Study on Distribution of Microbial and Diazotrophic *Azotobacter* Population in Five Different Rhizosphere Soil

Sornalatha.T[1], Mahalingam.P.U[2], Jansi.V[3]

Department of Biology, The Gandhigram Rural Institute - Deemed University, Gandhigram, Dindigul District, Tamil Nadu
*Corresponding Author E-mail: *sornalatha21@gmail.com

Abstract— *The current scenario encounters the steady demand of food supply and the application of fertilizer is become an indispensable in modern agriculture. Hence, the present study is focused on distribution of microbial and diazotrophic Azotobacter population in rhizosphere soil sample. Total microbial population viz, bacteria, fungi, actiomycetes and diazotrophic Azotobacter were enumerated from five different rhizosphere soil samples and compared the distribution of diazotrophic Azotobacter with other three microbial groups. The results of enumeration of total microbial population reveals that comparatively the bacterial population found to be higher followed by Azotobacter population in various rhizosphere soil. Based on the predominant growth, five Azotobacter strains (one isolate from each sample) were isolated and authenticated through morphological and biochemical characteristics.*

Keywords— Diazotrophs, Microbial population, Nitrogen fixation, Azotobacter.

I. INTRODUCTION

Soil is the blend of minerals, natural matter, gasses, fluids, and the incalculable living beings that together bolster life on Earth. Soil microorganisms advance physicochemical changes in the dirt through the adjustment of soil natural matter, nitrogen obsession and different modifications in soil properties essential for plant growth[1].

Nitrogen is extremely plenteous in nature and frequently restrains plant[2]. Microorganisms are essential segments of soil and straightforwardly or in a roundabout way impact the dirt's wellbeing through their useful or deterimental exercises. Rhizosphere microorganisms intercede soil forms through deterioration, supplement assembly and mineralization, nitrogen obsession and denitrification. Most by far of life forms have adjusted to process N by different ecological sources[3].

So as to accomplish the consistent interest of nourishment supply, use of compost is key in present day farming. Consistent utilization of compound manure prompts genuine ecological concerns[4]. Accordingly, a requirement for finding an option technique for manageable horticultural practices. Biofertilizer consider to be one such significant option for horticulture practices[5].

Biofertilizers are comprehensively named nitrogen fixers (N-fixers), potassium solubilizer (K-solubilizers) and phosphorus solubilizers (P-solubilizers)[6]. The real biofertilizers being used were Rhizobium, *Azotobacter*, Azospirillum and phosphate solubilizing bacteria[7]. *Azotobacter* is has a place with the family *Azotobacteriaceae* and regularly found in the dirt. It is extremely compelling for the change of soil ripeness and harvest efficiency. It can, fix nitrogen specifically from the environment that helps the plants for better grain production[8].*Azotobacter* application turn into a less expensive, low capital serious other than being eco-accommodating.

In perspective of every one of these focuses, present study is centered around conveyance of microbial and diazotrophic *Azotobacter* populace in five diverse rhizosphere soil tests.

II. MATERIALS AND METHODS

2.1 Collection of Sample:

Soil samples were collected from the rhizosphere zone in the cultivated land at the depth of 0-15cm around the five selected crops species via, Ground nut, Cotton, Onion, Brinjal and Banana. The collected soil samples were kept in the polythene bags and brought to the laboratory for further analysis.

2.2 Study on Distribution of Total Microbial and Diazotrophic *Azotobacter* Population in Rhizosphere Soil:

One gram of each rhizosphere soil samples was taken in a 250 ml sterile conical flask containing 100 ml of distilled water and shaken in a vortex mixture for 30 minutes. From this stock, various dilution were prepared from 10^{-2} to 10^{-6} with sterile

distilled water and the diluted samples were used for the analysis of total colony forming unit(CFU) of bacteria, fungi, actinomycetes and *Azotobacter* population using standard procedures[9].

The following procedure was adopted for enumeration of total microbial CFU using pore plate technique

Organism Studied	Culture medium used	Sample dilution tested	Sample Volume (ml)	Growth temperature (^{0}C)	Incubation day(s)
Bacteria	Nutrient Agar	10^{-6} &10^{-7}	1	37	1
Fungi	Martins Rose Bengal Agar	10^{-2} & 10^{-3}	1	28	5
Actinomycetes	Kanknight's Agar	10^{-2} & 10^{-3}	1	37	7
Azotobacter	Wak's mann medium	10^{-4} &10^{-5}	1	28	5

The Total CFU was calculated using standard formula:

$$\text{CFU} = \frac{\text{Number of colonies}}{\text{Vol of sample} \times \text{diluton factor}}$$

2.3 Identification of diazotrophic *Azotobacter*

The five *Azotobacter* isolates(one isolates from each soil sample) were selected based on their abundances growth in the N2 free medium and were identified through morphological and biochemical characteristics according to Bergey's Manual of Systematic bacteriology [10].

The five *Azotobacter* bacterial isolates were identified based on colony morphology, grams staining, motility and various biochemical properties like indole production, methyl red reaction, voges-proskaure reaction , citrate utilization, catalase reaction, oxidase reaction, urease production, gelatine hydrolysis and nitrate reduction. The results of cultural and biochemical characteristics of five *Azotobacter* isolates were compared with Bergey's Manual of Determinative Systematic Bacteriology for genus conformation.

III. RESULT AND DISCUSSION

As of late, a large portion of the area misfortunes its richness because of the consistent aggregation of substance manures. So as to accomplish Sustainable farming practices, the biofertilizer application presented over the globe. Subsequently, the present study was centered around circulation of microbial and *Azotobacter* populaces in rhizosphere soil tests and the ID of the potential diazotrophic *Azotobacter*.

The outcome exhibited in Table-1, plainly obvious that the wealth nearness of bacterial populace took after by *Azotobacter* (a gram negative microbes) in the second most noteworthy populace in the rhizosphere soil tests. Our outcomes are in concurrence with a prior report of [11], where a higher diazotrophic populace was seen in rhizosphere soils.

TABLE 1
DISTRIBUTION MICROBIAL AND DIAZOTROPHIC *AZOTOBACTER* POPULATION IN RHIZOSPHERE SOIL.

S.No	Source of Rhizosphere Soil Samples	Bacteria population ($\times10^7$ CFU g^{-1}soil)	Fungal population ($\times10^4$ CFU g^{-1}soil)	Actinomycetes population ($\times10^3$ CFU g^{-1}soil)	*Azotobacter* population ($\times10^4$CFU g^{-1}soil)
1	Brinjal	23.4	16.9	15.08	54.55
2	Ground nut	7.72	5.27	2.54	32.04
3	Cotton	2.31	8.68	27.4	45.81
4	Onion	6.36	18.6	15.6	29.22
5	Banana	10.8	9.31	54.54	22.72

In this study, five *Azotobacter* strains (one life form from each rhizosphere soil test) were secluded and distinguished through morphological and biochemical qualities as appeared in Table 2. By contrasting these outcomes and the Bergey's Manual of Systematic bacteriology five diazotrophic separates were affirmed as bacterial family *Azotobacter*. When all is said in done, Gram negative microbes more prevalent than the gram positive microscopic organisms in the rhizosphere soil[12]. Specialists have disengaged a strain of endophytic, nitrogen altering Burkholderia sp. connected with maize [1].

TABLE 2
CULTURAL AND BIOCHEMICAL CHARACTERISTICS OF FIVE *AZOTOBACTER* ISOLATES

S.No	Characteristics test	Strain-1	Strain-2	Strain-3	Strain-4	Strain-5
1	Gram staining	Negative	Negative	Negative	Negative	Negative
2	Shape	Paired Cocco bacilli	Rod	Rod	Rod	Rod
3	Indole production	Negative	Negative	Negative	Negative	Negative
4	Methyl red production	Negative	Negative	Negative	Negative	Negative
5	Vogus Proskauer reaction	Negative	Negative	Negative	Positive	Positive
6	Urease	Negative	Negative	Negative	Negative	Negative
7	Nitrate reduction test	Positive	Positive	Positive	Positive	Positive
8	Catalase test	Positive	Positive	Positive	Positive	Positive
9	Oxidase test	Positive	Positive	Positive	Positive	Positive
10	Citrate utilization	Positive	Positive	Positive	Positive	Positive
11	Triple sugar ion	Alkaline butt	Alkaline butt	Alkaline butt	Alkaline butt	Alkaline
12	Pigment production	Brown to black	-	-	-	-

IV. CONCLUSION

In this study, five diazotrophic isolates were isolated from rhizosphere soil samples (one from each soil sample) Based on morphological and biochemical characteristics, and all five selected diazotrophic bacterial strains were authenticate as *Azotobacter* sp. In conclusion, the selected *Azotobacter* strains have the potential of biological nitrogen fixation and thus it could be used as biofertilizer for sustainable agriculture practice.

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