 0.001, FeSO4·7H2O - 0.01 and CaCO3 - 2.0, pH - 7.0-7.2 (Yadav and Mowade 2005). The cultures need to be incubated at 28 ± 2°C for 4-5 days to produce bacterial colonies which are white, translucent and circular. After testing the purity followed by this standard protocol for testing purity of bacterial culture by incubating nutrient broth and bromothymol blue, individual strains need to be cultivated in liquid broth (Azotobacter isolation media). Basing on higher growth rate and tolerance to different environmental variables strains can be selected for use as biofertilizer. The objective of the paper is not to give details how efficient strains responding to various environmental variables were obtained for use as biofertilizer, hence not given. For this purpose, loops of the respective colonies inoculated in sterile N2-free medium are grown for 5-7 days. This starter culture is inoculated into a 500-ml flask with a bacterial suspension of 10^7 CFU/ml and grown in rotary shaker at about 120 rpm for 5 days at 30°C. For field experiments, 20-days-old healthy seedlings are used; the roots are dipped in bacterial culture suspension for 20-30 min for proper attachment of microbes and then planted.

CONCLUSION

Azotobacter is a broad spectrum biofertilizer and can be used as inoculant for most agricultural crops. Earlier, its utility as a biofertilizer was not a priority due to its relatively low population in the plant rhizosphere. However, seedling treatment with Azotobacter of several crops brought about an increase in yield. Besides, because of its well known N2 nutritional function, it is now recognized to play a multiple role in helping crop plants to improve their growth potential, yield and maintenance of soil health for sustainable agriculture. Hence there is renewed interest in this rhizobacterium. However, quantitative understanding of the ecological factors that control the performance of biological N2 fixation systems of the bacterium in crop fields is essential for promotion and successful adoption of the biofertilizer production technology.
ACKNOWLEDGEMENTS

The authors thank the authorities of Utkal University, Bhubaneswar, Odisha and Visva-Bharati, Santiniketan, West Bengal for providing laboratory facilities, to U.G.C. Govt. of India for providing a Research Fellowship to one of us (SKS) and to DST, Govt. of India for financial assistance. The authors also thank Dr. Jaime A. Teixeira da Silva for significant improvements to language and style.

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