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Isolation, Biochemical Characterization and Inoculation Effect of Azospirillum on the Growth of Wheat

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Abstract: ***Azospirillum is a free living plant growth promoting bacteria (PGPB) which affects the growth and yield of crops. Inoculation of azospirillum results in reduction in need for nitrogen fertilization and obvious increase in productivity of the inoculated crop. In the present study the organisms were screened from the crop soils and biochemical characterization was carried out. The effect of the isolated organism was studied on the germination of inoculated wheat. Azospirillum is a PGPB has been known to increase the productivity in terms of grain yield content and the growth of plant. The growth is stimulated because the organism also produces growth hormones like auxin. To study the effect of hormones on seed germination the seeds were inoculated with the organism and were allowed to germinate in 0.8 % agar medium. Two times increase in germinated seed shoot length was observed. Pot culture study on wheat plant was also carried out which demonstrated significant increase in the shoot length, root length, fresh weight and dry weight.***

Keywords: Azospirillum, wheat, plant growth, root length, shoot length, microcosms

1. Introduction

The growth of plant is governed by the microbial community that resides in the rhizhosphere and non rhizosphere soil. Some of these microbes affect plant root morphology and physiology by producing plant growth-regulating hormones and enzymes (Lugtenberg and Dekkers, 1995). The "rhizosphere effect,"first described by Hiltner (1904), assumes that many microorganisms are attracted to nutrients exuded by plant roots. Hiltner observed that the number and activity of microorganisms increased in the vicinity of plant roots (Noshin Ilyas, 2009). Genus *Azospirillum* (K-subclass of proteobacteria) is known for many years as plant growth promoting rhizobacteria (Okon, 1994; Okon and Vanderleyden, 1997). The studies by De Smedt *et al*., (1980) indicate that azospirilla belong to the same rRNA superfamily as *Rhizobium, Aquaspirillum, Acetobacter, Xanthobacter and Agrobacterium. Azospirillum* is considered the most important rhizobacterial genus involved in improvement of plant growth or crop yield worldwide (Bashan *et al*., 2004). The present study aims at the morphological and biochemical characterization of the organism and the effect on seed inoculation and also the pot culture experiment.

2. Materials and Methods

2.1 Identification of Bacterial Isolates

Azospirillum medium with 0.17% agar (twin pack) M518 Hi media was used for isolation. Media with following ingredients in gm /L were used: Part A: Malic acid 5.0, Dipotassium phosphate 0.5, $FeSO_4$ 0.5, $MnSO_4$ 0.01, $MgSO_4$ 0.2, NaCl 0.1, Bromothymol blue 0.002, Na Molybdate 0.002, $CaCl_2$ 0.02, Agar 1.75. Part B: KOH 4.0, Final pH adjusted to 6.8± 0.2 and the medium was autoclaved at 121°C for 20 min.

Cultural characteristics were observed after incubation at 30°C for upto 8 days.

Azospirillum species occur as free-living in soil or in association with the roots of cereal crops, grasses and tuber plants (Bergey's Manual of Determinative Bacteriology, 1994). Azospirillum species are plant-associated diazotrophs of the alpha subclass of Proteobacteria . Azospirillum Medium with 0.17% Agar is used for cultivation of Azospirillum species.Malic acid is used as the carbon source. Azospirillum species grow well in presence of Malic acid and are not overgrown by other nitrogen fixers. Dipotassium phosphate provides buffering effect and other inorganic salt ingredients provide necessary growth nutrients. Agar at 0.17% concentrations provides microaerophillic conditions necessary for nitrogen fixation by Azospirillum species (Bergey's Manual of Determinative Bacteriology, 1994).

Single colonies appearing on these plates were transferred on agar slants for further study. The morphology of the colonies (color and shape) was noted after 24 hours. To study the cell motility and shape, single colony from the agar plates was transferred to a drop of sterile water on glass slide and observed under light microscope at 100 x magnifications. The cells were observed as plump, polymorphic from slightly curved to short rods. In unfavorable conditions, such as desiccation and nutrient limitation, members of genus *Azospirillum* can convert into enlarged cyst-like forms (Lamm and Neyra, 1981; Sadasivan and Neyra, 1987). This morphological change is accompanied by the development of an outer coat of polysaccharides and by the accumulation of abundant poly-L-hydroxybutyrate granules, which can serve as C and energy source under conditions of stress and starvation (Tal and Okon, 1985; Tal *et al*., 1990). Pot culture experiment was performed by sterilizing the soil and keeping 1 set of pot as control and other as test which contained the seeds bacterized with Azospirillum.

3. Results and Discussion

Biochemical characterization: Results of various tests are mentioned in table1.

Table 1: Morphological and Biochemical characterization of the isolate

S. No.	*Tests*	*Isolate*
1.	Colonial characters	Large,mucoid, convex with entire margin
2.	Pigmentation	Nil
3.	Microscopic characters	Plump rods with polymorphic cells from curved to straight
4.	Gram's staining	Gram Negative
5.	Glucose fermentation	Positive
6.	fructose fermentation	Positive
7.	Sucrose fermentation	Negative
8.	Xylose	Negative
9.	Maltose	Positive
10.	Dextrose	Positive
11.	Lactose	Positive
12.	Mannitol	Positive
13.	Indole production	Negative
14.	Voges-proskaeur test	Negative
15.	Citrate utilization	Positive
16.	H_2S production	Negative
17.	Urease production	Positive
18.	Catalase test	Positive
19.	Oxidase test	Positive
20.	Gelatin liquefaction	Negative
21.	Acid production	Positive

3.1 Effect of Azospirillum inoculation on plant growth

To study the effect of hormones on seed germination the seeds were inoculated with the organism and were allowed to germinate in 0.8 % agar medium. Plants being inoculated with *Azospirillum* are characterized by changes in root growth and morphology, such as enhancement of root elongation, root dry weight, promotion of root hair growth and root branching (Hadas and Okon 1987). Inoculation with *A. brasilense* was shown to improve the mineral uptake and the water status of wheat, maize and sorghum in the greenhouse and in the field (Okon and Kapulnik, 1986; Sarig *et al*. 1988). *Azospirillum* effects depend on inoculum concentration, plant species, timing of inoculation and environmental conditions (Okon and Kapulink, 1986). In general, the optimum concentration is of about 10^7 colony forming units (CFU) seed–1 (Okon and Vanderleyden 1997) although it varies for every plant species. Relatively high inoculum concentrations might cause the inhibition of root elongation (Okon and Kapulnik 1986) and plant growth (Burdman *et al*. 1997), and a reduction in fresh root weight (Vedder- Weiss *et al*. 1999). By soaking seeds in culture containing bacteria, the seeds are effectively inoculated and the interaction between young root and bacteria is established earlier as compared to other method of inoculation (Afzal and Asghari, 2008). Plants inoculated with *Azospirillum* exhibit enhanced root system with more lateral roots and enlarged root hairs, therefore occupying an enhanced soil volume (Bertrand *et al*., 2000).

Viable cell count method

Viable cell count was done for determination of bacterial growth. Decimal dilutions of isolated strains were made in sterilized distilled water. About 100µl from each dilution was dispensed on respective culture media agar plates. The cultures were incubated at 30^0C for 24 h and the colonies appearing on the plates were counted. The number of viable cells was calculated according to the formula given by James (1978):

Viable cell count (CFU/g) = (number of colonies× dilution factor/volume of inoculum)

3.2 Inoculation of wheat by Azospirillum isolates

Growth promoting effects of *Azospirillum* were studied on wheat. The pots were sterilized by soaking them in 10% Chlorox overnight followed by successive washing with sterilized water. Seeds were surface sterilized with $HgCl_2$ (0.1% w/v) for 5 min, rinsed with tap water, and successively washed several times with sterilized water (Liu *et al*., 2004). Seeds were soaked overnight in 7 d old cultures of *Azospirillum* isolates having OD 0.99 at 600nm and10^7 cfu/ml and were sown in pots (14x 14 cm^2) filled with sterilized soil. Pots were placed on a plastic sheet on ground in a green house. Three replicates were used for each treatment.

Following treatments were carried out in this experiment.

3.3 Treatments

Initially the soil was sterilized by fractional sterilization for 3 days. Control was the pot un-inoculated and kept well watered. Test (Inoculated with *Azospirillum*) and kept well watered. After one week plants were harvested and growth parameters (plant shoot length, root length, fresh and dry shoot weight, and fresh and dry root weight) were recorded for each case and compared. Root and shoot lengths were measured for the entire contents of pot by hand. Plants were harvested 45 days after sowing. The results obtained are as:

Table 2: Effect of Azospirillum inoculation in Microcosms experiment of wheat

Treatment	*Root System*				*Shoot System*			*%*
	Root length (cm)	*Fresh Weight (g)*	*Dry Weight (g)*	*% increase*	*Shoot length (cm)*	*Fresh Weight (g)*	*Dry Weight (g)*	*Increase*
Control	20	0.07	0.05	-	26	0.5	0.1	-
Test	22	0.18	0.07	40	32	0.9	0.2	100

4. Conclusion

The Azospirillum inoculated seeds of wheat demonstrated significant increase in the growth of plant. The root length of inoculated seeds was increased by 2 cm, shoot length increased by 6 cm. % increase in the root dry weights is 40% and shoot dry weight is 100% which is a significant value. The present study indicates positive effect of Azospirillum on growth of wheat. As multiple mechanisms are involved in host plant interaction, further studies are needed to clarify the accurate role of inoculants under study. The study will contribute to make agriculture practices more productive with less harm to the environment and thus help in sustainable agricultural development.

5. Future Scope

The organism appears to be promising candidate for the use as biofertilizer. After studying all optimization it may be utilized as biofertilizer as an individual or in consortium to improve the productivity of soil and also the soil health as its use may limit the need to use chemical fertilizers.

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Author Profile

Dr. Pragya Rathore is an energetic, ambitious and self motivated person. She has a mature and responsible approach to any task she undertakes. She can handle the situation that she is presented with, as she possesses problem solving skills. She has an enriched experience of 17 years of research and teaching with excellent track record. She has published about 25 papers in National & International journals and 3 books as a sole author to my credit.