Int.J.Curr.Microbiol.App.Sci (2026) *15*(1): 103-111

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 1 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1501.012

Isolation, Purification and Characterization of *Azotobacter* isolates isolated from Rhizosphere soil of African Marigold (*Tagetes erecta* L.) collected from different locations of Kolhapur District, India

Pramila Gaikwad*, V. M. Karade, M. D. Raut, and S. J. Waghamare

Rajarshee Chhatrapati Shahu Maharaj College of Agriculture, Kolhapur
Division of Plant Pathology and Agricultural Microbiology
Mahatma Phule Krishi Vidyapeeth, Rahuri. 413-722, India

**Corresponding author*

Keywords

Azotobacter, African marigold *Tagetes erecta* L. Kolhapur district

Article Info

Received:
15 November 2025
Accepted:
22 December 2025
Available Online:
10 January 2026

A B S T R A C T

The laboratory studies were conducted at Division of Plant Pathology and Agricultural Microbiology, Rajarshee Chhatrapati Shahu Maharaj College of Agriculture, Kolhapur for the isolation, purification and characterization of isolates obtained from rhizosphere soil of Marigold fields completely randomized design (CRD) was used for laboratory studies. Ten isolates were obtained from fifteen soil samples collected from rhizosphere soil of marigold fields from different locations of Kolhapur district. They were designated as $ATSS_1$, $ATSN_2$, $ATSA_3$, $ATHM_1$, $ATHH_2$, $ATHA_3$, $ATHN_4$, $ATKK_1$, and $ATKB_2$ and $ATKM_3$. All the isolates were studied for the morphological, cultural and biochemical characterization. In the morphological studies all the isolates found Gram negative and stained pink in color, positive in motility and also showed positive results to KOH test. All of these isolates showed the white creamy to white color, out of 10 isolates 8 circular were (cocci) shaped with irregular wavy margin and flat elevation and 2 were rod shaped. All the isolates were tested for biochemical tests namely starch hydrolysis, gelatin hydrolysis, gas production, H_2S production, oxidase, methyl red test and catalase test. All of them showed the positive results to all the test except $ATHH_2$ which gave negative results to gelatin hydrolase and starch hydrolase. These isolates were cultured on five different medias for their cultural characterization which has resulted that the isolates showed superior growth on Ashby's media and isolates $ATHM_1$ and $ATKM_3$ showed superior growth with colony diameter of 2.50cm and 2.27cm respectively. Nitrogen fixation in N free broth isolate $ATHM_1$ significantly showed highest nitrogen fixation (18.68mg/ml) followed by $ATKM_3$(15.60mg/ml) and selected as efficient isolates. Out of them two isolates ($ATHM_1$ and $ATKM_3$) were found superior over other isolates which were collected from Male village, Hatkanagle tehsil and Mudshingi village, Karvir tehsil of Kolhapur district respectively.

Introduction

Azotobacter is genus of Gram negative motile, oval, or spherical bacteria that are thick-walled cyst. They are aerobic, free living soil microbes that play an important role in nitrogen fixation. Cells of *Azotobacter* genus are relatively large (2 - 4 µm in diameter). They are usually oval, may take various forms from rods to spheres. In fresh cultures, cells are mobile due to the numerous flagella. They produce large quantities of capsular slime. Development and germination of the *Azotobacter* sp. appear as cyst. While, growing *Azotobacter* produce flat, slimy paste like colonies with a diameter of 5 – 10 mm, which may form films in liquid nutrient media. The growth is favoured at a temperature of 20-30 ºC. It produces pigments. For example, *Azotobacter chroococcum* forms a dark-brown water soluble pigment melanin and other species produce pigments from yellow-green to purple colours. *Azotobacter* is free-living nitrogen fixing bacteria which fix 20- 25 kg nitrogen per hectare. It can fix at least 10 µg of nitrogen per gram of glucose consumed. The optimal pH required for nitrogen fixation is 7.0 – 7.5 but growth is sustained in the range from 4.8 to 8.5. Being non symbiotic nitrogen fixing bacteria they are highly diverse and globally widespread in soils. This bacterial group may represent the dominant natural source of N in ecosystem lacking SNF (Choudhary and Kennedy, 2004; Das and Saha, 2007). It also produces hormone like IAA & GA_3, Vitamin like biotin (Vit B_7) & folic acid and with judicious use of organic matter ensures good growth and increase productivity, (Kader, 2002 and Jadhav *et al.,*2014). Other important feature of *Azotobacter* sp. is the association with root.

Materials and Methods

Isolation and maintenance of Azotobacter isolates

Collection of soil samples

Soil samples were gathered from random locations in Kolhapur District from Marigold growing sites, where the samples taken from the marigold rhizosphere soil. Total fifteen soil samples were collected from fifteen different locations (five villages from each Tehsil) of Shirol, Karvir and Hatkanangle of Kolhapur District. Collected soil samples were kept in sterile polythene bags, labeled appropriately, brought to laboratory, and stored at 4ºC for further experiment. Collection of soil sample represented in table – 3.1.

Table.1 Details of soil samples collected from different locations of Kolhapur District

Sample No.	Sample code	Tehsil	Village
1	$ATSS_1$	Shirol	Shirol
2	$ATSN_2$	Shirol	Narsihwadi
3	$ATSA_3$	Shirol	Alas
4	$ATSG_4$	Shirol	Ganeshwadi
5	$ATSS_5$	Shirol	Shedshal
6	$ATHM_1$	Hatkanangle	Male
7	$ATHH_2$	Hatkanangle	Halondi
8	$ATHA_3$	Hatkanangle	Atigare
9	$ATHN_4$	Hatkanangle	Nimshirgaon
10	$ATHH_5$	Hatkanangle	Herle
11	$ATKK_1$	Karvir	Koparde
12	$ATKB_2$	Karvir	Bhamate
13	$ATKM_3$	Karvir	Mudshingi
14	$ATKK_4$	Karvir	Kuditre
15	$ATKK_5$	Karvir	Koge

Preparation of culture media

Ashby's Mannitol Agar medium was prepared for isolation and maintenance of *Azotobacter* isolates. Culture medium with following composition were used and sterilized at 15lbs pressure for 15 min at 121ºC temperature. After that the molten media used for culturing the bacterium.

Isolation, purification, and maintenance of *Azotobacter* isolates

Isolation of *Azotobacter* from collected soil samples from rhizosphere soil of African marigold was done by serial dilution method by preparing 10^{-4} to 10^{-6} dilution of each collected sample. Out of that 1ml of diluted solution was pipette out and transfer on Petri plates after that Ashby's agar media then poured in aseptic condition in laminar air flow cabinet and incubated at 28°C ± 2°C for 3days in incubator. After that culture plates were examined daily and each colony that appeared was considered one colony forming unit (cfu). After enumeration of colony count individual colony was re-cultured on fresh Ashby's agar plates for another 3 days and the individual colonies

were transferred on Ashby's agar slant (test tube) for getting pure culture. That pure culture was maintained on Ashby's agar slant and stored at 4℃ for further use and study.

Characterization and identification of *Azotobacter*

Morphological characterization of *Azotobacter* isolates

Simple staining

Fresh culture of 48 hrs old bacterial culture was used for simple staining. In thin film 48 hrs old bacterial culture transferred on glass slide and smeared by adding water drop on slide. Then it was allowed to air dry and heat fixed by passing over flame gently. Crystal violet used for simple staining. It was applied on the smear for 1-2 minutes and washed with water gently and allowed it to well dry. Then slides were examined under microscope for determination of shape and arrangement of cells (Cappuccino and Sharman, 1987).

Gram staining

Fresh culture of bacterium was smeared on glass slide and heat fixed. Then smear was stained with crystal violet (0.5%) for 1 min followed by rinsing with tap water and drained off excess water. Iodine solution used as mordant flooded on smear and allowed for 1 minute. Excess amount of mordant was drained out and smear was decolorized by using 95% alcohol for 30 seconds followed by counter staining with Safranin for 30 seconds followed by washing and air drying. The Gram-negative bacteria stained with color of counter stain (safranin) pink to red whereas Gram positive bacteria retained the color of crystal violet i.e., blue. (Cappuccino and Sharman, 1987).

KOH (Potassium Hydroxide) test

A loop full culture of bacteria was taken from a week-old colony on clean glass slide and mixed with a drop of 3% aqueous KOH solution and stirred for 5-10 seconds in quick circular motion with the help of toothpick. Observed for formation of stands of viscid material for confirmation of Gram reaction. Thread like slime formation when picked the toothpick indicated the presence of Gram-negative bacterium (Suslow *et al.,* Schaad, 1980; Ryu, 1940).

Motility test

Test for Motility in screw-top test tubes, prepare an agar semisolid medium. Pick a well-isolated colony with a sterile needle and stab the medium to within 1 cm of the tube's bottom to test for motility. When inserting and removing the needle from the medium, make sure it stays in the same line. Incubate for 18-48 hours at 27°C, or until growth is visible. A diffuse cloud of growth away from the inoculation line indicates a positive motility test (Tittsler and Sandholzer, 1936).

Cultural characterization of *Azotobacter* isolates

Cultural characteristics of *Azotobacter* were studied by growing it on five different solid media by following the standard procedures (Manual of microbiological methods, 1957). Media were sterilized by autoclaving at 15 lbs psi for 20 minutes at 121ºC.The medium was then poured aseptically in three sterile petri dishes and allowed to solidify followed by inoculation of isolates on that media aseptically. These plates were kept for incubation for seven days at room temperature (27ºC ±2ºC). The isolates were observed for their color, size, shape, surface, elevation, and texture of colony was recorded at seven days. Recorded data of colony size of *Azotobacter* isolates on different media was analyzed statistically with factorial completely randomized design (CRD) using three replications of each treatment to found out the significance of such treatments.

Biochemical characterization of isolates

For each biochemical test, inoculation of loop full culture of five days old culture streaked on Ashby's agar medium. The inoculated plates were kept for incubation at 28ºC ±2ºC for 3-5 days. For each test, three replicates and controls were used. Ultimately, the reaction of each *Azotobacter* isolate was determined as (+) positive or (-) negative.

Catalase test

A drop of suspension of *Azotobacter* culture was taken on clean glass slide. Few drops of 3% hydrogen peroxide (H_2O_2) were added on the culture. The evolution of air bubbles from the suspension is indicates the positive results (Dickey and Kelman, 1988).

Methyl red (MR) test

Some bacteria can use glucose and convert it to stable acids such as lactic acid, formic acid, and acetic acid as end products. As a result of the acids are produced, the pH drops to 4.5 or lower, as evidenced by a change in the color of methyl red from yellow to red. MR broth was prepared and sterilized it. The medium was inoculated with 18-24 hrs pure culture and incubated aerobically at 37ºC for 24 hrs followed by 24 hrs of incubation aliquot 1ml of broth to clean test tube. Re-incubated the remaining broth for additional 24 hrs. Immediately examine the red color when adding 2-3 drops of methyl red indicator to the aliquot

Starch hydrolysis test

The starch hydrolyzing ability of bacterium was studied by growing of bacterium on Starch agar plates nutrient agar containing 1% soluble starch. These agar plates were inoculated with culture of isolates and these plates were incubated in inverted position at room temperature 28ºC ± 2ºC for 7 days. After 7 days plates were flooded with Lugol's iodine solution observed for appearance of clear zone of hydrolysis around the bacterial growth indicates starch has been hydrolyzed (Cowan, 1974; Lelliot and Stead 1987).

Gelatin hydrolysis test

The tube containing gelatin medium (yeast extract 3g, peptone 5g, gelatin 120g) allowed to solid to stand in water for 15 min for dissolved by heating. Dispense into test tube of the depth of 5 cm (sterilized by autoclaving at 15 lbs for 20 min at 121ºC) were stab inoculated with bacterial isolates and incubated for 7-14 days record the liquefaction of the medium. On the final day, tubes were cooled at 5ºC for 30 minutes before the reading the result. If the test culture solidifies remove the culture and indicates in a titled position at room temperature or at 30ºC in a incubator for 30 minutes, if form slant indicates test is positive and no form slant only stab indicates negative test (Lelliot and Stead, 1987).

Oxidase test

Oxidase test was conducted to determine the oxidase activity of isolates. Trypticase Soy agar medium was prepared, sterilized, and poured into sterile petri plates and allowed to solidify followed by the inoculation of isolates on medium plates and incubated at 28 ±ºC for 2 days. After successful incubation period 2-3 drops of oxidase reagent were added on surface of colony and observed for colour change. The colour formation indicates the positive test.

Estimation of nitrogen fixing ability of *Azotobacter* isolates

The nitrogen fixing ability of *Azotobacter* isolates were estimated by Micro – Kjeldhal method in terms of the amount of total nitrogen fixed in 25 ml broth culture in 7 days under controlled condition. For that purpose, nitrogen free broth of Jensen's broth was used. After pouring 25 ml broth in 150 ml conical flask and plugged with cotton plugs then flask was autoclaved. After cooling, each flak was inoculated by 0.1 ml broth culture of each strain and the flask were kept on rotary shaker for continuous shaking of culture for 7 days at 28°C 2°C. From each flask 10 ml culture was taken for estimation of total nitrogen by Micro- Kjeldhal method as described Jackson (1967).

$$N_2(mg/g) = \frac{\text{ml of } H_2SO_4 \times \text{N of } H_2SO_4 \times 14.0}{\text{Weight of the sample}}$$

Results and Discussion

Morphological characterization

All the isolates were studied for their morphological characters. Out of 10 isolates 8 where cocci in shape 2 isolates were rod shaped, ,6 arranged in chains, 4 scattered chain and all the isolates Gram negative, motile and KOH test positive.

Biochemical characterization

Different biochemical tests *viz.,* methyl red test, catalase test, starch hydrolase, gelatinase, gas production, H_2S production and oxidase test were also used to characterise isolates. Biochemical characterization of *Azotobacter* isolates shown in Table-2. All the isolates passed all the test, except for isolate 5($ATHH_2$), which failed the starch hydrolase and gelatine tests.

Table.2 Morphological and biochemical characters of *Azotobacter* isolates of marigold field collected from different location of Kolhapur.

Sr. No	Isolate code	Morphological characters of *Azotobacter* isolates						Biochemical characters of *Azotobacter* isolates						
		Gram reaction	Stain colour	Cell shape	Cell Arrangement	Motility Test	KOH Test	Methyl red test	Catalase Test	Starch hydrolase test	Gelatine hydrolase test	Gas Prod.	H_2S Prod.	Oxidase
1	$ATSS_1$	-ve	Pink	Round	Scattered single	+ ve	+ ve	+	+	+	+	+	+	+
2	$ATSN_2$	-ve	Pink	Round	Arranged in chain	+ ve	+ ve	+	+	+	+	+	+	+
3	$ATSA_3$	-ve	Pink	Round	Arranged in chain	+ ve	+ ve	+	+	+	+	+	+	+
4	$ATHM_1$	-ve	Pink	Round	Arranged in chain	+ ve	+ ve	+	+	+	+	+	+	+
5	$ATHH_2$	-ve	Pink	Round	Scattered single	+ ve	+ ve	+	+	-	-	+	+	-
6	$ATHA_3$	-ve	Pink	Round	Arranged in chain	+ ve	+ ve	+	+	+	+	+	+	+
7	$ATHN_4$	-ve	Pink	Rod	Scattered single	+ ve	+ ve	+	+	+	+	+	+	+
8	$ATKK_1$	-ve	Pink	Rod	Arranged in chain	+ ve	+ ve	+	+	+	+	+	+	+
9	$ATKB_2$	-ve	Pink	Round	Scattered single	+ ve	+ ve	+	+	+	+	+	+	+
10	$ATKM_3$	-ve	Pink	Round	Arranged in chain	+ ve	+ ve	+	+	+	+	+	+	+

Legends: Prodn. - Production, (+) Positive result, (-) negative result, (D) partially positive result

Table.3 Colony size (cm) on different solid culture media and nitrogen fixing ability of *Azotobacter* isolates in N free broth.

Sr. No.	Isolate	Colony size (cm) of *Azotobacter* isolates on different solid culture medium					Mean colony size in cm	N fixation in N free broth (mg / ml)
		Ashby's media	Jensen's agar media	Burk's media	NA media	PDA Media		
1	$ATSS_1$	1.12	1.12	1.31	0.43	0.89	**0.97**	11.21
2	$ATSN_2$	1.00	1.02	1.23	0.47	0.97	**0.94**	10.27
3	$ATSA_3$	1.12	1.14	1.28	0.52	0.79	**0.97**	11.86
4	$ATHM_1$	2.50	2.12	1.56	0.64	2.08	**1.78****	18.68**
5	$ATHH_2$	0.80	0.85	1.09	0.52	0.71	**0.79**	11.68
6	$ATHA_3$	1.13	1.13	1.10	0.46	0.77	**0.92**	10.74
7	$ATHN_4$	0.94	0.98	1.51	0.51	0.64	**0.92**	10.28
8	$ATKK_1$	0.88	0.91	1.18	0.52	0.56	**0.81**	11.68
9	$ATKB_2$	0.80	0.92	1.04	0.55	0.67	**0.80**	12.05
10	$ATKM_3$	2.27	1.83	1.53	0.56	1.55	**1.55****	15.60**
SEm ±		**0.04**	**0.03**	**0.03**	**0.01**	**0.03**	**0.18**	**0.33**
CD at 1 %		**0.10**	**0.10**	**0.10**	**0.04**	**0.08**	**0.51**	**0.99**

Table.4 Cultural characterization of *Azotobacter* isolates on different solid media

Media	Sr. No	Isolates	Colony characters of *Azotobacter* isolates				
			Colour	Shape	Texture	Margin	Elevation
1. Nutrient Agar Media	**1**	$ATSS_1$	Whitish creamy	Irregular	Mucoid	Wavy	Flat
	2	$ATSN_2$	Yellowish creamy	Irregular	Mucoid	Wavy	Umbonate
	3	$ATSA_3$	Dull white	Irregular	Slime	Wavy	Flat
	4	$ATHM_1$	White	Irregular	Slime	Smooth	Umbonate
	5	$ATHH_2$	Whitish creamy	Round	Slime	Smooth	Flat
	6	$ATHA_3$	Whitish creamy	Irregular	Slime	Wavy	Umbonate
	7	$ATHN_4$	Whitish creamy	Irregular	Mucoid	Irregular	Umbonate
	8	$ATKK_1$	White	Round	Slime	Smooth	Flat
	9	$ATKB_2$	Yellowish creamy	Irregular	Mucoid	Wavy	Flat
	10	$ATKM_3$	White	Round	Mucoid	Wavy	Umbonate
2. Potato dextrose	**1**	$ATSS_1$	White creamy	Irregular	Slime	Wavy	Flat
	2	$ATSN_2$	Yellowish	Circular	Mucoid	Smooth	Flat
	3	$ATSA_3$	Yellowish creamy	Irregular	Slime	Wavy	Flat
	4	$ATHM_1$	White	Irregular	Slime	Smooth	Flat
	5	$ATHH_2$	White creamy	Irregular	Mucoid	Wavy	Flat
	6	$ATHA_3$	White creamy	Irregular	Slime	Smooth	Flat

agar media	**7**	$ATHN_4$	Yellowish creamy	Circular	Slime	Smooth	Convex
	8	$ATKK_1$	Dull white	Irregular	Slime	Wavy	Flat
	9	$ATKB_2$	Creamy yellow	Irregular	Dry	Wavy	Flat
	10	$ATKM_3$	Yellowish creamy	Round	Mucoid	Wavy	Crateriform
3. Ashby's Media	**1**	$ATSS_1$	Creamy	Irregular wrinkled	Slime	Wavy	Flat
	2	$ATSN_2$	White creamy	Irregular	Mucoid	Wavy	Hilly
	3	$ATSA_3$	Opaque white	Round	Slime	Smooth	Drop like
	4	$ATHM_1$	White	Circular	Slime	Smooth	Umbonate
	5	$ATHH_2$	Transparent White	Circular	Slime	Smooth	Umbonate
	6	$ATHA_3$	White	Circular	Slime	Smooth	Drop like
	7	$ATHN_4$	White	Irregular	Slime	Wavy	Umbonate
	8	$ATKK_1$	White	Circular	Slime	Smooth	Convex
	9	$ATKB_2$	White	Irregular	Slime	Wavy	Flat
	10	$ATKM_3$	White creamy	Circular	Mucoid	Wavy	Convex
4. Burk's media	**1**	$ATSS_1$	Creamy white	Complex	Mucoid	Wavy	Crateriform
	2	$ATSN_2$	Creamy white	Irregular	Mucoid	Wavy	Umbonate
	3	$ATSA_3$	Opaque white	Round	Slime	Smooth	Drop like
	4	$ATHM_1$	White	Circular	Slime	Wavy	Convex
	5	$ATHH_2$	White	Round	Slime	Scalloped	Umbonate
	6	$ATHA_3$	Creamy White	Irregular	Slime	Wavy	Drop like
	7	$ATHN_4$	Creamy White	Irregular	Slime	Wavy	Umbonate
	8	$ATKK_1$	White	Irregular	Mucoid	Wavy	Flat
	9	$ATKB_2$	White creamy	Wrinkled	Slime	Wavy	Flat
	10	$ATKM_3$	Creamy yellow	Round	Mucoid	Wrinkled	Crateriform
5. Jensen's media	**1**	$ATSS_1$	Creamy Yellow	Irregular	Mucoid	Scalloped	Hilly
	2	$ATSN_2$	Creamy white	Round	Slime	Wavy	Umbonate
	3	$ATSA_3$	White	Round	Slime	Smooth	Umbonate
	4	$ATHM_1$	Transparent white	Irregular	Mucoid	Wavy	Umbonate
	5	$ATHH_2$	Creamy yellow	Irregular	Mucoid	Wavy	Umbonate
	6	$ATHA_3$	Creamy white	Irregular	Slime	Wavy	Hilly
	7	$ATHN_4$	Creamy White	Round	Slime	Smooth	Crateriform
	8	$ATKK_1$	Creamy	Round	Mucoid	Wavy	Flat
	9	$ATKB_2$	Yellowish creamy	Irregular	Mucoid	Wavy	Crateriform
	10	$ATKM_3$	Creamy white	Irregular	Slime	Wavy	Umbonate

Cultural characterization

All the isolates were studied for cultural characters by growing on five different solid medias *viz.,* Ashby's media, Jensen's media, Burk's media, Potato dextrose agar media, and Nutrient agar media for colony characters such as colony colour, shape, margin, texture, elevation, and diameter. The result revealed that most of the *Azotobacter* isolates showed whitish creamy colour of colony, circular to irregular in shape, smooth Mucoid or slimy texture with Umbonate to flat elevation and highest colony diameter and best growth on Ashby's media followed by Jensen's media and Burk's media. The isolate $ATHM_1$ and $ATKM_3$ showed best growth on cultural Medias and colony diameter 2.50 cm and 2.27 cm on Ashby's media respectively followed by 2.12 cm and 1.83 cm on Jensen's media respectively. The obtained results mentioned.

Estimation of nitrogen fixing ability of *Azotobacter* isolates

Efficient isolates were selected based on nitrogen fixing ability in nitrogen free broth by using the Micro - Kjeldhal method. Isolate $ATHM_1$ significantly showed the highest nitrogen fixation (18.68mg/ml) followed by $ATKM_3$ (15.60mg/ml) and selected as an efficient isolate. The selected efficient isolates were further named as Strain-A and Strain-B respectively. Following Table-3 showed the results of nitrogen fixing ability.

In conclusion, ten isolates were obtained from fifteen soil samples collected from rhizosphere soil of marigold fields from different locations of Kolhapur district. They were designated as $ATSS_1$, $ATSN_2$, $ATSA_3$, $ATHM_1$, $ATHH_2$, $ATHA_3$, $ATHN_4$, $ATKK_1$, and $ATKB_2$ and $ATKM_3$. All the isolates were studied for the morphological, cultural, and biochemical characterization. In the morphological studies all the isolates found Gram negative and stained pink in color, positive in motility and showed positive results to KOH test. All these isolates showed the white creamy to white color, circular (cocci) shape with irregular wavy margin and flat elevation. All the isolates were tested for biochemical tests namely starch hydrolysis, gelatin hydrolysis, gas production, H_2S production, oxidase, methyl red test, and catalase test. All of them showed the positive results to all the test except $ATHH_2$ gave negative results to gelatin hydrolase and starch hydrolase.

These isolates were cultured on five different medias for their cultural characterization which resulted that the isolates showed superior growth on Ashby's media and isolates $ATHM_1$ and $ATKM_3$ showed superior growth with colony diameter 2.50cm and 2.27cm respectively. Nitrogen fixation in N free broth isolate $ATHM_1$ significantly showed highest nitrogen fixation (18.68mg/ml) followed by $ATKM_3$ (15.60 mg /ml) and selected as efficient isolates. Out of them two isolates ($ATHM_1$ and $ATKM_3$) were found superior over other isolates.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

P. H. Gaikwad: Investigation, analysis, V. M. Karade: writing original draft, M. D. Raut: Methodology, investigation, S. J. Waghamare: Conceptualization, methodology, writing.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Akhter, M. S.; Hossain, S. J.; Hossain, A., and Datta, R. K. 2012. Isolation and characterization of salinity tolerant *Azotobacter* species *Gree. J. Biolo. Sci.,*2 (3): 43 – 51.

Ambesh, B., Roy A., Ngomle, S. Bhattacharya P., Meena, V. 2017. Isolation and evaluation of *Azotobacter* spp. from different crop rhizosphere. *Int.Jr. Curr. Microbiol. App. Sci.,*6 (4): 883 –888.

Andhare, A., Poudel, A., Deshmukh, A., and Dargad, J. 2019. Isolation of *Azotobacter* and study of its effect as a liquid formulation on seed germination and growth parameters of Green gram (*Vigna radiata* L.). *The Phar. Inno. Jr.*8 (4): 336- 341.

Anonymous, 1957. 'Manual of microbiological methods'.

Aqualanti, L., Favilli F., and Clamenti, F. 2004.) Gave the comparison of different strategies for isolation and preliminary identification of *Azotobacter* from soil samples. *Soil Biol and Biochem.*36(9):1475-1483.

Cowan, S. T., 1974. Cowan & Steel's manual for the identification of medical bacteria, 2nd ed^{n}. Cambridge University Press, Cambridge.

Islam, M.Z. Sharif, D. I. and Hossain, M. A. 2008. A comparative study of A*zotobacter* species from different soil samples. *J. Soil. Nature*. 2 (3):16-19.

Jimenez, D. J., Montana, J. S. and Martinez, M. M. 2011. Characterization of free nitrogen fixing bacteria of the genus *Azotobacter* in organic vegetable-grown Colombian soil. *Brazili. J.of Microbio.*42:846-858.

Kaviyarasan, G., Shricharan, S. and Kathiravan, R. 2020. Studies on isolation, biochemical characterization and nitrogen fixing ability of *Azotobacter* sp. isolated from agricultural soils. *Int. J. of Sci. Engi. and Appl. Sci.* 6 (11): 2395 – 3470.

Kizilkaya, R. 2009. Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. *J. Environ. Biol.*30 (1): 73 – 82.

Lele, A. B., Pawar, N. B. and Kolse, S. V. 2009. Studies on *Azotobacter* from rhizosphere of gerbera (*Gerbera jamesonii* H.). *J. Mahara. Agric. Univ.*, 34 (3): 298-300.

Lelliott, R. A. and Stead, D. E., 1987. Method for the Diagnosis of Bacterial Diseases of Plants. *British Soc. for Plant Pathol.* 2*:*216.

Martinez-Toledo, M. V.; Gonzalez-Lopez, J., T. de la Rubia and A. Ramos Cormenzana 1985.Isolation and characterization of *Azotobacter chroococcum* from the roots of *Zea mays. FEMS Micro. Eco.* 31*:*197-203.

Patil, V.R., Potdukhe, S.R., Guldekar, D.D. and Ghate, A.M. 2014. Morphological and biochemical characterization of *Azotobacter chroococcum* from soils of different locations of Nagpur district. *J. Soils and Crops* 24 (1) 148-153.

Ryu, E., 1940. A simple method of differentiation between Gram-positive and Gram-negative organisms without staining. *Ixilasato Archives of Hxpcr. Medicine*, 17: 58-63.

Schaad, N.W., 1980. Laboratory guide for identification of plant pathogenic bacteria. *Dept. Plant pathology Univ. of Georgia*, :28.

Suslow, T. V., Schroth M. N. and Iska, M 1982. Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology,* 72: 917-918.

Tejera, N., Lluch, C., Martinez-Toledo, M. V. and Gonzalez-Lopez, J. 2005. Isolation and characterization of *Azotobacter* and *Azospisillum* strains from the sugarcane rhizosphere. *Plant and soil.*270 (1):223-232.

Tittsler R. P. and Sandholzer L. A. (1936). The use of semi-solid agar for the detection of, bacterial motility. *J. Bacteriol.* 31 (6): 575-580.

Upadhyay S., Narendra K., Singh, V. K and Singh A. 2015. Studied the isolation, characterization, and morphology of *Azotobacter* isolates. *J. of Appl. and Nat. Sci.*7 (2): 984– 990.

How to cite this article:

Pramila Gaikwad, V. M. Karade, M. D. Raut, and Waghamare, S. J. 2026. Isolation, Purification and Characterization of *Azotobacter* isolates isolated from Rhizosphere soil of African Marigold (*Tagetes erecta* L.) collected from different locations of Kolhapur District, India. *Int.J.Curr.Microbiol.App.Sci.* 15(1): 103-111.
doi: https://doi.org/10.20546/ijcmas.2026.1501.012