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Selection of Efficient EPS Producing and Cyst Forming *Azotobacter* Isolates under Stress Condition

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Abstract: ***Azotobacters are unique biofertilizers to maintain the N level in agricultural soil and synthesize the plant growth promoting hormones as indole acetic acid and gibberellins. Azotobacter EPS in the soil habitat play key roles in ecosystem functioning through controlling nutrient cycling reactions essential for maintaining soil fertility and also contributing to the genesis and maintenance of soil structure under conventional, biotic, and abiotic stressed soil environment. In the present investigation, attempts have been made to isolate efficient strains of Azotobacter from different locations of Tamil Nadu for the production of exopolysaccharide and cyst under induced stress conditions. Based on exopolysaccharide production, three efficient Azotobacter isolates viz., AztRMD2 (1.755 g/50ml), AztPMK1 (1.56 g/50ml) and AztPMK2 (1.353 g/50ml) were selected for studying the cyst formation. The effect of limiting conditions of specific single micronutrient, combination of two micronutrients, reduced sucrose concentrations and addition of n- butanol as a carbon source on the induction of cyst formation was evaluated. The AztRMD2 isolate showed maximum cyst formation of around 50 - 85 % with elimination of magnesium from the growth medium, followed by AztPMK1. The results indicated that AztRMD2 can withstand upto 4% NaCl concentration whereas maximum polysaccharide production was noticed in the broth containing 0.02% of NaCl concentration. For artificial soil aggregation, AztRMD2 showed maximum pore space percentage (52.5%) and minimum bulk density (1.12g/cc). The aggregation stability (45stable aggregates) was also high in this isolate compared to Control. The results indicated that AztRMD2 performed better than the standard strains.***

Keywords: *Azotobacter*, exopolysaccharide, cyst, salinity tolerance, soil aggregation

1. Introduction

Nowadays, microbial polysaccharides are of great interest due to its wide industrial application and lack of structural complexity without extensive branching. The production of polysaccharides by microorganisms was first reported in 1880s. Applications of exopolysaccharides (EPS) are of continuing interest and have found a good commercial market in food and health care industry. The viscous exopolysaccharides produced by *Azotobacter* is well characterized (Gauri *et al*., 2009). *Azotobacter* is a free living, asymbiotic nitrogen fixer and mostly abundant in plant rhizosphere and phyllosphere region. The EPS of *Azotobacter* are copolyuronans ubiquitous nature of alginates (Elsayed *et al.*, 2013) commercially used in plant tissue culture to produce insoluble artificial seeds, immobilizing enzymes by entrapment, as food and wound dressing substances. Thus the industrial application of alginate is well understood (Hay *et al*., 2010). Aside from industrial application, *Azotobacter* alginate plays a key role in encystment, to provide protection against desiccation and predation by protozoa or phage attack (Hynes *et al*., 2008), or affect the penetration of antimicrobial agents and toxic metals (Aleem *et al*., 2003). This polysaccharide also protects nitrogenase against high oxygen concentration (Bhattacharyya and Jha, 2012) and also participates in interaction between plants and bacteria (Mandal *et al*., 2008).

The role of alginate like EPS production in natural environments by *Azotobacter* is not well understood but the structural integrity and route of biosynthesis have been well defined. There are few recent findings providing new insights into biofilm conditions, the environmental factors and novel regulatory mechanisms controlling EPS production that maintains their life cycle strategies (Segura *et al*., 2003). EPS is more effective in binding or aggregating the soil. It also reduces the bulk density of Greenfield sandy loam and increased hydraulic conductivity in neutral soil. Moreover, the polysaccharide rich soil provides a cementing action to increase stability for soil aggregation (Flouri *et al.*, 1990). Soil aggregate stability increases biomass and decomposition of organic materials by PGPRs (Ahemad and Khan, 2012c; Tank and Saraf, 2010). This increased soil porosity and reduced bulk density allows retention and exchange of air and water. This pore space provides zones of weakness through which plant roots can grow easily.

This study will help to investigate the role of exopolysaccharide in sustainable agricultural system and also to the survival in its own environments. Hence, the present study have been made to isolate efficient EPS producing and cyst forming *Azotobacter* from different locations of Tamil Nadu for better performance under different concentration of NaCl on the growth and exopolysaccharide production.

2. Materials and Methods

All the laboratory experiments were conducted at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore. All the enzymes and primers used in molecular studies were obtained from M/s. Bangalore Genei(P) Ltd., Bangalore and Sigma Aldrich, USA. The Analytical Reagent Grade (AR) chemicals were obtained from M/s. Himedia, M/s. Qualigens, M/s. Merck, M/s. BDH and M/s. Sigma and were used for media preparation and biochemical studies.The standard strain of *Azotobacter chroococcum* (Ac1) maintained at the Biofertilizer Production and Quality Control Laboratory, TNAU and the standard strain of *Azotobacter venilandii* (MTCC2460) was obtained from Microbial Type Culture Collection and Gene Bank, India were used in the study.

2.1 Collection of soil sample

The rhizosphere soil samples were collected from different rice growing places such as Paramakudi, Madurai, Ramnad and different region of Kanyakumari and Nilgiri districts. Rhizosphere soil samples were collected carefully by uprooting the root system and placed in a cool box for transport and stored at 4° C.

2.2 Isolation and enumeration of *Azotobacter* from rhizosphere soil.

Azotobacter was enumerated and isolated by following pour plate method (Allen, 1953). The Waksman No 77 medium was used for enumeration and isolation of *Azotobacter*.

One gram of soil from each sample was aseptically weighed, transferred to 100ml sterile water blank and shaken (120 rpm) form 0 min to get 10^{-2} dilution. After thorough shaking, one ml of diluents from 10^{-2} was transferred to 9 ml water blank to get 10^{-3} dilution. Likewise the sample was diluted serially with 9 ml water blanks until the appropriate dilution was obtained. Aliquots (1ml) from the serially diluted samples (10^{-3} to 10^{-6}) were added to N-free media in Petri plates and kept in an incubator at 30°C for isolation and enumeration.

The selected isolates were subjected to a set of morphological and biochemical tests for the purpose of identification. The cellular morphology like shape and cell arrangement, Motility test, Gram staining, colony characters *viz.*, colour, elevation, shape were observed under microscope and pigment production, polysaccharide production in Waksman No 77 media were recorded. The biochemical tests *viz.*, catalase test, Oxidase, Indole formation test, Starch hydrolysis test, Gelation liquefaction was carried out for identification of genus.

2.3 Cell dry weight determination (Jarman *et al*.,1978**)**

A mixture of culture **(40** ml), ***5*** M-NaCI **(0.8** ml) and ***0-5*** M Na,EDTA **(0.8** ml) was centrifuged at 24000 g for 40 min. The supernatant obtained was decanted, and the sediment was resuspended in distilled water and centrifuged at 24000g for 20 min. The final sediment was transferred to a preweighed porcelain dish and dried to constant weight at 105 ° ***C.***

2.4 Polysaccharide determination (Jarman *et al*.,1978**)**

The supernatant (25 ml) obtained from the first centrifugation described above was added to propan-2-01 (75 ml) and the mixture was shaken vigorously. After 10 min, the precipitate obtained was filtered on to a predried and preweighed Whatman **GF/A** filter disc and washed with 100 ml of propan-2-ol/water (3 : 1, vlv). The filter disc plus precipitate was dried under vacuum at 45 °C for 24 h, reweighed and the concentration of polysaccharide in the culture broth was calculated.

2.5. Studies on cyst formation by *Azotobacter* (Socolofsky and Wyss, 1962)

2.5.1. Induction of cyst formation under specific micronutrient limiting conditions

The effect of omissions of specific micronutrient such as calcium, iron, magnesium, and molybdenum from the growth medium on the induction of cyst formation was studied. *Azotobacter* was grown in four sets of flasks, each of which contained 50 ml of Burk's nitrogen free medium normal in every respect except that one of the micronutrients mentioned above was omitted from the growth medium in each sets of flasks. As a Control, *Azotobacter* was grown in a fifth sets of flasks containing medium from which none of the minerals had been omitted. In Burk's nitrogen free medium the micro nutrients such as calcium was added in the form of calcium chloride at 0.0025 g per 50 ml of medium, iron in the form of ferric sulfate at 0.00043 g per 50 ml of medium, magnesium in the form of magnesium sulfate at 0.01 g per 50 ml of medium, and molybdenum in the form of sodium molybdate at 0.00043 g per 50 ml of medium. All media were made with triple glass-distilled, deionized water and sterilized by filtration to eliminate precipitation of any minerals due to heat. Each flask was incubated on a rotary shaker at 30° C for 18 to 24 hr. A sample was then taken from each flask and diluted 1:1,000 with distilled water. One drop of a saturated alcoholic solution of methyl violet 6-B was then added to 10 ml of the diluted sample. The preparation was gently heated, allowed to cool, and then a sample was introduced into the chambers of a standard hemocytometer. The total number of cells was determined by the conventional counting technique with this counting chamber. The number of cysts was then determined, and the percentage of cysts in each of the flasks was calculated. Counts were made in triplicate.

2.5.2. Induction of cyst formation under limiting condition of combination of two micronutrients

The effect of elimination of combination of two micronutrients on cyst formation was also studied. The procedure described above was followed, except that two micronutrients were not added to the medium in each sets of flask as designated below. A set of flasks containing medium from which none of the micronutrient had been omitted again served as Controls. All flasks were incubated on a rotary shaker at 30° C for 18 to 24 hr. Triplicate samples were then taken from each of the flasks and counted as described earlier in section

2.5.3. Induction of cyst formation under reduced concentration of sucrose in the growth medium

Azotobacter was grown in three sets of flasks each containing 50 ml of Burk's nitrogen free medium. Three different concentration of sucrose *viz*., 0.5 % (normal concentration) and two reduced concentrations viz., 0.1% and 0.05 % were maintained in each set of flaks. The normal 0.5% sucrose served as Controls. The flasks were incubated at 30° C on a rotary shaker, and samples were taken and described earlier in section 3.9.1.

2.5.4. Induction of cyst formation with n-butanol as carbon source

Attempts were also made in this study to induce cyst formation in liquid Burk's medium containing either n - butanol or n – butanol agar extract as the carbon source. The butanol-agar extract was prepared by mixing butanol and agar and then heating the mixture. The butanol was then separated by filtration. Burk's medium containing 0.3 % of either n-butanol or the n- butanol agar extract as the carbon source, in the place of sucrose, was then inoculated with *Azotobacter*. The cultures were incubated at 30 °C for periods ranging from 18 hr to 72hours. At the end of the incubation period, samlples were taken from each flask and the percentage of cysts was determined as described earlier in section 3.9.1.

2.6 Salt tolerance

Waksman No 77 broth containing different concentration of NaCl viz. 0%, 2%, 4%, 6% and 10% were inoculated and incubated at 28 ºC for 48 hours. The growth of isolates at different concentrations of NaCl was then compared with the Control.

2.7 Study of artificial soil aggregates

Artificial aggregates was produced by the method described by Ramasawmy *et al*. (1992). One hundred gram of soil sample was taken and mixed with 33 ml culture filtrate of *Azotobacter* isolates.

2.7.1. Determination of bulk density and pore space

Twenty gram of soil was weighed and inoculated with the cell free extract of Azotobacter isolates, incubated for 3 days and air dried for 2 days. The soil was transferred to a 100 ml measuring cylinder with glass stopper and tapped gently (Muthuvel and Udhayasoorian, 1997). The volume was noted after completely transferring the soil in to the cylinder.Known volume of water (50 ml) was added along the sides of the cylinder using pipette till the entire soil was completely soaked. At least 5 ml of water should above the soil surface after the addition of water and the level should be below 100 ml mark.The cylinder with soil and water was kept in undisturbed condition for 30 minutes. So the entire pore space was completely filled with water.

2.7.2. Determination of aggregation stability (Ramasawmy *et al*.1992)

One hundred gram of soil was taken and mixed with 33 ml of culture filtrate, and shaken in bottom plate of the Petri dish to allow aggregate formation.Uniform sized aggregates were selected and placed at 1cm interval on circles drawn in 40 cm dia Whatman No. 3 filter paper. This setup was placed on 'Z' glass rod support with absorbant cotton padding, after assembling water was added to the bottom plate and allowed to ascent on to filter paper. Saturation was reached in an hour. Such a setup was covered with the top lid of the Petri plate and allowed to saturate for further 24 hr. The rates of disintegration were observed and recorded. The data generated from the experiment were subjected to statistical analysis as suggested by Panse and Sukhatme (1976) using AGRESS software package

3. Results and Discussion

Plant associated nitrogen-fixing bacteria have been considered as one of the possible alternatives for inorganic nitrogen fertilizer for promoting plant growth and yield. The variety of nitrogen fixing bacteria have been isolated from the rhizosphere region of various crop plants. In the present study, attempts were made to isolate the *Azotobacter* from the rhizosphere soil samples of rice. The isolates were purified and characterized. Selected isolates were screened for polysaccharide production, cyst formation, ability to form soil aggregates. The major findings of these experiments are presented below.

3.1 Exopolysaccharide production by *Azotobacter* isolates

Many polysaccharides produced by bacteria have characteristic properties in rheology and physiological activity different from natural gums and synthetic polymers. *Azotobacter sp* is more suitable for the production of exopolysaccharide in view of its latent utilization as a food stabilizer. For these reasons, some bacterial polysaccharides are produced on industrial scales and used as raw materials for food processing, medical and industrial preparations (Elsayed, 2013 ; Lee *et al*., 2012). In this present study, ten isolates were selected based on production of exopolysaccharide, pigment production and biochemical characters.

The bacterial isolates such as AztPMK1, AztPu1, AztPMK2, AztPa, AztMDU1, AztKa, AztMDU2, AztMDUT1, AztRMD1, AztRMD2 were selected and the production of exopolysaccharide was compared with the standard cultures, AztTNAU (*Azotobacter chroococchum*) and AztMTCC 2460 (*Azotobacter venilandii*). The isolate AztRMD2 recorded maximum polysaccharide production in Waksman No 77 broth. Preliminary experiments were performed in order to determine the incubation time for optimum recovery of exopolysaccharides in *Azotobacter* cultures. The result was in accordance with the Lozano *et al*. (2011). Thus, culture media samples were removed at intervals and quantitative extractions of EPS were made. According to these results maximum EPS recovery could be obtained in 5-day-old cultures. Hence the incubation period was standardized as five days for EPS extraction and quantification in further experiments. The yield of exopolysaccharide production is negatively correlated with biomass production and positively correlated with carbon source consumption. The yield of exopolysaccharide increases when the biomass production decreases (figure 1). And also the specific yields of EPS production were higher as the initial concentration of substrates increased (Hay *et al*., 2010).

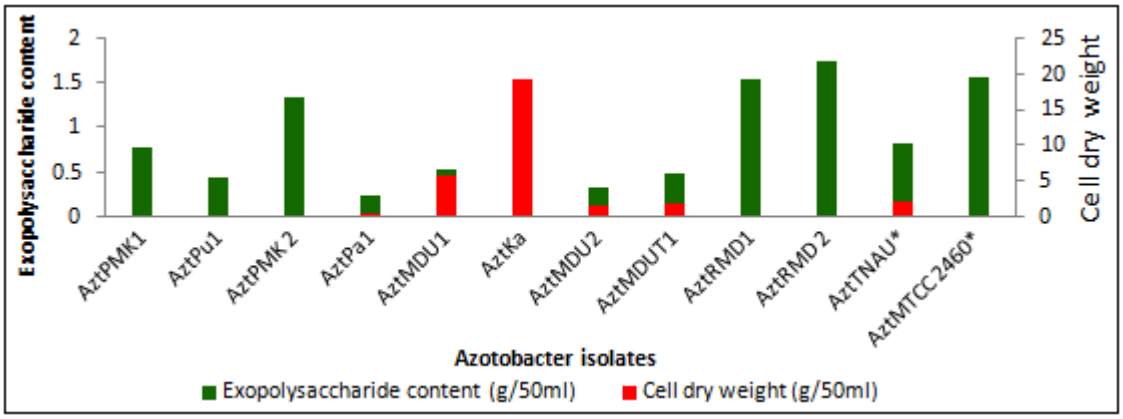

Figure 1: Cell dry weight and exopolysaccharide production of *Azotobacter* isolates after 5 days of growth in Waksman No 77 broth

3.2. Factors influencing cyst formation in *Azotobacter* isolates

Segura (2003) found that cyst formation can be induced in *Azotobacter* by addition of 0.3% n-butanol as the carbon source to the medium on which the organisms are grown. However, they were unable to induce cyst formation in liquid Burk's medium with sucrose as the carbon source. Thus, the view that cyst formation in *Azotobacter* does not occur in media containing sucrose as the carbon source has gained widespread acceptance in recent years.

The present study however shows this view to be untenable, since cyst formation was induced in liquid Burk's medium, with sucrose as the carbon source, by elimination of one or more of the micronutrients normally present in the medium. In the resent study, elimination of combination of two micronutrients from the medium was found to bring about much greater increase in the number of cysts formed than elimination of a specific micronutrient. Thus, the effects of micronutrients deficiencies in inducing cyst formation seem to be additive (figure 2 & 3). Another interesting observation is that the number of cysts formed in response to elimination of combination of two micronutrients is very nearly the same, no matter what two micronutrients are eliminated. This may indicate that one or more of the micronutrients are able to substitute for one another.

During the course of this study, it was also shown that cyst formation can be induced by reduction of the sucrose content (Fig 4) of the medium in which the organisms are grown and, as was already shown (Gauri *et al*., 2011), incorporation of 0.3% n-butanol as the carbon source into the solidified Burk's medium on which the organisms are grown also induces cyst formation (Fig 5). Thus, it would seem that, the *Azotobacter* cyst may represent a survival state formed in response to a variety of detrimental changes in the environment. It would further seem that induction of cyst formation in response to mineral deficiencies, as was accomplished in the present study, would come closer to representing the mechanisms controlling cyst formation in nature than would cyst formation in response to the presence of such compounds as butanol in the environment.

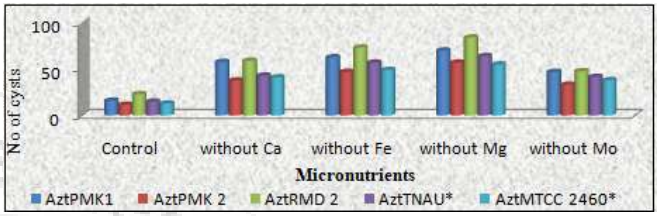

Figure 2: Effect of elimination of specific micronutrient from Burk's nitrogen free broth on cyst formation by *Azotobacter* isolates

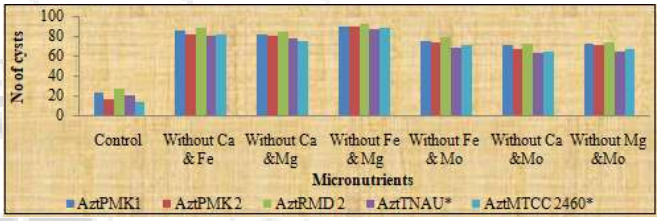

Figure 3: Effect of elimination of combination of two minerals from Burk's nitrogen free broth on cyst formation by *Azotobacter* isolates

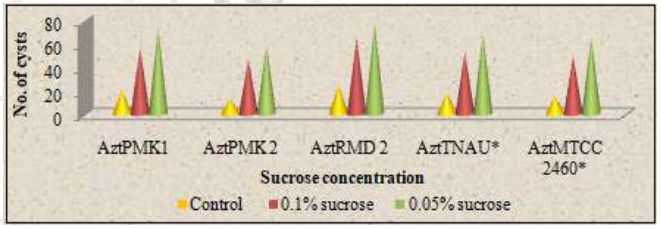

Figure 4: Effect of reduced concentration of sucrose in Burk's nitrogen free broth on the cyst formation by *Azotobacter* isolates

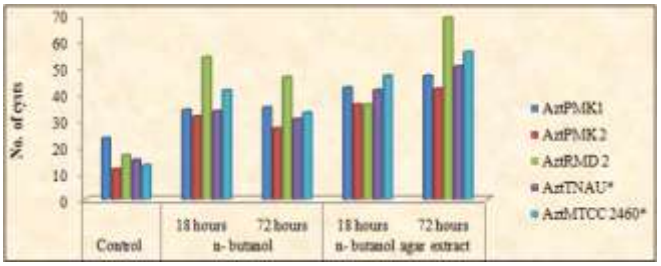

Figure 5: Effect of n- butanol and n- butanol agar extract on the induction of cyst formation in *Azotobacter* isolates

3.4 Effect of salt concentration on the production of exopolysaccharide

Salt stress affects both bacteria and plants in two ways: it induces ionic stress due to the high concentration of ions and also osmotic stress through the change in the solute concentration around the cells, producing water deficit and desiccation. Salt stress may inhibit the initial steps of the symbiosis (nodule initiation, nodule infection, and development) but it also has a depressive effect on nitrogen fixation (Yao, 2010). *Azotobacter* subjected to salt stress may undergo morphological alterations, leading to changes in cell morphology and size or modifications in the pattern of extracellular polysaccharides (EPS) (Vanderlinde *et al*., 2010) and lipopolysaccharides (LPS) (Sousi *et al*., 2001; Vanderlinde *et al*., 2009). In the present study, exopolysaccharide production was determined in different salt concentration (Table1). Some strains of *Azotobacter* are able to grow at NaCl concentrations as high as 4 % and others cannot grow, when NaCl concentration is above 6%. The result was in accordance with the Kohler and Caravaca, (2010).

Table 1: Effect of different concentration of NaCl on the growth of Azotobacter isolates

Azotobacter Isolates	NaCl concentration	Time intervals (Hours) OD $_{600}$nm				
		6	12	24	48	72
AztRMD2	C	0.927	1.181	2.618	2.736	2.869
	0%	0.378	0.701	1.969	2.145	2.606
	2%	0.495	0.504	0.629	2.294	2.869
	4%	0.194	0.231	0.342	0.629	1.247
	6%	0.109	-	-	-	-
	10%	-	-	-	-	-
AztTNAU*	C	0.715	1.987	2.335	2.408	2.655
	0%	0.578	1.663	1.806	2.377	2.573
	2%	0.043	0.579	0.582	0.665	0.867
	4%	-	-	-	-	-
	6%	-	-	-	-	-
	10%	-	-	-	-	-
AztMTCC 2460*	C	0.766	1.751	2.225	2.634	2.756
	0%	0.521	1.677	2.261	2.440	2.598
	2%	0.463	0.890	0.921	1.843	1.936
	4%	-	-	-	-	-
	6%	-	-	-	-	-
	10%	-	-	-	-	-

During the course of this study it was also shown that the polysaccharide production was minimum in the 4 % salt concentration (Table 2). It is mainly due to the loss of intracellular water, which imposes a water deficit because of osmotic effects on a wide variety of metabolic activities (Fatnassi *et al.*, 2011). And the maximum polysaccharide production was noticed in the mdium containing NaCl in the normal concentration. These results are in conformity with the findings of Alavi *et al*. (2013).

Table 2: Effect of different concentrations of NaCl on cell dry weight and exopolysaccharide production of Azotobacter isolates

Isolates	Cell dry weight (g/50ml)						Exopolysaccharide production (g/50ml)					
	NaCl Concentration (%)						Salt Concentration (%)					
	Control	0	2	4	6	10	**Control**	**0**	**2**	**4**	6	10
AztRMD2	0.04	0.169	0.192	1.147	1.146	0.115	3.00	2.07	0.51	0.49	-	-
AztTNAU*	0.247	0.213	0.033	0.050	1.045	0.828	1.57	0.36	0.49	-	-	-
AztMTCC 2460*	0.062	0.128	0.162	0.175	0.445	0.3	2.94	0.83	0.64	-	-	-

3.5. Studies on the role of *Azotobacter* on artificial soil aggregation:

3.5.1. Effect of *Azotobacter* isolates on pore space of rice field soil

Soil structure has a strong impact on a range of processes influencing crop yield. The basic units of soil structure, named aggregates, comprise solid material and pores. These aggregates determine the mechanical and physical properties of soil such as retention and movement of water, aeration, and temperature (Kohler and Caravaca, 2010). Aggregate formation is an important factor controlling germination and root growth.

Several studies have shown that formation of stable aggregates strongly depends on both the nature and the content of organic matter. Unstable aggregates generally have a lower content of organic matter than do stable ones. Plant roots contribute to soil organic material, and thereby to soil aggregate stability, directly through the root material itself and indirectly through stimulation of microbial activity in the rhizosphere. It is generally believed that microbial action on soil aggregation is due to the production of exopolysaccharide (EPS). This is supported by experimental observations demonstrating that the amendment of soil with microbial EPS results in an increased soil aggregation.

In the present investigation *Azotobacter* isolates were examined for improving aggregation of rice field soil. The results indicated a great influence of *Azotobacter* on soil physical conditions at varying degree of aggregation treatments. The improvement in the structure of treated soils was assessed by the measurements of soil porosity and bulk density. Among the isolates, AzRMD2 performed well recording higher pore space in soil. The pore space had increased in all the treatments, when compared to Control. In soil, pore space increased to 62.5% by inoculating AzRMD2 isolate over the Control. The pore space may be increased due to the aggregation by the influence of microorganisms. In a similar study, a characteristic improvement of macro pores (porosity, pore size distribution, pore continuity, stability of the pore system, resiliency), infiltration, drainage and aeration by inoculation of bacteria was observed by Freitas *et al*. (2011)

3.5.2. Effect of *Azotobacter* isolates on bulk density

The addition of organic matter to the soil improves the soil physical condition. The bulk density was reduced to 0.5g/cc with the inoculation of AzRMD2 isolate. Reduction of bulk density may be due to the addition of materials that would automatically decrease bulk density, because the added material is of lower density. Further, the development of good aggregate and structure by the binding substances produced by the added bacterium might have reduced the volume of soil solids and consequently the bulk density. These result confirm the findings of Maqubela *et al*.(2009), who observed a decrease in bulk density of soil on the addition of organic matter. This reduction might occur directly by dilution of the soil matrix with a less dense material or indirectly by the improvement of aggregate stability.

3.5.3. Stability of soil aggregates formed by *Azotobacter* isolates

In the present study the aggregate stability was higher (45 drops) in the soil, with the inoculation of AztRMD2 isolate (Table 3). This is confirmation with the result of Tikhonovich and Provorvo (2011),who found that addition of organic matter improved the aggregation stability. The exopolysaccharide from *Azotobacter* augment the aggregate stability. It is imperative that the artificially formed aggregates had more stability and will throw more information, if tested for crop production and soil characteristics than applied in soil.

Table 3: Effect of cell free extract of Azotobacter on pore space, bulk density and aggregate stability of rice soil

S. No	Treatments	Pore space %	Bulk dencity(g/cc)	Aggregate stability
1	Soil + AztRMD2	62.5	0.5	45 drops
2	Soil + AztTNAU	60.0	0.66	32 drops
3	Soil+ AztMTCC2460	50.0	0.86	27 drops
4	Soil + sterile water	37.0	1.3	19 drops

4. Conclusion

This study will help to investigate the role of exopolysaccharide in sustainable agricultural system and also to the survival in its own environments. Cyst of Azotobacter is of great interest for liquid biofertilizers production. liquid biofertilizers have some more advantages over than solid biofertilizers as cyst contains special nutrients that ensure longer shelf life, improved survival rate on seeds and soil, drought tolerance, very high enzymatic activity, contamination free and easy handling.

References

[1] Ahemad, M. and M.S. Khan. 2012c. Evaluation of plant growth promoting activities of rhizobacterium *Pseudomonas putida* under herbicide-stress. **Ann. Microbiol., 62:** 1531–1540.

[2] Ahemad, M. and M.S.Khan.2012a. Effect of fungicides on plant growth promoting activities of phosphate solubilizing *Pseudomonas putida* isolated from mustard (*Brassica campestris*) rhizosphere. **Chemosphere., 86:** 945–950.

[3] Ahemad, M., and M.S.Khan. 2010a. Influence of selective herbicides on plant growth promoting traits of phosphate solubilizing *Enterobacter asburiae* strain PS2. **Res. J. Microbiol., 5:** 849–857.

[4] Ahemad, M., and M.S.Khan.2010b. Plant growth promoting activities of phosphate-solubilizing *Enterobacter asburiae* as influenced by fungicides. **Eurasia. J. Biosci., 4:** 88–95.

[5] Ahemad, M., and M.S.Khan.2012e. Alleviation of fungicide-induced phytotoxicity in greengram [*Vigna radiata* (L.) Wilczek] using fungicide-tolerant and plant growth promoting *Pseudomonas* strain. **Saudi J. Biol. Sci., 19:** 451–459.

[6] Aleem, A., J. Isar and A. Malik.2003. Impact of long-term application of industrial wastewater on the emergence of resistance traits in *Azotobacter chroococcum* isolated from rhizospheric soil. **Bioresour. Technol., 86:**7–13.

[7] Alavi, P., M.R. Starcher, C. Zachow, H. Müller and G. Berg. 2013. **Root-microbe systems: the effect and mode of interaction of stress protecting agent (SPA) Stenotrophomonas rhizophila DSM14405^{T}.** ***Front. Plant Sci.*****, 4:**141.

[8] Bhattacharyya, P.N. and D.K. Jha. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. **World J. Microbiol. Biotechnol., 28:** 1327–1350.

[9] Chandler, D., G.Davidson, W.P. Grant, J. Greaves and G.M. Tatchell. 2008. Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. **Trends Food Sci. Tech., 19:** 275–283.

[10] Elsayed, N.S., M. Aboulwafa, K. Aboshanab and N. Hassouna. 2013. PHB production in *Azomonas*, *Acinteobacter* and *Bacillus* species: isolation, screening and identification. **Arch. Clin. Microbiol.,** In press**.**

[11] Fatnassi, I.C, S.H.Jebara and M. Jebara. 2011. Selection of symbiotically efficient and high salt-tolerant rhizobia strains by gamma irradiation. **Ann Microbiol., 61:** 291-297.

[12] Freitas, F., V.D. Alves and M.A.M. Reis. 2011. Advances in bacterial exopolysaccharides: from production to biotechnological applications. **Trends Biotechnol., 29:**388–398.

[13] Gauri, S.S., S. Archanaa, K.C. Mondal, B.R. Pati, S.M. Mandal and S. Dey. 2011. Removal of arsenic from

aqueous solution using pottery granules coated with cyst of *Azotobacter* and portland cement: characterization, kinetics and modeling. **Bioresour. Technol., 102:**6308–6312.

[14] Gauri, S.S., S.M.Mandal, K.C.Mondal, S. Dey and B.R. Pati. 2009. Enhanced production and partial characterization of an extracellular polysaccharide of an newly isolated Azotobacter SSB81. **Bioresour Technol., 100:** 4240–4243.

[15] Hay, I.D., Z.U. Rehman, A. Ghafoor and B.H.A. Rehm. 2010. Bacterial biosynthesis of alginates. **J. Chem. Technol. Biotechnol., 85:**752–759.

[16] Hynes,R.K., G.C.Leung, D.L. Hirkala and L.M. Nelson.2008. Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil and chickpea grown in Western Canada. **Can. J. Microbiol., 54:** 248–258.

[17] Kohler. J. and F. Caravaca. 2010. **An AM fungus and a PGPR intensify the adverse effects of salinity on the stability of rhizosphere soil aggregates of Lactuca sativa Roldan.** ***Soil Biol. Biochem*****., 42:**429-434.

[18] Lee,M.C., Y.C.Chen and T.C. Peng. 2012. Two-stage culture method for optimized polysaccharide production in *Spirulina platensis*. **J. Sci .Food Agric., 92:**1562–1569.

[19] Looijesteijn,P.J., L. Trapet, E.de-Vries, T. Abee and J. Hugenholtz. 2001. Physiological function of exopolysaccharides produced by *Lactococcus lactis*. **Int. J. Food Microbiol., 64:**71–80.

[20] Lozano, E., E. Galindo and C.F. Pena. 2011. Oxygen transfer rate during the production of alginate by *Azotobacter vinelandii* under oxygen limited and non oxygen-limited conditions. **Microb. Cell Factories., 10:**13.

[21] Mandal, S.M., B.R. Pati, A.K.Das and A.K. Ghosh. 2008. Characterization of a symbiotically effective *Rhizobium* resistant to arsenic: Isolated from the root nodules of *Vigna mungo* (L.) Hepper grown in an arsenic-contaminated field. **J. Gen. Appl. Microbiol., 54:**93–99.

[22] Maqubela,M.P., P.N.S.MnkenI, O.Malam issa, M.T.Pardo, and L.P.Dacqui. 2009. *Nostoc* cyanobacterial inoculation in South African agricultural soils enhances soil structure, fertility, and maize growth. **Plant Soil., 315:**79-92.

[23] Muthuvel, P. and C. Udayasooriyan. 1997. Determination of bulk density and pore space. In**: soil, water and chemical analysis**. Tamil Nadu Agricultural university, Coimbatore, pp.81-82.

[24] Ramasawmy, K., V.Gomathy and R. Subramanian.1992. Isolation and characterization of microbial products involved in stable soil aggregation. **In:** Final report submitted to ICAR, Adhoc Research Project Dept. of environmental sciences, TamilNadu Agrl. University, Coimbatore, India.p 115.

[25] Rehm, B.H.A. 2010. Bacterial polymers: biosynthesis, modifications and applications. **Nat Rev Microbiol 8:**578–592.

[26] Segura, D., J. Guzmaan and G. Espin.2003. *Azotobacter vinelandii* mutants that overproduce poly-beta-hydroxybutyrate or alginate. **Appl. Microbiol. Biotechnol., 63:**159–163

[27] Somers, E., J. Vanderleyden and M. Srinivasan. 2004. Rhizosphere bacterial signalling: a love parade beneath our feet. **Crit. Rev. Microbiol., 30:**205–240.

[28] Soussi, M., M. Santamaria, A. Ocana and C. Lluch. 2001. Effects of salinity on protein and lipopolysaccharide pattern in a salt-tolerant strain of *Mesorhizobium ciceri*. **J. Appl. Microbiol., 90:**476-481.

[29] Tank, N. and M. Saraf. 2010. Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. **J. Plant Interact., 5:** 51–58.

[30] Tikhonovich, I.A. and N.A. Provorvo. 2011. Microbiology is the basis of sustainable agriculture: an opinion. **Ann. Appl. Biol., 159:** 155–168.

[31] Vanderlinde, E.M., A. Muszynski, J.J. Harrison, S.F. Koval, D.L.Foreman, H. Ceri, E.L. Kannenberg, R.W. Carlson and C.K.Yost. 2009. *Rhizobium leguminosarum* biovar viciae 3841, deficient en 27-hydroxyoctacosanoate-modified lipopolysaccharide, is impaired in dessication tolerance, biofilm formation and motility. **Microbiol. SGM., 155:**3055-3069.

[32] Vanderlinde, E.M., J.J. Harrison, A. Muszynski, R.W. Carlson, R.J. Turner and C.K. Yost. 2010. Identification of a novel ABC-transporter required for desiccation tolerance and biofilm formation in *Rhizobium leguminosarum* bv. *viciae* 3841. FEMS **Microbiol. Ecol., 71:** 327–340.

[33] Yao. L., Z. Wu, Y. Zheng, I. Kaleem and C. Li. 2010. **Growth promotion and protection against salt stress by Pseudomonas putida Rs-198 on cotton.** ***European J. Soil Biol*****., 46:**49-54.

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