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Isolation and characterisation of Nitrogen Fixing Bacteria (*Azotobacter* Sp.) from Tea field soil of Dooars and Darjeeling region of North Bengal, India.

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Abstract : *Different strains of Azotobactersp were isolated and charaterised morphologically, folowing their resistance activities against antibiotic and Nacl from Dooars and Darjeeling region of North Bengal.The soil sample studied were DS-1, DS-2 and DJ-1. DS-1 have 50 isolates, DS-2 have 45 isolates and DJ-1 have 50 isolates. Among morphlogical parameter colony colour, shape and size has been considered for this study. Most of the colony are white many shows colour, circular, colony diameter ranges from 1-4.5 cm . Four strains namely DS-1-18, DS-1-25,DS-2-10,DJ-1-45 shows resistance against anibiotic 35mg/l). Nacl tolerance activities of seveteen promising clone has studied, among them three strains namely DS-1-16, DS-1-17and DS-2-18 shows salt tolerance of upto 4.5%.Nitrogen fixing capacityof five strains has been carried out for highest antibiotic resistance starins, among them DS-2-10 shows maximum N_2 content (0.006 %)*

Keywords: *N2 fixing soil bacteria, Azotobacter sp., Antibiotic Resistance assay, Salt tolerance. Kjeldhal.*

I. Introduction

Tea is the most popular and inexpensive beverage produced from the shoots of commercially cultivated tea plants (*Camellia sinensis* (L.) O. Kuntze). Tea is grown in more than 50 countries, mostly inlarge plantations. India is the largest producer in the World.

The use of chemical fertilizer and chemical pesticides in crop field makes soil infertile and the demand of cash crop like Tea becoming decline . To search an alternative approach the use of bio fertilizer is gaining momentum in crop field . The demand of " **Organic crop** " in India and abroad is highly increasing day by day . Biofertilizers may be defined as the preparations containing the living cells of different strains of microorganisms that help in enhancing the nutrient uptake by the plants and hence enriches the nutrient quality of the soil by their interaction in the rhizosphere when these biofertilizers applied either on the top soil or through seed inoculations. Isolation of *Azotobacter*sp and cost effective production of biofertilizer reported by **Gomare** *et al.,* 2013. Biofertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. The use of biofertilizer is steadily increased in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. So Biofertilizers are low cost renewable sources of plant nutrients which supplement chemical fertilizers.Biofertilizers generate plant nutrients like nitrogen and phosphorous through their activities in the soil or rhizosphere and makes them available to the plants on the soil. Biofertilizersare gaining an importance in use because of the proper maintenance of soil health minimize environmental pollutions and cut down the use of chemicals. For the last one-decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status and for enhancement of crop production by their biological activity in the rhizosphere. Application of beneficial microbes in agricultural practices started 60 years ago and there is now increasing evidence that these beneficial microbial populations can also enhance plant resistance to adverse environmental stresses, e.g. water and nutrient deficiency and heavy metal contamination (Wua *et al*, 2004)
In agriculture , the importance of nitrogen fertilizer is immense to meet the demand of nitrogen to the plant . Generally different inorganic salts of nitrogen are being used to replenish the demand of agricultural crop, like urea, ammonium sulfate etc . India requires 29 million tons of NPK of which 13 million tons of Nitrogen is required to produce 220 million tons of food grain in a year . Effect of these inorganic fertilizers is a matter of great concern today. They have a negative impact on the soil -fertility . Secondly, they are also acting as environment pollutant by depleting the ozone layers or to decrease the micro flora of the soil .

The altitude of Dooars area ranges from 90 to 1750m.,many rivers go through these fertile plain. The average rain fall of the area is about 3500mm. Monsoon generally start from the middle of May and continues till the end of September. Summer is mild and winter is too cold and foggy. Darjeeling is located in the Lesser Himalaya at an elevation of 6,700 ft (2,042.2 m) The soil is chiefly composed of sandstone and conglomerate formations, which are the solidified and upheaved detritus of the great range of Himalaya. However, the soil is often poorly consolidated (the permeable sediments of the region do not retain water between rains) and is not

considered suitable for agriculture. Darjeeling has a temperate climate with wet summers caused by monsoon rains. The annual mean maximum temperature is 14.9 °C (58.8 °F) while the mean minimum temperature is 8.9 °C (48.0 °F), with monthly mean temperatures ranging from 6 to 18 °C (43 to 64 °F) (wikipedia)Among the free-living nitrogen-fixing bacteria, those from genus *Azotobacter* have an important role, being broadly dispersed in many environments such as soil, water and sediments (Mirjana *et al*, 2006). *Azotobacter sp*, are free-living aerobic bacteria dominantly found in soils, present in alkaline and neutral soils. They are nonsymbiotic heterotrophic bacteria capable of fixing an average 20kg N/ha/year. Besides, it also produces growth promoting substances and are shown to be antagonistic to pathogens. *Azotobacter sp*. are found in the soil and rhizosphere of many plants and their population ranges from negligible to 104 g-1 of soil depending upon the physico-chemical and microbiological (microbial interactions) properties (Ridvan, 2009). Besides, nitrogen fixation, *Azotobacter* also produces, thiamin, riboflavin, indole acetic acid and gibberellins. When *Azotobacte*r is applied to seeds, seed germination is improved to a considerable extent, so also it controls plant diseases due to above substances produced by *Azotobacter*. The exact mode of action by which *Azotobacteria* enhances plant growth is not yet fully understood. Three possible mechanisms have been proposed: N2 fixation; delivering combined nitrogen to the plant; the production of phytohormone-like substances that alter plant growth and morphology, and bacterial nitrate reduction, which increases nitrogen accumulation in inoculated plants (Mrkovac & Milic, 2001).

The effect of *Azotobacter chrococcum* as Nitrogen biofertilizer on the growth and yield of *Cucumis sativus* has been investigated by Salhia 2001. But such type of study specially on Tea crops is little or no, and also the characterization of *Azotobacter* sp from Tea field soil is scanty or little.From the perusal of literature it has been revealed that there are scanty reports on isolation and characterization of bio fertilizer (non symbionts-Azotobacter sp) in Tea Crops specially in Dooars and Darjeeling area. Keeping the background information the present study has been undertaken to isolation and characterization of soil borne efficient N_2 fixing bacteria in Tea garden soil mainly of Dooars and Darjeeling region .

II. Material And Methods

Materials:

Soil samples were collected from two different region of West Dooars and one region of Darjeeling tea garden , three different location of each then mix,10- 30cm below from the soil surface by aseptic manner in 3 sterilized polythene bags. The soil samples were brought to the laboratory and kept in a thermocol box for further processing.

Media Preperatuion:

The isolation of *Azotobacter*sp has been carried out in Selected media (Mannitol Agar media: MA).For preparation of one liter of MA media Di potassium phosphate (1g/L),Magnesium sulphate(0.2g/L), Sodium Chloride(0.2g/L) Ferrous Sulphate (0.05g/L0 mannitlo(20g/L), Nitrogen free Agar(15g/L) has been weigh and dissolved in double distilled water and make the volume to one liter, P^H Was adjusted to 5.0 with 1N(HCL)/1N (NaOH).

Isolation of Bacteria:

Collected soil sample (1g of each of Dooars and Darjeeling soil Viz. DS-1,DS – 2 and DJ – 1) was added to 10 ml of sterile distilled water in a sterile culture tube shake well by allowing to mix it properly. From the top of the suspension, sample(1 ml) was then transferred to sterile distilled water (9 ml) in another tube, shake well and allowed to stand for 30 minutes. In this way, samples were diluted up to 10^{-5} dilution fraction. So five serial dilutions were done for each of the three sample (total 15 dilutions). One ml of sample suspension (from 10^{-1} to 10^{-5} fraction) was taken in a sterilized Petri Plates containing approximately 15-20 ml melted Mannitol Agar medium and then incubated at 28±2ºC temperature for about 48 hours. After incubation, the individual colonies were appeared on the medium. The well separated distinct colonies on these plates were used for further study.

Pure Culture Preparation:

Well developed and separated colonies which were identified on Mannitol Agar media were marked and then these separated colonies were chosen and by the help of toothpicks the colonies were transferred and streaked separately on 3 plates aseptically (DS-1,DS-2,and DS-3) containing Mannitol Agar media. These plates were considered as Master Plate.

Bacterial colony identification and morphology :

Using the spread plate technique, the bacterial colony identification and external morphology(e.g. color, shape and size of the colony) were studied. Then the serial dilution of $10^{-1}$to 10^{-5} were chosen and from

that 0.5 ml of bacterial culture was transferred from each serially diluted test tubes and spreaded on the petriplates by means of spreader. Then petriplates were kept in incubation for 28°C for 2 days for the incubation and growth of bacteria. Two types of assay have been done for the characterization of soil bacterium - NaCl and Antibiotic assay.

NaCl tolerant assays:

Sodium chloride is a salt which is responsible for the salinity of the soil. The main aim of NaCl assay is determining the optimum condition of salinity at which the bacteria can survive. Five different concentration have been made ranging from 0.5% to 5 % of NaCl in Mannitol Agar media then were poured on petriplates. Isolated bacteria's were then inoculated into the petriplates by the help of toothpick followed by incubation for 2 days at 28^0 C .

Antibiotic resistance and susceptibility assay :

The activity (concentration) of antibiotic may be demonstrated under suitable conditions by their inhibitory effect on microorganisms . Antibiotic sensitivity test usually done for determining the level of sensitivity of bacteria strain against a particular antibiotic .In this investigation three antibiotic each of which seven concentration were used namely Chloramphenicol , Rifampicin , Tetracycline . Seven different concentration were made in Mannitol Agar media followed by streaking the selected bacterial culture.

N estimation of pure culture : By Kjeldhal method:

It is a method for the quantitative determination of organic nitrogen in chemical substances like ammonia developed by Johan Kjeldahl in 1883 . On the basis of highest resistance, against Chloramphenicol (35mg/l),mainly strain DS-1-18, DS-1-25 and DS-2-10, Tetracyclin (35mg/l), mainlyDS-2-10, DJ-1-45 and Rifampicin (35mg/l) mainly DS-2-10 were selected for this study. The nitrogen in Bacterial cultures (three days) were estimated by kjeldhal method (Williams and Wasington,1996). The acid digested sample was distilled and subjected to quantification of nitrogen through titrimetric method using 0.0N NaOH.

Total Nitrogen was calculated and expressed as % (percentage) using the formula:

$$\text{Total N \%} = \frac{(\text{Blank value} - \text{Titrated value}) \times \text{Normality of NaOH} \times 0.014}{\text{Sample Weight taken}} \times 100$$

III. Result And Discussion

Bacterial colony identification and characterization:

After incubation of soil sample in *Azotobacter* selected media, total 145 colony has been thoroughly characterized on the basis of colony color, shape and diameter of the colony. Out of them the characteristics features of some (20) selected colony has been summarized (Table:1) Most of the bacterial colony isolated are circular (even) in shape and most of the bacterial colony are whitish in colour and the size ranges between 1.0 – 4.0 mm in size (Table: 1) **.** Others bacterial colonies isolated are spindle(even) in shape and circular(undulated) and others bacterial colony are translucent white with central black dot yellowish, and creamy . The fifty isolates of Dooars (DS-1) region has been studied and characterised, among them six promising isolates has been considered for NaCl assay and six promising isolates has been considered for antibiotic assay .

The forty-five isolates of Dooars(DS – 2) region has been studied and characterised, among them six promising isolates has been considered for NaCl and antibiotic assay The fifty isolates of Darjeeling (DJ-1) region has been studied and characterised , among them seven promising isolates has been considered for NaCl and six promising isolates has been considered for antibiotic assay .

NaCl tolerant assay:

Among 19 isolates , three isolates (DS – 1 – 17 , DS – 1 – 18 , DS – 2 – 18) were exhibits highest NaCl tolerance at4% and moderate tolerance at 4.5%,and three isolates (DS – 1 – 16 , DJ –1 – 35, DJ-1-46) shows minimum NaCl (1.5 %) tolerance. The isolates number DS-1-16 shows no tolerance at 2%, DS-1-25 shows no tolerance at 2.5%, DS-2-17 shows no tolerance at 1%, DJ-1-11 shows no tolerance at 2.55, DJ-1-22,23 shows no tolerance at 3% and DJ-1-46 shows no tolerance at 3.5%(Table:2) Salt tolerance properties of *Azotobacter* has been investigated by Akhter et al 2012,five isolates of *Azotobacter* were found which could tolerate 6% NaCl concentration whereas only two isolates *of Azotobacter* were found which could stand for at 10% NaCl concentration, which may become a promising source for further study regarding salinity tolerant *Azotobacter* sp.

Antibiotc assay:

Among 18 isolates , three isolates (DS – 1 – 18 , DS – 1 – 25 , DS – 2 – 10) has been shown maximum resistance 30 mg / L of chloramphenicol . Isolates DS – 2 – 17 has been shown no resistance

properties . Three isolates shows minimum resistance properties . Three isolates shows minimum resistance (20 mg / L) properties against chloramphenicol .(Table : 5) Among 18 isolates , two isolates (DS – 2 – 10 , DJ –1 – 25) has been shown maximum resistance 30 mg / L of tetracycline . Three isolates shows minimum resistance properties (15mg/L). Three isolates shows minimum resistance (20 mg / L) properties against tetracycline . (Table : 6] Among 18 isolates , four isolates (DS – 1 – 25 , DS – 2 – 10 , DS – 2 – 17 , DS – 2 - 19) has been shown maximum resistance 30 mg / L of Rifampicin . Isolates DS – 2 – 18 has been shown no resistance properties . Three isolates shows minimum resistance properties . Three isolates shows minimum resistance (20 mg / L) properties against Rifampicin . (Table: 7). The antibiotic resistance and susceptibility of Asymbiotic N_2-fixers (*Azotobacter*) and symbiotic N_2-fixers (*Rhizobium* spp against seven different antibiotics, nalidixic acid, cloxacillin, chloramphenicol, tetracycline, amoxycillin, methicillin, doxycyclinehas has been studied by Shahin andMalik 2003.

N estmation:

Kjeldhal method was performed to see the nitrogen estimation by two antibiotic resistance bacteria (DS-1-25, DS-2-10). The two tested strains DS-1-25 and DS-2-10 has been shown maximum nitrogen uptake capacity. Nitrogen fixation potentiality of the selected isolates of *Azotobacter* sp was estimated by Akhter et al (2012) ,ranging from 04.95 to 10.55 mg N/g substrate.

IV. Conclusion

The use of chemical fertilizer and chemical Pesticides in crop field makes soil infertile and the demands of cash crop like Tea becoming decline. To Search an alternative is gaining momentum in Crop field. The demand of organic Crop in India and abroad is highly increasing day by day .The present study has revealed that some strain of *Azotobacter* sp especially those have high salt tolerant, antibiotic resistance and contains high percentage of N are of importance to have the use as a Biofertiliser in Tea field. Further study can also be made for their molecular characterization (on the basis of 16srRNA analysis).

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References

[1]. Gomare K.S., Mese.M, Shetkar.Y. Isolation of *Azotobacter* sp and cost effective production of biofertilizer.*Indian Journal of Applied Research .3(5)* 2013 P:54-56

[2]. Akhter S.M, Hossain S.J, Hossain S. A , Datta R .K Isolation and Characterization of Salinity Tolerant *Azotobacter* sp. *Greener Journal of Biological Sciences 2 (3)*, 2012 pp. 043-051.

[3]. Ridvan Kizilkaya, (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),* 2009. p:73-82 .

[4]. Mirjana Jarak, Rade Protiã, Snezana Jankoviã, and Jovan Èolo.. Response of wheat to Azotobacter - actinomycetes inoculation and nitrogen fertilizers. Romanian
Agricultural Research,23, 2006 p:37-40.

[5]. Wua, S.C., Caob, Z.H., Lib, Z.G., Cheunga, K.C, and Wonga, M.H Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma 125*. 2004. p:155.166.

[6]. Shafiani Shahin and , Malik Abdul Tolerance of pesticides and antibiotic resistance in bacteria isolated from wastewater-irrigated soil. *World Journal of Microbiology and Biotechnology, 19,(9),* (2003) pp 897-901

[7]. Mrkovac, N. , Milic, V. Use of *Azotobacter chroococcum* as potentially useful in agricultural application. *Annals of Microbiology, 51*, 2001. p:145-158.

[8]. Salhia Basel M The Effect of *Azotobacter chrococcum* as Nitrogen biofertilizer on the growth and yield of *Cucumis sativus* thesis submitted for Master of Biological Sciences, The Islamic University-Gaza. 2001.

[9]. AOAC, *In : Official methods of Analysis, 15th edition (eds.)* S. williams and D.C Washington 1996 Pp: 105-107.

Table:

Table 1: Morphological Characteristics of selected colony of DS-1 , DS-2 and DJ-1 soil sample(Azotobacter sp):

Sample code	Colony number	Dilution	Colour(opacity)	Shape(margin)	Size(in mm)
DS-1	16	10^{-3}	Translucent with central white dot	Circular(even)	2.0
DS-1	17	10^{-3}	Transparent with central white dot	Circular(even)	1.0
DS-1	18	10^{-3}	White(translucent)	Circular(even)	1.0
DS-1	19	10^{-3}	White(translucent)	Circular(even)	1.0
DS-1	20	10^{-3}	White(translucent)	Circular(even)	0.5
DS-1	21	10^{-3}	White(translucent)	Circular(undulated)	1.0
DS-1	25	10^{-2}	Bluish(iridescent)	Circular(even)	3.0

DS-2	10	10^{-3}	White(translucent)	Circular(even)	3.5
DS-2	14	10^{-3}	Translucent white with central black dot	Circular(even)	4.0
DS-2	17	10^{-3}	Translucent white with central black dot	Circular(even)	2.0
DS-2	18	10^{-3}	White(translucent)	Circular(even)	1.5
DS-2	19	10^{-3}	Yellowish(opaque)	Circular(even)	2.0
DS-2	22	10^{-3}	White(translucent)	Circular(even)	2.0
DJ-1	11	10^{-4}	Orange(translucent)	Circular(even)	3.0
DJ-1	22	10^{-3}	Greenish yellow(translucent)	Circular(even)	2.5
DJ-1	23	10^{-3}	Orange(translucent)	Circular(even)	3.0
DJ-1	24	10^{-3}	Orange(translucent)	Circular(even)	2.0
DJ-1	35	10^{-2}	Greenish yellow(translucent)	Circular(even)	1.5
DJ-1	45	10^{-2}	Orange(translucent)	Circular(even)	1.5
DJ-1	46	10^{-2}	Creamy(translucent)	Circular(even)	2.5

Table 4: NaCl tolerant properties of Azotobacter sp :

Sample Code	Colony Number	0.5 %	1.0 %	1.5 %	2.0 %	2.5 %	3.0 %	3.5 %	4.0 %	4.5 %	5.0%
DS - 1	16	+++	+++	++	-	-	-	-	-	-	-
DS – 1	17	+++	+++	+++	+++	+++	+++	+++	+++	++	-
DS -1	18	+++	+++	+++	+++	+++	+++	+++	+++	++	-
DS -1	20	+++	+++	+++	+++	+++	+++	++	++	+	-
DS -1	21	+++	+++	+++	+++	++	++	+	+	+	
DS -1	25	+++	+++	+++	++	-	-	-	-	-	
DS -2	10	+++	+++	+++	+++	+++	+++	++	-	-	
DS -2	14	+++	+++	+++	+++	+++	+++	+++	++	+	
DS -2	17	+++	-	-	-	-	-	-	-	-	-
DS -2	18	+++	+++	+++	+++	+++	+++	+++	+++	++	-
DS -2	19	+++	+++	+++	+++	+++	+	+	-	-	
DS -2	22	+++	+++	+++	+++	++	-	-	-	-	-
DJ-1	11	+++	+++	+++	+++	-	-	-	-	-	-
DJ-1	22	+++	+++	+++	++	++	-	-	-	-	-
DJ-1	23	+++	+++	+++	+++	++	-	-	-	-	-
DJ-1	24	+++	+++	+++	+++	+++	+++	++	+	+	-
DJ-1	35	+++	+++	++	++	+	-	-	-	-	-
DJ-1	45	**+++**	**+++**	**+++**	**+++**	**++**	**+**	**+**	**-**	**-**	**-**
DJ-1	46	**+++**	**+++**	**++**	**++**	**+**	**+**	**-**	**-**	**-**	**-**

Table 5: Antibiotic (Chloramphenicol) tolerance propertics of Azotobactor sp :

Sample Code	Colony Number	10 mg/L	15 mg/L	20 mg/L	25 mg/L	30 mg/L	35 mg/L	40 mg/L
DS – 1	**16**	**+++**	**+++**	**+++**	**+++**	**++**	**-**	**-**
DS – 1	**17**	**+++**	**+++**	**+++**	**+++**	**++**	**+**	**-**
DS – 1	**18**	**+++**	**+++**	**+++**	**+++**	**+++**	**++**	**++**
DS – 1	**20**	**+++**	**+++**	**+++**	**+++**	**++**	**-**	**-**
DS – 1	**21**	**+++**	**+++**	**++**	**+**	**+**	**-**	**-**
DS – 1	**25**	**+++**	**+++**	**+++**	**+++**	**+++**	**++**	**+**
DS – 2	**10**	**+++**	**+++**	**+++**	**+++**	**+++**	**++**	**+**
DS – 2	**14**	**+++**	**-**	**-**	**-**	**-**	**-**	**-**
DS – 2	**17**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
DS – 2	**18**	**+++**	**+++**	**+++**	**+++**	**++**	**+**	**-**
DS – 2	**19**	**+++**	**+++**	**+++**	**+++**	**++**	**+**	**+**
DS – 2	**22**	**+++**	**+++**	**+++**	**+++**	**+**	**+**	**-**
DJ-1	**11**	**+++**	**+++**	**+++**	**++**	**+**	**-**	**-**
DJ-1	**23**	**+++**	**+++**	**-**	**-**	**-**	**-**	**-**
DJ-1	**24**	**+++**	**+++**	**++**	**+**	**+**	**-**	**-**
DJ-1	**35**	**+++**	**+++**	**+++**	**++**	**++**	**+**	**-**
DJ-1	**45**	**+++**	**+++**	**+++**	**++**	**++**	**+**	**+**
DJ-1	**46**	**+++**	**+++**	**++**	**-**	**-**	**-**	**-**

Table 6 : Antibiotic (Tetracyclin) tolerance propertics of Azotobactor sp .

Sample Code	Colony Number	10 mg/L	15 mg/L	20 mg/L	25 mg/L	30 mg/L	35 mg/L	40 mg/L
DS – 1	**16**	**+++**	**-**	**-**	**-**	**-**	**-**	**-**
DS – 1	**17**	**+++**	**-**	**-**	**-**	**-**	**-**	**-**
DS – 1	**18**	**+++**	**-**	**-**	**-**	**-**	**-**	**-**
DS – 1	**20**	**+++**	**+++**	**-**	**-**	**-**	**-**	**-**
DS – 1	**21**	**+++**	**+++**	**+**	**-**	**-**	**-**	**-**
DS – 1	**25**	**+++**	**+++**	**++**	**+**	**-**	**-**	**-**

DS – 2	**10**	+++	+++	+++	+++	+++	++	+
DS – 2	**14**	+++	+++	+++	-	-	-	-
DS – 2	**17**	+++	+++	-	-	-	+	-
DS – 2	**18**	+++	+++	+++	+++	+	+	-
DS – 2	**19**	+++	+++	+++	+++	++	-	-
DS – 2	**22**	+++	+++	+++	+	-	-	-
DJ-1	**11**	+++	+++	+++	+++	-	-	-
DJ-1	**23**	+++	+++	+++	++	-	-	-
DJ-1	**24**	+++	+++	+++	++	+	-	-
DJ-1	**35**	+++	+++	+++	+++	-	-	-
DJ-1	**45**	+++	+++	+++	+++	+++	++	+
DJ-1	**46**	+++	+++	++	++	-	-	-

Table 7 : Antibiotic (Rifampicin) tolerance propertics of Azotobactor sp .

Sample Code	Colony Number	10 mg/L	15 mg/L	20 mg/L	25 mg/L	30 mg/L	35 mg/L	40mg/L
DS – 1	**16**	+++	+++	+++	++	-	-	-
DS – 1	**17**	+++	+++	++	-	-	-	-
DS – 1	**18**	+++	-	-	-	-	-	-
DS – 1	**20**	+++	+++	+++	++	-	-	-
DS – 1	**21**	+++	+++	++	+	-	-	-
DS – 1	**25**	+++	+++	+++	+++	++	-	-
DS – 2	**10**	+++	+++	+++	+++	+++	++	+
DS – 2	**14**	+++	+++	+++	++	-	-	-
DS – 2	**17**	+++	+++	+++	+++	++	+	-
DS – 2	**18**	-	-	-	-	-	-	-
DS – 2	**19**	+++	+++	+++	+++	++	-	-
DS – 2	**22**	+++	+++	+++	-	-	-	-
DJ-1	**11**	+++	+++	+++	-	-	-	-
DJ-1	**23**	+++	+++	-	-	-	-	-
DJ-1	**24**	+++	+++	+++	++	++	+	+
DJ-1	**35**	+++	+++	+++	-	-	-	-
DJ-1	**45**	+++	+++	+++	-	-	-	
DJ-1	**46**	+++	+++	++	-	-	-	-

Table 8 : N estimation of Azotobacter strain (Kjeldhal Method)

Sample code	N Content
DS-1-18	**0.0002%**
DS-1-25	**0.005%**
DS-2-10	**0.006%**
DJ-1-45	**0.005%**