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Azotobacter Isolation and Screening Methods–A Review

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Abstract: Sustainable organic farming is one of the best methods to be adapted for the good agricultural practice. To maintain the ecological balance and to protect the diverse microbial growth we have to avoid the use of chemical fertilizers and pesticides. So as to protect ourselves and to overcome the adverse effects of these pesticides, we are in search of the useful soil microorganisms as biofertilizers. When inoculated in the farmyards they can increase the plant growth by supplying the nutrients useful for the crops and also protect them from the harmful pathogens. In this attempt plant growth regulating microbes are to be isolated and used. *Azotobacter* is a free living nitrogen fixing useful bacteria which can be used for the sustainable farming practices. In the present investigation a review is presented how different scientists have used methods in isolating these bacteria from soil and screen them using different morphological and biochemical tests.
Keywords: *Azotobacter*, agricultural practice, biofertilizers.

Introduction

Agriculture plays a crucial role in the food production. Population is growing rapidly, but land used for agricultural purposes is decreasing due to rapid urbanization. So enhance the production in limited available resources, farmers are looking for synthetic fertilizers and pesticides. Chemical fertilizers and pesticides usage in turn is increasing the soil and water pollution. Mathur [1] long back has reported that 76% of pesticide is used in India. These chemicals pose risk and unwanted side effects to the life on earth and environment [2, 3]. To overcome these adverse effects Organic farming is a sustainable and reliable option. Biofertilizers in this scenario are the best, for enriching soil fertility, and decreasing the biomagnification of toxic chemicals in the soil. Soil bacteria are playing a significant role in this sustainable agricultural and environmental development [4]. Microbes are omnipresent and those which are in rhizosphere can alter soil physicochemical properties and can increase the crop productivity. Nitrogen fixing bacteria (symbiotic or non-symbiotic), mychorrhiza and phosphorus solubilizing bacteria play a promising role in this regard.

Azotobacter is one of such soil bacteria which was found to increase the soil fertility by nitrogen fixing and can supply vitamins and plant growth hormones [5]. It is a free living nitrogen fixing bacteria and is distributed in diverse environmental conditions [6]. They are also having antifungal character with which they can protect crop plants [7]. In the present paper a review on the isolation and characterization methods of *Azotobacter* is presented.

Aquilanti et al. [8] first collected soil samples at a depth of 10-15 cm below the surface into sterile vials using Kole et al. method [9]. They have collected the samples from rhizosphere of corn, wheat and lawn grasses and majorly from uncultivated soils. Distribution of *Azotobacter* is greatly influenced by pH, these are mostly observed at pH above 6.5. They are rarely present in pH below 6 [10]. They have isolated the bacteria using 3 methods, 1) serial dilution of soil samples and plating on Brown N-free medium [11]. 2) placing soil in Winogradsky enrichment solution for 7-14 days

followed by streaking on Brown agar [12, 13]. 3) a combination of soil paste and the direct sowing of single soil grains [14, 15]. All isolates were purified by streaking on TSA plates (Tryptic soy agar plates). Screening of gram positive and negative was done using KOH-test [16]. For molecular analysis total DNA extraction was done and subjected to amplification of 16S rRNA using PCR. Identification was done using Amplified Ribosomal DNA Restriction Analysis (ARDRA). *Azotobacter* on selective Brown medium were slimy, smooth, glistening, weakly convex and whitish with 2-10mm in colonies diameter. They used two restriction enzymes Rsa I and Hha I. They have screened 196 Gram negative strains from 35 soil samples.

Upadhyay et al. [17] isolated 42 strains. They have collected soil samples during October to December from rhizosphere of different cereal crops. For culturing and identification they used Nitrogen free Jensen's medium. Gram staining test, catalase activity, carbon source utilization and cyst behavior were observed. Morphological and biochemical characteristics were compared with those in Bergey's manual [18].

Shaikh and Shakir [19] collected soil samples from various fields and orchards from a depth of 10-15 cm. They cultured using Enrichment in selective medium (Derx medium), isolated using Nitrogen free medium and observed morphology and growth of colonies for 3-5 days. Performed relevant tests like motility, Gram staining, Cyst forming ability and biochemical test like oxidase, catalase, amylase, nitrate reductase, urease, proteolytic activity and H_2S production. They also determined sensitivity to antimicrobial agents.

Conflicts of interest: There is no conflict of interest of any kind.

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