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Characterization of *Azotobacter* spp. from Gangapur, Dist. Aurangabad (MS)

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Abstract

Biological Nitrogen Fixation (BNF) is a critical microbial process that recycles environmental nitrogen and maintains nitrogen homeostasis within the biosphere. *Azotobacter*, a genus of free-living, non-symbiotic, heterotrophic diazotrophs, plays a vital role in augmenting soil nitrogen levels. In this study, 25 rhizosphere soil samples were randomly collected from diverse agricultural locations across the Gangapur taluka. Physiochemical analysis revealed soil pH values ranging between 7.2 and 7.8, alongside Water Holding Capacities (WHC) between 30.45% and 39.81%. Morphological and biochemical assays confirmed the presence of *Azotobacter* spp. in 18 of the 25 samples. We successfully isolated and identified three distinct species: *A. chroococcum*, *A. indicus*, and *A. paspali*. Biochemical characterization indicated that *A. paspali* lacked starch hydrolysis and nitrate reduction capabilities, whereas *A. indicus* tested negative for urea hydrolysis. Conversely, *A. chroococcum* exhibited positive results across all administered biochemical tests.

Keywords: *Azotobacter*, Rhizosphere, Water Holding Capacity (WHC), Gangapur, Diazotrophs

Introduction

The Gangapur region, situated along the Godavari River, benefits from significant irrigation infrastructure that supports extensive crop diversity. To sustain this agricultural productivity, Biological Nitrogen Fixation (BNF) provides a constant, natural input of nitrogen crucial for plant growth and development. While chemical fertilizers are frequently utilized, long-term soil productivity relies heavily on biological constituents to maintain natural fertility.

BNF is enzymatically mediated by nitrogenase—a biocatalyst naturally synthesized by free-living bacteria such as *Azotobacter*, *Acetobacter*, *Azoarcus*, and *Klebsiella*. While much of the historical literature focuses on symbiotic bacteria (e.g., *Rhizobium*), free-living, non-symbiotic diazotrophs have gained attention for their ability to associate with non-leguminous crops and promote growth. *Azotobacter* is a prominent, non-symbiotic heterotroph capable of fixing an average of 20 kg N/ha annually. Although it requires oxygen for metabolism, it demonstrates aeroadaptive capabilities and can thrive under low O_2 conditions. Furthermore, *Azotobacter* exhibits superior nitrogenase activity compared to other nitrogen fixers, especially when soils are amended with soluble organic substrates like glucose and sucrose.

This study aims to isolate, identify, and biochemically characterize the diversity of *Azotobacter* species present in the crop rhizospheres of the Gangapur taluka.

Materials and Methods

1. Soil Sampling and Physiochemical Analysis

Rhizosphere soil samples (n=25) were collected from varied agricultural sites across Gangapur taluka and stored in sterile zip-lock bags. Samples were maintained at 25°C under laboratory conditions for subsequent analysis. Soil pH was determined potentiometrically, and Water Holding Capacity (WHC) was calculated for each sample (Pawar *et al.*, 2014) [15].

2. Serial Dilution and Isolation

A 1g aliquot of each rhizosphere sample was suspended in 9 mL of sterile distilled water. Serial dilutions were performed across five test tubes to achieve the desired microbial concentration. The final dilutions were inoculated onto nutrient broth using the streak plate method and incubated for 48 hours at 25 ± 2°C (Rahmi *et al.*, 2015) [17].

3. Bacterial Culturing and Morphological Characterization

Bacterial colonies were sub-cultured for purification on a selective medium: *Azotobacter* Agar with Glucose (HiMedia- M371). Pure cultures were maintained on slants of the same medium. Morphological characterization included Gram staining, cell biometry, and colony morphology (color, shape, size, appearance, and margin).

4. Biochemical Characterization

Isolates were subjected to a suite of biochemical assays to identify specific metabolic pathways Hossain *et al.,* (2019) [7]. Tests included:

- Catalase, Citrate utilization, Starch Hydrolysis, and Indole Production.
- Urease (Urea Hydrolysis) and Nitrate Reduction Phalke *et al.,* (2016).

Results and Discussion

1. Soil Properties and Crop Distribution

A total of 25 rhizosphere samples were collected from various host crops. The predominant host was *Gossypium herbaceum* (Cotton, n=7), followed by *Zea mays* (Maize, n=6). Soil pH remained slightly alkaline across all sites, ranging from 7.2 to 7.8, with six samples exceeding a pH of 7.6. Water holding capacity varied from 30.45% to 39.81%

The chart illustrates the variety of host plants included in the study, with Cotton (*Gossypium herbaceum*) and Maize (*Zea mays*) being the most frequently sampled crops, representing 28% and 24% of the total samples respectively.

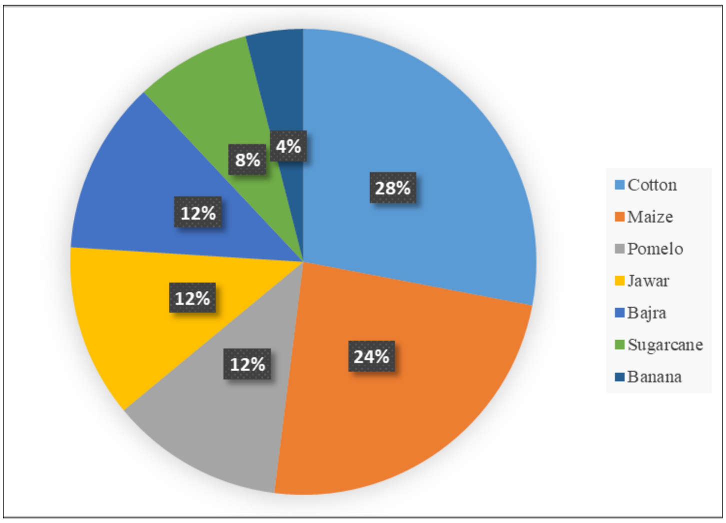

Fig 1: Crop Distribution of Samples (Gangapur Taluka)

Table 1: Physico-chemical Soil Properties and Distribution of Azotobacter Species

Sr. No	Sample Code	Soil pH	Water Holding Capacity (WHC)	Azotobacter Species Isolated
1.	GA01	7.7	30.45 %	*A. chroococcum*
2.	GA02	7.2	34.57 %	*A. indicus*
3.	GA03	7.6	31.25 %	*A. chroococcum*
4.	GA04	7.4	32.46 %	*A. indicus*
5.	GA05	7.6	33.58 %	*A. paspali*
6.	GA06	7.5	35.87 %	Not detected*
7.	GA07	7.8	33.56 %	*A. chroococcum*
8.	GA08	7.5	34.98 %	*A. indicus*
9.	GA09	7.5	35.64 %	Not detected*
10.	GA10	7.3	36.74 %	*A. paspali*
11.	GA11	7.2	36.57 %	*A. indicus*
12.	GA12	7.2	38.21 %	*A. indicus*
13.	GA13	7.4	38.47 %	Not detected*
14.	GA14	7.4	38.82 %	*A. indicus*
15.	GA15	7.3	34.87 %	Not detected*
16.	GA16	7.8	34.56 %	*A. chroococcum*
17.	GA17	7.4	39.81 %	*A. paspali*
18.	GA18	7.6	31.69 %	*A. chroococcum*
19.	GA19	7.3	33.97 %	Not detected*
20.	GA20	7.4	38.59 %	*A. paspali*
21.	GA21	7.4	35.88 %	Not detected*
22.	GA22	7.7	32.57 %	*A. chroococcum*
23.	GA23	7.4	34.66 %	Not detected*
24.	GA24	7.4	36.59 %	*A. indicus*
25.	GA25	7.5	35.92 %	*A. indicus*

2. Morphological Identification

Of the 25 samples, 7 isolates did not exhibit characteristic *Azotobacter* morphology and were excluded from further investigation. The remaining 18 isolates were identified as Gram-negative, rod-shaped bacteria belonging to the *Azotobacter* genus.

Based on colony and cellular biometry, three distinct species were categorized:

- ***A. paspali***: Exhibited yellow, circular, opaque colonies. Recorded the largest cellular structure, averaging 7.1 µm to 7.5 µm in length.
- ***A. chroococcum***: Displayed creamy white, translucent colonies with entire margins. Average cell length ranged from 4.0 µm to 4.4 µm.
- ***A. indicus***: Formed white, opaque colonies. Exhibited the smallest cellular profile, averaging 3.2 µm to 3.5 µm in length.

Table 2: Morphological Characteristics of Isolated Azotobacter Species

Morphological Characteristic	*A. chroococcum*	*A. indicus*	*A. paspali*
Colony Color	Creamy White	White	Yellow
Colony Shape	Circular	Circular	Circular
Colony Appearance	Translucent	Opaque	Opaque
Colony Margin	Entire	Entire	Entire
Gram Staining	Gram-Negative (-)	Gram-Negative (-)	Gram-Negative (-)
Cellular Shape	Rods / Coccoid	Rod-shaped	Rod-shaped
Mean Cell Length (µm)	4.0 – 4.4 µm	3.2 – 3.5 µm	7.1 – 7.5 µm

Morphological examination of the 18 isolates confirmed their placement within the genus Azotobacter. As detailed in Table 2, all isolates were Gram-negative and predominantly rod-shaped, though *A. chroococcum* exhibited a tendency toward a coccoid morphology in older cultures. Significant inter-species variation was observed in pigmentation and cell size; notably, *A. paspali* produced distinctive yellow colonies and possessed significantly larger cell lengths (up to 7.5 µm) compared to the smaller *A. indicus* (3.2–3.5 µm).

3. Biochemical Profiling

All confirmed *Azotobacter* isolates tested positive for Catalase, Citrate, and Indole production. However, key metabolic differentiators were observed among the species:

Biochemical Assay	**Target Enzyme/Metabolism**	***A. chroococcum***	***A. indicus***	***A. paspali***
Catalase	Hydrogen Peroxide Breakdown	(+)	(+)	(+)
Citrate	Carbon Source Utilization	(+)	(+)	(+)
Indole	Tryptophan Metabolism	(+)	(+)	(+)
Starch Hydrolysis	Amylase Activity	(+)	(+)	(-)
Nitrate Reduction	Anaerobic Respiration	(+)	(+)	(-)
Urease	Urea Degradation	(+)	(-)	(+)

Fig 2: Comparative Biochemical Profile of Isolated Azotobacter Species

The biochemical phenotyping (Figure 2) revealed distinct metabolic divergence among the isolates. While primary characteristics such as Catalase and Citrate utilization were conserved across the genus, specific enzymatic signatures provided clear taxonomic separation. Notably, the absence of amylase activity (Starch Hydrolysis) and nitrate reductase in *A. paspali* serves as a key diagnostic marker compared to the more metabolically diverse *A. chroococcum*.

Conclusion

The Gangapur taluka region possesses a robust population of indigenous, free-living, nitrogen-fixing soil bacteria. Through comprehensive morphological and biochemical characterization, this study successfully isolated 18 distinct *Azotobacter* strains, categorizing them into *A. indicus*, *A. chroococcum*, and *A. paspali*. The presence of these diazotrophs across various non-leguminous crop rhizospheres highlights their potential utility as natural biofertilizers, promoting sustainable agricultural practices and reducing reliance on synthetic chemical inputs in the region

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