IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 19, Issue 1 Ser. 1 (Jan. – Feb. 2024), PP 39-45
www.Iosrjournals.Org

Characterization Of Azotobacter Chroococcum Isolated From Maize Rhizosphere Of Darjeeling Hills For Presence Of Pgp Traits And Its Survivality Efficiency Under Different Growth Conditions

Deewa Basnett[1#], Projjwal Chandra Lama[2], Binod Chandra Sharma[3], Sitoshna Sinha[4]

[1](Department Of Botany, Balurghat College, Balurghat-733101, Dakshin Dinajpur, West Bengal, India)
[2](Department Of Botany, Darjeeling Government College, Darjeeling-734101)
[3](Department Of Botany, Abn Seal College, Coochbehar-736101)
[4](Department Of Microbiology, Darjeeling Government College, Darjeeling-734101)

Abstract
Composite soils from the rhizosphere region of maize crops of Darjeeling hills was screened for presence of free-living nitrogen fixing bacteria on Ashby's medium. The isolated strains were subjected to various morphological and biochemical tests. Among the various isolates one of the most potent isolates was selected and sent for 16 S rRNA sequencing at NCMR-NCCS, Pune and the report led to its identification as Azotobacter chroococcum. In-vitro production of IAA by the isolate was 31.5 µg/ml supplemented with tryptophan, Phosphate solubilization Index was 2.57. The maximum growth of the isolate was observed at 30˚C at neutral and alkaline pH. The isolate shows other Plant growth promoting activities too and hence can be developed as an indigenous biofertilizer.

Keyword*: Darjeeling hills, Azotobacter, rhizosphere, Plant Growth Promoting traits, biofertilizer*

Date of Submission: 12-01-2024 Date of Acceptance: 22-01-2024

I. Introduction

Plant growth promoting rhizobacteria (PGPR) are group of bacteria that actively colonize the area around plant roots to promote plant growth and yield (1). Numerous species of bacterial genera like *Azotobacter, Pseudomonas, Burkholderia, Arthrobacter, Bacillus, Enterobacter, Alcaligenes, Serratia, Azospirillum* and *Klebsiella* have been shown to promote plant growth (2,3,4,5). Plant growth is enhanced by PGPR through direct or indirect mechanisms. The production of phytohormones, nitrogen fixation and mineral solubilization are examples of direct growth promoting processes. Indirect growth promoting mechanisms take place when PGPR reduces or eliminates the harmful effects of plant pathogens on plants (6).

Azotobacter is a PGPR, as demonstrated by a number of studies, and it functions either directly as a biofertilizer, indirectly as a bioprotectant, or both. *Azotobacter* fixes atmospheric nitrogen non-symbiotically; it is a member of the Azotobacteraceae family. Numerous crops, including rice (*Oryza sativa* L.), maize (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.), bajra (*Pennisetum glaucum* L.), vegetables, and plantation crops, have been reported to harbour this organism in their rhizospheres (7). Lately, *Azotobacter* has gained recognition as a significant fertiliser that increases the availability of nitrogen and replaces chemical fertilisers (8). It also generates secondary metabolites, particularly phytohormones and exopolysaccharides, which are absent in chemical fertilisers.

The genus *Azotobacter*, which is widely distributed in various environments like soil, water and sediments is one of the most significant groups of free-living nitrogen fixing bacteria (9). Free-living aerobic bacteria, *Azotobacter* sp are primarily found in alkaline and neutral soils. These are heterotrophic, nonsymbiotic bacteria that can fix 20 kg of nitrogen per hectare annually on average. Furthermore, it has been demonstrated that it produces compounds that promote growth and are antagonistic to pathogens. Numerous plant species have soil and rhizosphere that contain *Azotobacter* sp. Depending on the physico-chemical and microbiological properties of the soil, the population of these bacteria can range from negligible to 10^4 g^{-1}(10).

Azotobacter chroococcum is the most common species found in the rhizosphere among the other species. Numerous studies have documented the advantages of *Azotobacter chroococcum* for the growth and productivity of a range of crops that are significant to agriculture. It helps plants in many ways as it can produce various growth substances like auxins, cytokinins and gibberellins that helps in root growth and absorb

nutrients. It produces vitamins and ammonia that improves in seed germination. It can inhibit phytopathogenic fungi by producing antifungal substances (11,12).

Darjeeling the northernmost district of West Bengal is situated between 27˚ 13'' N to 28˚ 31" N and 88˚ 53" E to 87˚ 59" E in the Eastern Himalayan region of India (13). The soil of this area is mostly categorized as brown forest soil due to their characteristic reddish-brown color, rich in organic carbon and potassium, but deficient in available phosphorus. The soil reaction varies from highly acidic to neutral in nature. Maize is one of the fastest growing cash crops and is commonly cultivated by the people of hills. In addition to its traditional uses, maize is a vital raw material in the food processing, poultry, dairy, meat, and ethanol industries.

The objective of this study was to isolate free-living nitrogen fixing bacteria from the rhizosphere soils of maize crops of Darjeeling hills and examine their ability to solubilize inorganic phosphate, IAA production and their *in-vitro* survival efficiency under different growth conditions.

II. Material And Methods

Collection of soil samples and bacterial isolation from rhizosphere of maize crop field:

The soil samples required for the study were collected from a depth upto 15 cm from the rhizosphere soils of maize field of Darjeeling hills. The sufficient amount of soil samples was collected in sterile Ziplock bags and tagged. The collected samples were brought to the laboratory for further analysis.

Ten grams (10gm) of soil sample was suspended in 90 ml of sterile double distilled water under aseptic conditions and serial dilutions were prepared until the 10^{-5} dilutions was obtained. Ashby's broth/agar medium [20.0 g Mannitol, 0.2 g potassium phosphate dibasic, 0.2 g magnesium sulfate, 0.2 g sodium chloride, 0.1 g potassium sulfate, 5.0 g calcium carbonate, 15 g agar (for solidification), distilled water 1000 ml, pH adjusted to 7.4±0.2] was used for isolation, enumeration and maintenance of isolates during studies. From 10^{-4} and 10^{-5} dilutions enrichment was done by inoculating 10 ml pre-sterilized Ashby's broth with 0.1 ml of sample dilution. Repeated transfer of 0.1 ml was made after few days of growth to fresh pre-sterilized Ashby's broth.

From 10^{-4} and 10^{-5} dilutions spread plates were made in Ashby's agar medium and incubated at 28ºC for 7 days for the development of visible colonies. The bacterial isolates were preliminary identified on the basis of their morphological and biochemical characteristics according to Bergey's manual of determinative bacteriology (14). The most potent isolate was sent to NCMR- NCCS Pune, for molecular characterization and identification using 16S rRNA method.

Morphological and biochemical characterization of the bacterial isolate:

The morphological characters like motility, cell shape, size, margin, colour, appearance and Gram nature was evaluated for the selected isolate. The strain was inoculated on Ashby agar plates and incubated at 28ºC for 48 hours. The freshly grown colonies were observed under microscope for morphological characterization.

The biochemical properties like carbohydrate fermentation, oxidase test, catalase, H_2S production, Indole test, citrate utilization, NO_2 reduction, urease and starch hydrolysis for the preliminary identification were carried out as per standard methods of Bergey's manual of determinative bacteriology (14).

***In vitro* screening of selected bacterial isolate for their plant growth promoting (PGP) activities:**

Indole acetic acid production

The bacterial isolate was grown in Jensen Medium supplemented with tryptophan (100mg/l) and incubated at 28º C for 7 days. After incubation for 7 days, 2 ml of culture was removed from each tube and centrifuged at 10,000 rpm for 15 mins. 1 ml of supernatant fluid was transferred to fresh tube to which 100 µl of 10 mM orthophosphoric acid and 2 ml of reagent consisting of 1 ml of 0.5 $FeCl_3$ in 50 ml of 35% $HClO_4$ was added. The absorbance of the developed pink colour was read at 530 nm after 25 min. The IAA concentration in the culture was determined by using a calibration curve of pure IAA as a standard, following linear regression analysis (15)

Phosphate solubilizing activity

Solubilization of inorganic phosphate by the isolate was done on Pikovskaya's agar (PKV) by Agar spot method. PKV agar plates were inoculated with the bacterial isolate by using Agar Spot technique and were incubated for 7 days at 28°C. The bacterial isolate produced transparent zone of clearing around the colonies and the diameter of halo zone alongwith colony was measured by using metric scale. Solubilization efficiency and solubilization index was also calculated using the formula. Solubilization Efficiency (SE) = Solubilization diameter/ Colony diameter x 100 and Solubilization Index (SI) = Colony diameter+ Halo diameter/ Colony diameter (16,17,18).

Screening of Nitrogen fixing activity

The nitrogen fixing activities of the bacterial isolate was observed by using glucose nitrogen free mineral (GNFM) agar medium containing bromothymol blue (BTB). The bacterial isolates were inoculated into GNFM prepared plates and incubated for 7 days at 28ºC and the change in the color of colonies was observed (19).

Screening of Siderophore production

Siderophore production by the bacterial isolate was assayed qualitatively by Chrome azurol S (CAS) agar medium. The bacterial isolate was spotted on the prepared CAS agar plate and incubated at 28°C for 3 days. Development of orange coloured halo zone around the colonies indicated the production of siderophore (20).

Production of Hydrogen cyanide

Cyanide production was tested following the method of Bakker and Schippers (21). Bacterial isolate was inoculated in a Luria-Bertani agar plate supplemented with 4.4 g/L glycine. Inoculated plate was overlaid with Whatman paper No.2 that was impregnated with 0.5% picric acid and 2% sodium carbonate. The plates were incubated at 28 °C for 2-4 days. Papers were saturated with the reagent, the change in the colour from the yellow to orange-brown on the filter paper indicated the production of cyanide.

Production of Ammonia

Bacterial isolate was tested for the production of ammonia in peptone water. After inoculating the bacterial isolate in peptone water, it was incubated at 28°C for 48 hours, and after that 0.5 ml of Nessler reagent was added to the culture. The development of yellow to dark orange colour indicated the production of ammonia (22).

Molecular Identification of bacterial strain MDH-01

The selected bacterial isolate was sent to sequencing facility of National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune for molecular identification using 16SrRNA method. The genomic DNA was isolated by the standard phenol/chloroform extraction method (23), followed by PCR amplification of the 16S rRNA gene using universal primers 16F27 [5'-CCA GAG TTT GAT CMT GGC TCA G-3'] and 16R1492 [5'-TAC GGY TAC CTT GTT ACG ACT T-3']. The isolate was identified as *Azotobacter chroococcum.*

***In-vitro* survivality tests of bacterial isolate under different growth conditions**

Salt tolerance:

A loopful of selected bacterial isolate grown overnight on broth culture was inoculated to Ashby broth media containing different concentrations of NaCl (0%, 2%, 4%, 6%, 8% 10%) and incubated at 28 °C. At each day interval 0.1 ml of the suspension was spread plated on nutrient agar plates and colony forming units (cfu/ml) was recorded up to 7 days (24).

pH tolerance:

A loopful of selected bacterial isolate from overnight grown broth culture was inoculated to Ashby broth adjusted to different pH (5, 7, 9) and incubated at 28 °C. At each day interval 0.1 ml of the suspension was spread plated on nutrient agar plates and colony forming units (cfu/ml) was recorded up to 7 days (24).

Temperature tolerance:

For the study of temperature tolerance of selected bacterial isolate, a loopful of selected bacterial isolate from 24 hours old culture broth was inoculated to freshly prepared Ashby broths and were allowed to grow at different temperatures (10ºC, 20ºC, 30ºC, 40ºC, 50ºC). At each day interval 0.1 ml of the suspension was spread plated on nutrient agar plates and colony forming units (cfu/ml) was recorded up to 7 days (24).

III. Result

One free living nitrogen fixing bacterial strain isolated from maize field of Darjeeling hills initially designated as MDH01 and later identified as *Azotobacter chroococcum* showed cell shape bluntly rod, oval or coccus, pleomorphic in nature, cells are often in pairs, cells size were 2- 4 µm, colonies were cream in colour, slimy, translucent, elevated smooth margin, brownish black pigments were observed in old cultures. Table 1 shows the morphological characteristic of the isolate.

The biochemical characterization of the bacterial isolate was given in Table 2. The bacterial isolate showed gram negative in nature. The strain showed positive utilization of mannitol, dextrose, mannose, sucrose

and raffinose. It shows negative result for H_2S production and urease test. The strain also showed positive result for oxidase, catalase, Indole test, nitrate reduction, citrate utilization and gelatin hydrolysis.

Table 1. Morphological characteristic of the isolate

Isolate	Shape	Surface	Margin	Colour	Texture	Pigment colour
MDH-01	Bluntly rod, oval, coccus	Smooth elevated, glistening	Entire	Creamy white	Mucoid	Brown

Table 2: Physical and Biochemical characterization of the isolate

Characteristics	Results
Gram's reaction	Gram negative
Motility	+
Utilization of sugars	
Mannitol	+
Dextrose	+
Rhamnose	-
Mannose	+
Sucrose	+
Raffinose	+
Starch hydrolysis	+
Oxidase test	+
Citrate Utilization	+
Nitrate reduction	+
Catalase test	+
Indole test	+
H_2S production	-
Urease test	-
Gelatin hydrolysis	+

Table 3. Molecular identification

Strain	Closest Neighbour	% Similarity	16 S rRNA fragment length	Accession no.
MDH-01	*Azotobacter chroococcum* AM 12666	99.51	1200 bp	AB175653

The detail of molecular identification is described in previous section.

Table 4. IAA production and Phosphate solubilization Index and Solubilization efficiency of bacterial isolate

PGP activities of the bacterial isolate

The amount of IAA produced was found to be 31.5 µg/ml by the isolate after 7 days of incubation. The SI and SE of bacterial isolate was found to be about 2.57 and 157 respectively (Table 4).

The isolate shows positive results for PGPR activities like Nitrogen fixing ability, HCN production, ammonia production and siderophore production (Table 5).

Table 5. Characterization of plant growth promoting traits of the isolate

Isolate	Nitrogen fixing activity	Siderophore producing ability	Hydrogen cyanide producing activity	Ammonia producing activity
MDH-01	Positive	Positive	Positive	Positive

***In-vitro* survivality test of bacterial isolate**

The bacterial isolate showed positive growth upto 6 % of NaCl concentration, the growth was decreased at 8% NaCl concentration and no growth was observed at 10% NaCl concentration (Fig. 1).

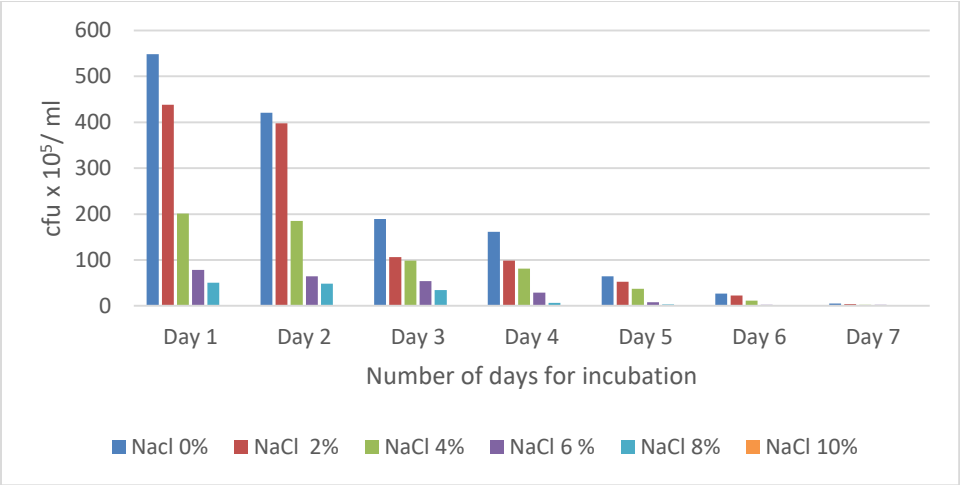

Fig 1. Survivality pattern of bacterial isolate at different concentration of NaCl

The bacterial isolate showed maximum growth (cfu/ml) at neutral pH (7) throughout the study period. The growth was observed slightly decreased at alkaline pH (9) and the minimum growth was observed at acidic pH (5) (Fig 2).

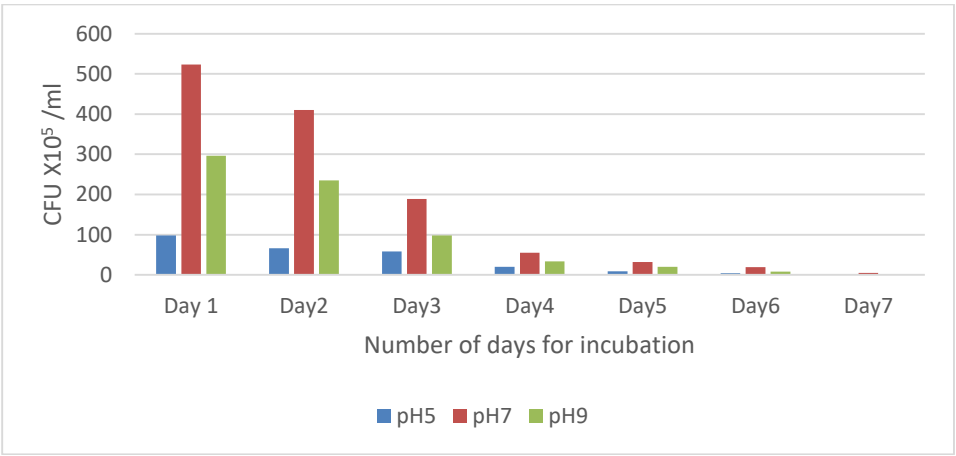

Fig 2. Survivality pattern of bacterial isolate at different pH

The bacterial isolate showed maximum growth at 30˚C temperature. Growth was reduced with increasing temperature and almost no growth at 50 ˚C (Fig 3).

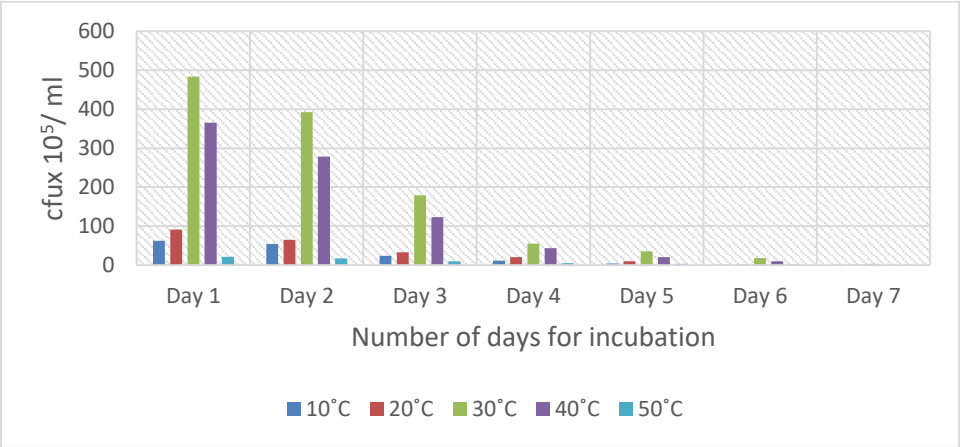

Fig 3. Survivality pattern of bacterial isolate at different incubation temperatures

IV. Discussion

The bacterial isolate MDH-01 isolated from maize rhizosphere of Darjeeling hills by preparing the serial dilution methods and characterized for their PGP traits and survivality efficiency under different growth conditions. The colony characterization and biochemical studies were made. The study result revealed that the bacterial isolate is gram negative in nature, bluntly rod and coccus in shape, cells were in pairs, mucoid, transparent, creamy in colour, the brown pigment was observed in older culture. Pigment production in *Azotobacter* species was reported in previous study (25).

Nitrogen fixation and IAA production are the direct mechanism thorough which *Azotobacter* contributes to plant growth. The amount of IAA production by the isolate MDH-01 falls in the range of IAA produced by six *Azotobacter* isolates i.e. 12-48.1 mg/ L in the medium supplemented with tryptophan as reported in previous study (26). Another study reported production of IAA in tryptophan amended media by 16 isolates of *Azotobacter* was up to 42.80-82.00 µg mL^{-1} (27)

The *Azotobacter* isolates were able to dissolve both inorganic and organic phosphate compounds as reported by previous workers (28, 29, 30). The solubilization index results obtained from the above study was almost similar to other findings (30,31).

The PGP activities of *Azotobacter* isolates were also investigated previously (32). According to the report (33) the majority of isolates from the maize rhizosphere had PGPR traits, meaning they ought to be considered as possible biofertilizers.

One of the most crucial traits of *Azotobacter* is salt tolerance capacity for areas where salinity is a problem.The studied isolate shows growth upto 6% of NaCl and growth decreases at 8% of NaCl with no growth at 10% of NaCl. The results co-relate with the findings reported earlier (34).

One of the most significant characteristics of *Azotobacter* for regions where pH is a factor is its ability to tolerate pH variations. According to the opinion of earlier report (24), the genus *Azotobacter* is widely distributed in nature and can grow in environments with pH ranges of 6.0 to 9.0 and varying climatic temperatures. The isolates exhibited the highest CFU at pH 7, a slight decrease at pH 9, and a minimum at pH 5, which is comparable to the previous finding (35), where it has been noted that the maximum population of *Azotobacter* spp. in soil with pH 7.8 and 8.0, and that the counts declined as pH increased. Knowles investigated the growth of *Azotobacter* species in acidic and alkaline conditions (36).

The isolates show maximum growth at temperature of 30˚C and no growth at 50˚C. Previous research indicated that the highest density of *Azotobacter* spp. occurs at approximately at 28˚C, while another study discovered that approximately 30˚ C is the ideal temperature for *Azotobacter* growth. (37,38,39).

The isolate MDH-01 was identified by 16S rRNA sequencing method at NCMR, NCCS Pune. The closest neighbour of the isolate is *Azotobacter chroococcum* which has 99.51 % similarity (Table 3).

V. Conclusion

The result of the present study has shown that the isolate MDH-01from the rhizosphere soils of maize crops of Darjeeling hills has the capability of IAA production, phosphate solubilization and have different PGP traits. The isolate has shown their survivality efficiency under different growth conditions like salt concentration, pH and temperature. Further field studies are required to evaluate for their development as an indigenous biofertilizer organisms to be used in upland agricultural systems.

Acknowledgements

The authors would like to thank Dr. Arnab Sen, Professor, Department of Botany, North Bengal University and Dr. Dorjay Lama, Associate Professor, Department of Botany, St. Joseph College, Darjeeling for their unconditional support and guidance in completion of this study.

References

[1]. Davies Pj. Plant Hormones: Physiology, Biochemistry, And Molecular Biology. Kluwer Academic Publishers, Netherlands, 1995.
[2]. Anjum Ma, Sajjad Mr, Akhtar N, Qureshi Ma, Iqbal A, Jami Ar, And Hasan Mu. Response Of Cotton To Plant Growth Promoting Rhizobacteria (Pgpr) Inoculation Under Different Levels Of Nitrogen. Journal Of Agricultural Research, 2007; 45 (2): 135-143.
[3]. Pichu R. Free-Living Bacterial Inoculate For Enhancing Crop Productivity. Trends In Biotechnology.1989; 7: 39-44.
[4]. Okon Y And Labandera-Gonzalez Ca. Agronomic Applications Of Azospirillum In Improving Plant Productivity With Rhizosphere Bacteria, Australia. Commonwealth Scientific And Industrial Research Organization, 1994.
[5]. Glick Br. The Enhancement Of Plant Growth By Free Living Bacteria. Canadian Journal Of Microbiology.1995; 41:109-114.
[6]. Munees A And Mohammad Sk. Functional Aspect Of Plant Growth Promoting Rhizobacteria. Insight Microbiology. 2001; 1:39-54.
[7]. Mazid M And Khan Ta. Future Of Bio-Fertilizers In Indian Agriculture: An Overview. International Journal Of Agricultural And Food Research. 2015; 3(3).
[8]. Subedi R, Khanal A, Aryal K, Chhetri L And Kandel B. Response Of Azotobacter In Cauliflower (Brassica Oleracea Var. Botrytis) Production At Lamjung, Nepal. Acta Scientifica Malaysia. 2019; 3:17-20.
[9]. Jarak M, Protia R, Jankovia S, And Eolo J. Response Of Wheat To Azotobacter - Actinomycetes Inoculation And Nitrogen Fertilizers. Romania Agricultural Research. 2006; 23:37-40

[10]. Kizilkaya R. 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. 2009; 30(1):73-82.
[11]. Sharma Pk And Chahal Vps. Antagonistic Effect Of Azotobacter On Some Plant Pathogenic Fungi. J. Res. Punjab Agri.Univ.1987; 24: 638-640.
[12]. Varma A, Kukreja K, Pathak Dv, Suneja S And Nanda N. In Vitro Production Of Plant Growth Regulators By A. Chroococcum. Indian Journal Of Microbiology. 2001;41: 305-307.
[13]. Chhetri Dr, Basnett D, Chiu Pf, Kalikotay S, Chhetri G And Parajuli S. Current Status Of Ethnomedicinal Plants In Darjeeling Himalaya. Current Science. 2005; 89(2):264-268.
[14]. Holt Jg, Krieg Jn, Sneath P, Staley J, Williams S. Bergey's Manual Of Determinative Bacteriology. Williamson And Wilkins. Baltimore, 1994: 786-788.
[15]. Bano N And Mussarat J . Characterization Of A New Pseudomonas Aeruginosa Strain Nj-15 As A Potential Biocontrol Agent. Curr Microbiol. 2003; 46:324-328.
[16]. Gaur Ac. Phosphate Solubilizing Microorganisms As Biofertilizers, Omega Scientific Publisher, New Delhi 1990; 176.
[17]. Nguyen C, Yan W, Le Tacon F And Lapeyrie F. Genetic Variability Of Phosphate Solubilizing Activity By Monokaryotic And Dikaryotic Mycelia Of The Ectomycorrhizal Fungus Laccaria Bicolor (Msire) P.D. Orton. Plant Soil 1992; 143: 193-199.
[18]. Vazquez Pg, Holguin Me, Puente A, Lopez-Cortes And Bashan Y. Phosphate-Solubilizing Microorganisms Associated With The Rhizosphere Of Mangroves Growing In A Semiarid Coastal Lagoon. Biol. Fertil. Soils. 2000;30: 460-468.
[19]. Kyaw Ep, Yu Ss And M.M. Oo. Purification And Characterization Of Cellulases From Wild Type And Mutant Strains Of Cellulytic Nitrogen Fixing Bacteria Isolated From Soil In N Free Media. International Conference On Sustainable Development.2008.
[20]. Schwyn B And Neilands Jb. Universal Chemical Assay For The Detection And Determination Of Siderophores. Analytical Biochemistry. 1987; 160 (1): 47-56.
[21]. Bakker Aw And Schippers B. Microbial Cyanide Production In The Rhizosphere In Relation To Potato Yield Reduction And Pseudomonas Spp-Mediated Plant Growth-Stimulation. Soil Biol. Biochem. 1987;19: 451-457.
[22]. Cappuccino Jc, Sherman N. Microbiology. In: A Laboratory Manual. 3rd Eds. New York, Benjamin/Cummings Pub. Co. 1992; 125–179
[23]. Sambrook J, Fritsch Ef And Maniatis T. Molecular Cloning A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.1989.
[24]. Chennappa G, Naik Mk, Adkar-Purushothama Cr, Amaresh Ys And Sreenivasa My. Pgp Potential, Abiotic Stress Tolerance And Antifungal Activity Of Azotobacter Strains Isolated From Paddy Soils. Indian Journal Of Experimental Biology. 2016; 54:322-331.
[25]. Jimenez Dj, Montana, Js And Martinez Mm. Characterization Of Free Nitrogen Fixing Bacteria Of The Genus Azotobacter In Organic Vegetable-Grown Colombian Soils. Brazilian Journal Of Microbiology. 2011; 42(3) :846-58.
[26]. Patil V. Production Of Indole Acetic Acid By Azotobacter Sp. Recent Research In Science And Technology. 2011; 3(12).
[27]. Kumar A, Kumar K, Kumar P, Maurya R, Prasad S And Singh Sk. (2014). Production Of Indole Acetic Acid By Azotobacter Strains Associated With Mungbean. Plant Archives. 2014; 14(1):41–42
[28]. Kumar V And Narula N. Solubilization Of Inorganic Phosphates And Growth Emergence Of Wheat As Affected By Azotobacter Chroococcum Mutants. Biol Fertil Soils. 1999; 28:301-305.
[29]. Garg Sk, Bhatnagar A, Kalla A And Narula N. In Vitro Nitrogen Fixation, Phosphate Solubilization, Survival And Nutrient Release By Azotobacter Strains In An Aquatic System. Bioresource Technology. 2001; 80:101-109
[30]. Farajzadeh D, Yakhchali B, Aliasgharzad N, Sokhandan-Bashir Nand Farajzadeh M. Plant Growth Promoting Characterization Of Indigenous Azotobacteria Isolated From Soils In Iran. Curr Microbiol. 2012; 64:397-403.
[31]. Nosrati R, Owla P, Saderi H, Rasooli Iand Malboobi Ma. Phosphate Solubilization Characteristics Of Efficient Nitrogen Fixing Soil Azotobacter Strains. Iran J Microbio. 2014; 6(4): 285–295.
[32]. Miljaković D, Marinković J, Branislava T, Sonja T Ž, Mrkovacki N, Nastasic A. Screening Of Azotobacter Isolates For Pgp Properties And Antifungal Activity. Zbornik Matice Srpske Za Prirodnenauke. 2015; 65-72.
[33]. Suresh A, Pallavi P, Srinivas V, Kumar Vp, Chandra Sj And Reddy Sr. Plant Growth Promoting Activities Of Fluorescent Pseudomonads Associated With Some Crop Plants. Afr. J. Microbiol. Res. 2010; 4: 1491–1494.
[34]. Islam Mz, Sharif Di And Hossain Ma. A Comparative Study Of Azotobacter Spp. From Different Soil Samples. J.Soil.Nature. 2008; 2(3): 16-19.
[35]. Khullar S And Chahal Vps. Distribution Of Azotobacter In The Punjab Soils. J. Res. 1975; 13: 398-404.
[36]. Knowles Sd. Adaptation Of Azotobacter And Azospirillum In Soil. Ind. J. Soil Microbiol. 2008; 56: 211-217.
[37]. Saribay Gf. Growth And Nitrogen Fixation Dynamics Of Azotobacter Chroococcum Nitrogen-Free And Omw Containing Medium. M.Sc. Thesis In Graduate School Of Natural And Applied Sciences, Middle East Technical University, Turkey.2003.
[38]. Dhanasekar R. Poly (3-Hydroxy Butyrate) Synthesis From Mutant Strain Azotobacter Spp. Utilizing Glucose In A Batch Reactor. J. Biochem. Eng.2003; 16: 1-8.
[39]. Jadhav Gg, Salunkhe Ds, Nerkar Dp And Bhadekarrk. Isolation And Characterization Of Salt-Tolerant Nitrogen-Fixing Microorganism From Food. J. Eur. Asia. Bio. Sci. 2010; 4: 33-40.