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PRODUCTION OF LIQUID BIOFERTILIZER BY USING *AZOTOBACTER* SPECIES AND THEIR EFFECT ON PLANTS GROWTH

Patel Shraddha[1], Singh Shruti[2]
[1,2]Dolat-Usha Institute of Applied Sciences and Dhiru- Sarla Institute of Management & Commerce, Valsad, Gujarat, India
*Corresponding author: singhshruti.242@gmail.com

ABSTRACT
Biofertilizer have been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. The present study was aimed for the production of liquid biofertilizer by using *Azotobacter* species and their effect on plant growth. In the present study, soil sample were collected from different Rhizospheric regions of south Gujarat namely Valsad, Bilimora, chikhli, Navsari and were investigated for *Azotobacter* species by isolating on Ashby's mannitol agar plate. All the isolates were identified as *Azotobacter* species by performing morphological analysis and various enzymatic analysis including catalase test, nitrate reduction test, phosphate solubilizing test. 10 *Azotobacter* species were isolated and were found to have catalase, phosphate solubilizing and nitrate reductase test activities. The seed germination assay reveals that the inoculation of the *Azotobacter* species potentially increase the root and shoot length of seedling and can serve as a potential source of Biofertilizer. The effect of prepared liquid biofertilizer was studied by performing pot experiment. The morphological parameter such as shoot length, root length were analysed at different time intervals respectively.

KEY WORDS: *Azotobacter*, liquid Biofertilizers, Seed germination, Plant growth.

INTRODUCTION

In recent year salinization, soil erosion and ground water contamination are the most important ecological concerns which affect the agriculture land causing it to become unsuitable for crop production. Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or to soil. Biofertilizer includes mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms. Biofertilizer benefiting the crop production are *Azotobacter*, *Azospirillum*, blue green algae, *Azolla*, phosphate solubilizing microorganisms, *Myco-rrhizae*[1]. The genus *Azotobacter* belonging to family Azotobacteraceae represented the main group of heterotrophic, non symbiotic nitrogen fixing bacteria principally inhabiting neutral or alkaline soils[2]. *Azotobacter* is gram negative, motile, pleomorphic aerobic bacterium which produces catalase, oval or spherical that form thick-walled cysts and may produce large quantities of capsular slime. It is one of the plant growths promoting rhizobacteria which is proved to promote the growth of plant producing growth hormones like thiamine, riboflavin, nicotine, indole acetic acid and gibberellins[3,4].

MATERIALS AND METHODS

Sample collection and isolation

Soil samples were collected from different rhizospheric region of South Gujarat namely Valsad, Bilimora, chikhli, Navsari from the depth of 10-15 cm. All the samples were investigated for *Azotobacter* species.

Samples were serially diluted where 0.1 ml of sample aliquots were taken and spreaded on the Ashby's mannitol agar plate. The plates were then incubated at room temperature for 24-72 hours. All the isolates were purified by using streak plate technique on Ashby's mannitol agar plate[5].

Identification

All the isolates obtained were subjected for various morphological and enzymatic analysis[6].

I Morphological analysis

For morphological analysis, Gram staining and Capsule staining (Maneval's method) was performed.

II Enzymatic analysis

Various enzymatic properties of the obtained isolates were studied. It includes catalase test, nitrate reduction test, phosphate solubilizing test.

Catalase test

Some microbes degrade hydrogen peroxide by producing catalase enzyme. Slide test was carried out for the confirmation of catalase enzyme. The colony isolate was placed on the glass slide, 2-3 drops of hydrogen peroxide was added and the formation of bubbles was observed.

Nitrate reduction test

One of the main characteristics of *Azotobacter* is nitrogen fixation by reducing the nitrate to nitrite. The test was performing to determine the ability of the obtained isolates to convert nitrate to nitrite. The isolates were inoculated in 1% peptone and incubated at 25˚-28˚C for 24 hours and next day result was observed**.**

Phosphate solubilizing test

Pikovskaya's agar medium was used for the phosphate solubilizing test**.** Isolates were checked for their ability to solubilizing phosphate by line streaking on the

Pikovskaya's agar medium, and was then incubated at 25˚-28˚C for 36-72 hours. Clear zone around the bacterial colony indicates phosphate solubilizing ability of bacteria[7].

Seed germination experiment

Wheat seed germination assay was done by applying cultures of obtained isolates. The cultures were in sterile Ashby's mannitol broth with 1.0 OD at 530 nm. Seeds were surface sterilized with 0.1% $HgCl_2$ for 3 minutes and washing with distilled water 6 times. Surface sterilized seeds of wheat were bacterized with inoculums for 30 minutes. The seeds were then transferred on moist sterilized filter paper in Petri plates and were incubated at room temperature and left undisrupted. Seeds soaked in sterile distilled water were used as negative control. The specified distilled water addition was followed every day until 7th day. The root and shoot lengths were measured and were then compared[7].

PRODUCTION OF BIO FERTILIZER

Liquid Biofertilizers

Azotobacter strain, isolated from various rhizospheric regions, was used for the present study. Isolated strain was transferred to the liquid broth of selective N- free Mannitol broth. The broth was then placed on the rotatory shaker for 8-9 days to provide continuous agitation and to prepare starter culture. When the cell count reached 108-109 cells/ml, the broth was then used as Biofertilizer[8].

POT ASSAY

The present investigation was carried out during the season of winter at microbiology department, Valsad. The experiment consists of 2 pots treated with or without Biofertilizer to check its effect on seeds of wheat. First agriculture soil was collected in polythene bags and processed for pot laboratory experiment. The wheat seed were soaked in to the H_2SO_4 for 5 minutes and washed thrice with sterile water. Then above prepared Biofertilizer was mixed with soil. Control pot was left un-inoculated. The Seeds were removed from water and seeds were transplanted in each pot. Plants were watered as and when required and allowed to grow for about 21 days. Pot was observed regularly and after plant harvested the length of shoot and root after every seven days with the help of ruler were noted down.

RESULT AND DISCUSSION

In the present study, 10 *Azotobacter* species were isolated and were studied for their morphological, biochemical characteristics and enzymatic activities.

Morphological characteristics

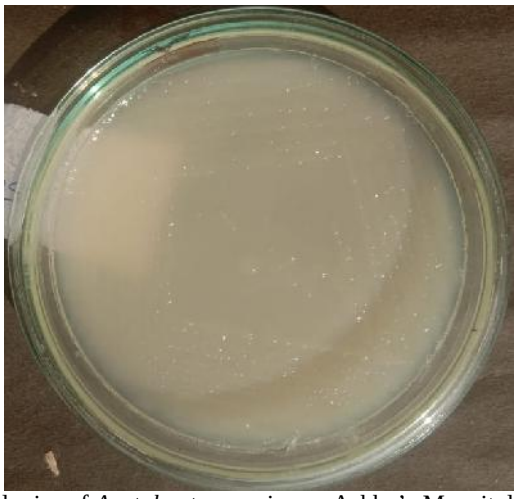

FIGURE 1: Colonies of *Azotobacter* species on Ashby's Mannitol salt agar plate.

All the isolates were found with small, round, convex and mucoid colonies with an entire margin, when streaked on fresh same Ashby's mannitol agar plate (Bergey's Manual of Determinative Bacteriology). The *Azotobacter* isolates were microscopically studied by performing Gram staining and special staining i.e. capsule staining (Figure-1). All the isolates were gram negative short rods, capsule former and motile. The similar findings were also obtained by Akhter *et al*. (2012) [9] and Gomare *et al*. (2013) [8].

Enzymatic properties:

All the isolates were further studied for their enzymatic activity (Table 1). In the present study, all the isolates were found positive for nitrate reductase test, phosphatase test and catalase test. In 2015, Nawadkar *et al* has also reported the similar results[6].

FIGURE 2: Nitrate reduction properties of *Azotobacter* isolates

TABLE 1: Enzymatic properties of Isolates

Enzyme Assay	Isolates									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Catalase test	+	+	+	+	+	+	+	+	+	+
Nitrate reduction Test	+	+	+	+	+	+	+	+	+	+
Phosphate solubilizing Test	+	+	+	+	+	+	+	+	+	+

Seed germination assay

FIGURE 3: The root and shoot growth of the seed bacterized with isolates

TABLE 2: Wheat Root and shoot length after 7 days of incubation

Isolates	Shoot length	Root Length (cm)
Control	2.3	1.3
S1	4.5	2.6
S2	4.3	2.6
S3	4.8	2.7
S4	3.1	2.5
S5	3.2	2.4
S6	2.9	2.3
S7	2.9	2.1
S8	3.5	2.4
S9	2.4	2.2
S10	3.5	2.5

The seed germination, performed with wheat seed, results were obtained after 7 days incubation. The maximum length of shoot obtained was 4.8 cm and maximum length of root obtained was 2.7 cm which was obtained by S3 isolates (Table 2).

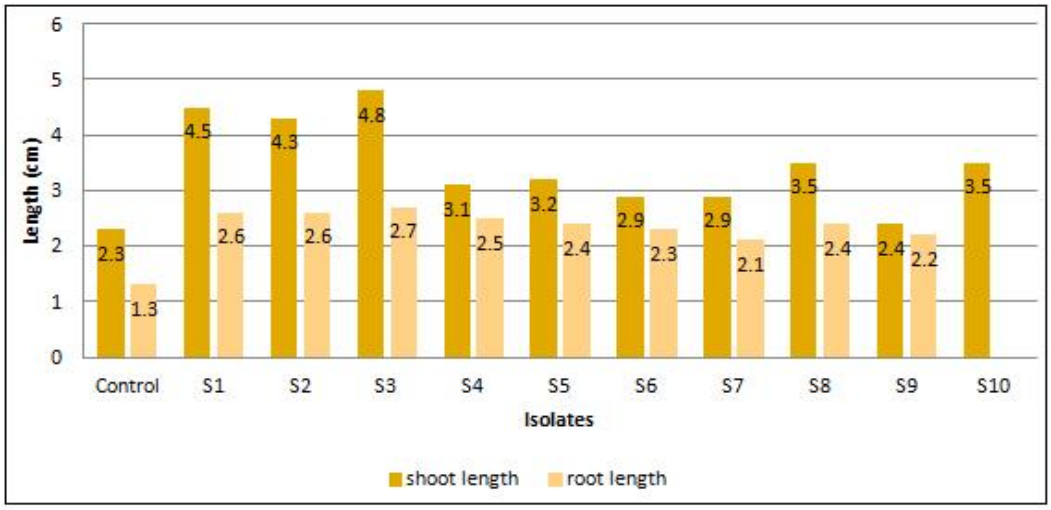

Figure 4: Root and shoot elongation in wheat seed after 7 days of incubation.

Seed germination experiment was performed by the process mentioned by Rodge *et al.* (2016)[7]. In the present study, control which is devoid of any culture, all the seeds bacterized by the isolates had flourished very well. However comparing among the bacterized seeds, maximum growth was observed in the seeds bacterized with S3, which shows its ability to act as biofertilizer**.** Barley is a suitable cereal for study because the processes involved in germination have been studied in detail. Rhizospheric microorganism, particularly beneficial bacteria can improve plant performances under environmental stress and consequently enhance the yield[10]. The major limitation to a more widespread use of seed inoculation has generally been the variability in effects in both field and laboratory studies [11,12].

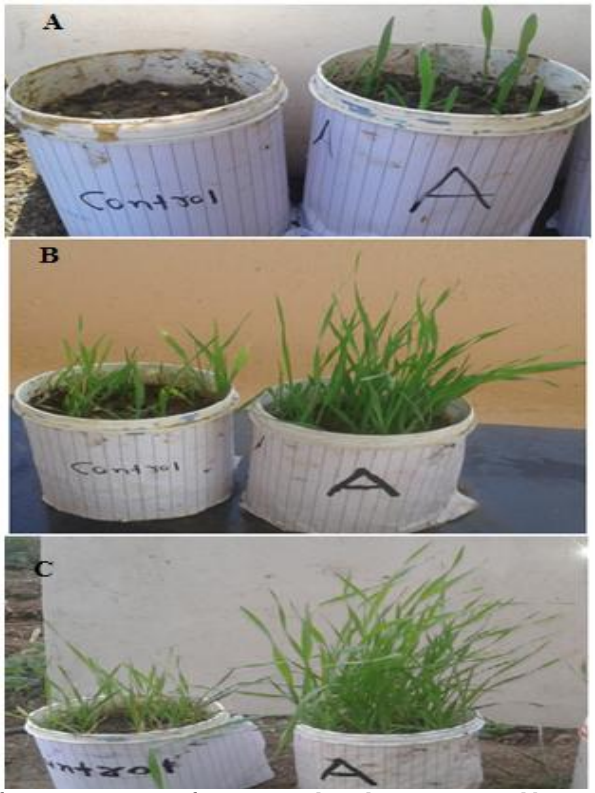

FIGURE 5: Results of pot experiments after 7, 14 and 21 days represented by A, B and C respectively.

TABLE 3: Results of Pot experiments

DAYS	7 DAYS		14 DAYS		21 DAYS	
	Shoot length	Root length	Shoot length	Root Length	shoot Length	Root Length

	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)
Control	1	1	10.3	10.1	13.1	12.1
Liquid Biofertilizer (laboratory)	4.9	5	26.5	27.2	35.6	30.1

The effect of *Azotobacter* species on wheat plant was observed and the high ratio of the biofertilizer gave good result. In 2013, Gomare *et al*. has also reported that economical and better plant growth production is better with the use of liquid biofertilizer of *Azotobacter* [8].

CONCLUSION

In the present study, *Azotobacter* species were isolated from the Rhizospheric region of South Gujarat and were found gram negative rods, motile, capsules formers and were found to have catalase, phosphate solubilizing and nitrate reductase enzymatic activities. The current study reveals that the inoculation of the *Azotobacter* species potentially increase the root and shoot length of the seedling by performing the seed germination assay. The *Azotobacter* species can serve as a potential source of Biofertilizer that offers an environmentally sustainable approach to boost crop yield. Liquid Biofertilizer serves as a better supplement and can improve the growth and yield of crops.

REFERENCES

[1]. Selvakumar, G., Lenin, M., Thamizhiniyan, P. & Ravimycin, T. (2009) Response of biofertilizer, on the growth and yield of blackgram (*Vigna mungo* L.) *Recent Research in Science and Technology*, 1(4).

[2]. Sartaj, A.W., Subhash, C. and Tahir, A. (2013) Potential Use of *Azotobacter chroococcum* in Crop Production: An Overview. *Current Agriculture Research Journal*. 1(1), 35-38.

[3]. Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I. (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol. 60: 579-598.

[4]. Farajzadeh, D., Yakhchali, B., Aliasgharzad, N., Sokhandan- Bashir, N. and Farajzadeh, M. (2012) Plant growth promoting characterization of indigenous Azotobacteria Isolated from Soils in Iran. Curr Microbiol. 64:397403.

[5]. Islam, M.Z., Sharif, D.I. and Hossain, M.A. (2008) A comparative study of Azotobacter spp. from different soil samples. J. Soil. Nature, 2(3): 16-19.

[6]. Nawadkar, R.B., Jadhav, D.B., Shaikh, N.R. (2015) Isolation of *Azotobacter* spp from saline soil and its applications on wheat (*Tritium aestivum*) plant for use in reclamation of saline soil with wheat plant. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 1(1), 62-73.

[7]. Rodge, S.P., Sable, S.K., Salve, S.K., Sawant, S.A. & Patil, N.P. (2016) Isolation and Characterization of PGPR from Roots of Ficus religiosa growing on Concrete Walls and its Effect on Plant Growth in Drought Condition. *International Journal Current Microbiol. Applied Science*, *5*(9), 583-593.

[8]. Gomare, K.S., Mese, M., Shetkar Y. (2013) Isolation of Azotobacter and cost effective production of biofertilizer. Indian J. Appl. Res., 3(5): 54 56.

[9]. Akhter, M.S., Hossain, S.J., Amir-Hossain, S.K. & Datta, R.K. (2012) Isolation and characterization of salinity tolerant *Azotobacter* sp. *Greener J Biol*ogical *Science*, *2*, 42-051.

[10]. Dimkpa, C., Weinand, T. & Asch, F. (2009) Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant cell & environment*, 32(12), 1682-1694.

[11]. Mishustin, E.H. & Shilnikova, U.K. (1969) Free-living nitrogen-fixing bacteria of the genus *Azotobacter*. In Soil Biology, pp. 72-1 09, Paris: U.N.E.S.C.O.

[12]. Brown, M.E. (1974) Seed and root bacterization. *Annual Review of Phytopathology*, *12*(1), 181-197.