ISOLATION AND IDENTIFICATION OF *PSEUDOMONAS AERUGINOSA* FROM *SCUTELLOSPORA ARENICOLA* AND ITS ASSOCIATION WITH HOST PLANT AS *ALLIUM CEPA*

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Abstract

The rhizosphere soil is important for the plant growth by regulating soil organic matter decomposition and through nutrient cycling. The rhizosphere soil contains mostly Gram-negative bacteria. The growth of plants is more efficient when spores with bacteria like organism (BLO) colonize the roots. Arbuscular mycorrhizal fungi along with BLO help in plant nourishment. The study shows the symbiotic relationship of spores with BLO in the host plant Allium cepa. The spores were identified as Scutellospora arenicola and BLO were identified as Pseudomonas aeruginosa through morphology characteristics. Using trap culture, symbiotic association of spores with BLO was indicated. It says that, the Pseudomonas aeruginosa along with Scutellospora arenicola shows high percentage of colonization than Scutellospora arenicola associated with host plant Allium cepa.

Keywords: *Allium cepa, Scutellospora arenicola**,** Pseudomonas aeruginosa**,** symbiotic association, arbuscular mycorrhizal fungi.*

1. INTRODUCTION

Mycorrhiza is a fungus root that describes the interdependent, a dependent relationship between fungi and also the plant roots. Most mycorrhiza are symbiotic i.e., the mycorrhiza replenish soil sources to plants while plant transpose for photosynthesis. Arbuscular mycorrhiza is a type of mycorrhiza, are the most epidemic fungal symbionts of plants that are being related with more than 80% of terrestrial land plants [1]. Mycorrhiza's are used for abounding reasons, but they are essential for plant production, due to the absorption of nutrients from the soil and transit into the roots [2]. Mycorrhiza gets connected with plant roots through their hyphal branching. Arbuscular mycorrhizal fungi accept plants through MYC factor, that yield by hyphae from germinating spore of mycorrhiza [3].·The role of mycorrhiza is prerequisite since the terrestrial plants are colonized with mycorrhizal fungi to transpose nutrients [4].· Mycorrhizal fungi are able to grow in Rhizospere soil and also in the roots of a plant. These fungi have arbuscules that are form inside the plant root that has many small projections into the root cell and in the hyphae outside the roots [5]· Most

of the plant grows in natural conditions that are of a symbiotic organism that ingest nutrients and water from the muddy that consist of a root and fungus tissue [6]. The symbiosis association between the arbuscular mycorrhizal fungi and plant roots were widespread among natural environment and they are favor to the plants in several ways by enhancing the nutrition, drought resistance, forbearance of heavy metals and effects in soil structure [7]. Arbuscular mycorrhizal fungi increase the supply of water and nutrients such as phosphate and nitrogen to the plant while plant deliver 20% of fixed carbon to the fungus. This type of nutrient intake carried out by the arbuscules [8]. Mycorrhizal fungi live in Rhizospere soil which is heir for plant growth, development, stress tolerance, food reliability and agriculture maintainable. Mycorrhizal fungi support a plant by spreading their hyphae network to enrinch the plant growth by nutrient intake [9]. Arbuscular mycorrhizas are widespread among rhizosphere soil that forms a symbiotic relationship between fungus-root which enhancement in an increase of plant phosphate, physiology and development of the host plant [10]. The biotrophic arbuscular mycorrhizal fungi in the root that colonize by carbon supply that reminiscent the mycorrhiza pertain directly with a root pathogen that has the unique tropical requirements [11]. Rhizospere serve as the hotspot for mycorrhiza that helps plant to prevent the roots from pathogens.The Mycorrhiza mainly divided into two major types: Ectomycorrhiza (outside the root), Endomycorrhizae (inside the root). Development of endomycorrhiza is by ectomycoorhiza through undeveloped intracellular hyphal structure [12]. BLOs are abbreviated as bacteria like organelles. The bacteria like organelles adhere to the hyphae of mycorrhizal fungi and enrich the plant growth. The fungal spores and the hyphae provide sites for the bacterial population. The bacteria like organelles (BLOs) ensue in spores, hyphae, and arbuscular protoplasm. There are three interpretations of bacteria like organelles (BLOs); they are temporary cytobionts that can constantly be acquired or lost by fungi; prokaryotic cytobionts that establish an obligate association with the host; permanent and non-prokaryotic characteristic certain fungi and are organelles of unknown origin and function [13]. The plant attaches to the arbuscular mycorrhizal fungi which increases the uptake of phosphate from the soil. The stages are; initiation of arbuscular mycorrhizal fungi (AMF); penetrating and inception of infection; prolong the infection in roots; retaliation of plants and mechanism of arbuscular mycorrhizal fungi; the carbon sinks, ease to the fungus; inequality in the symbiosis [14].

2.MATERIALS AND METHODS

Isolation of spores and bacteria

The debris were compiled from the soil of the root hairs and the spores were identified by wet sieving and decantation method and through the trap culture with *Allium cepa* as a host plant spores were collected [15]. Roots were compiled and cut into 2 to 3cm size and kept at 60ºC in 10% KOH solution for 10 min and washed with 0.2 N HCl. Then slides were prepared with trypan blue strain and viewed under a 40X microscope to know about the percentage assessment of spores.

Identification of bacteria associated with spores

Identification of BLO was performed based upon morphological, biochemical, molecular characteristics. The Gram staining, biochemical test such as IMViC, TSI, carbohydrate test, catalase, Oxidase, Indole and MR-VP was performed.

Isolation of DNA

Transfer 1.5 ml of the overnight culture to an Ependorff tube and centrifuge. Resuspend the cell pellet in 600 µl Lysis buffer and incubate it for an hour. Add an equal volume of Phenol and mix well by inverting the tube. Spin at maximum speed for 5 minutes at Room temperature. There is a White layer in

the aqueous. Carefully transfer the upper aqueous phase. To remove phenol, add an equal volume of Chloroform to the aqueous layer. Mix well by inverting the tube. Spin at maximum speed for 5 minutes. Remove the aqueous layer. To precipitate the DNA, Add 2.5 or 3 volumes of cold 200 proof Ethanol and mix gently. Incubate the tube at 20° C for 30 minutes. Spin at maximum speed for 15 minutes at 4° C. Cleanse the DNA pellet with 1 ml of 70% Ethanol. Spin at maximum speed for 2 minutes. Dry the DNA pellet at 37° C in Incubator. Resuspend DNA in TE buffer. The DNA is then run in agarose gel electrophoresis in 1% with ethium bromide.

Identification of bacteria by gene sequencing

The 16 s rRNA gene sequence was performed to determine the molecular characteristics. The PCR was performed using universal forward and reverse primer pair to amplify 16S rRNA gene. The end product was analyzed and observed through UV-transilluminator. To detect the phylogenetic analysis of the sequence BLAST tool is used.

Symbiotic association of *Scutellospora arenicola* in the presence of *Pseudomonas aeruginosa*

The host plant used is *Allium cepa* in trap culture. The three pots were made; *Scutellospora arenicola* associated with *Allium cepa*, *Scutellospora arenicola* with *Pseudomonas aeruginosa* associated with *Allium cepa* and soil with *Allium cepa.* Percentage of association was calculated by:

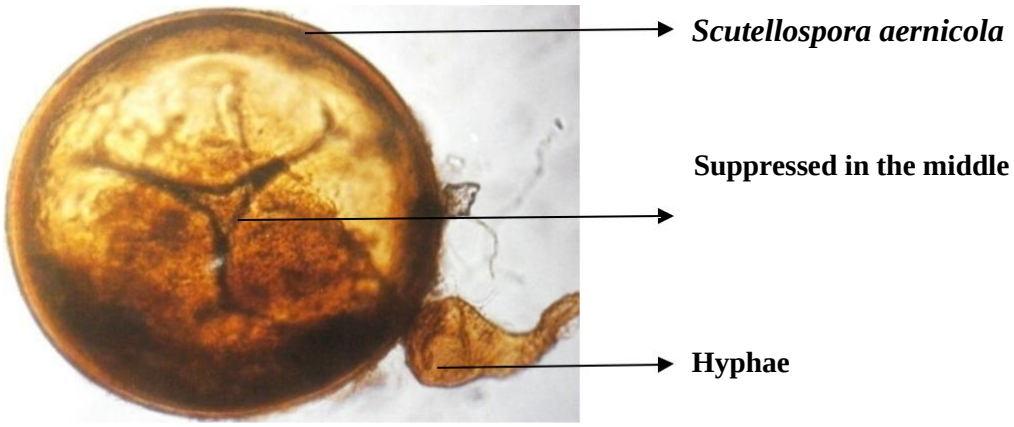
$$\frac{\text{Mycorrhiza associated with cell}}{\text{Total number of cells viewed through microscope}} \times 100$$

Statistical analysis using bar diagram

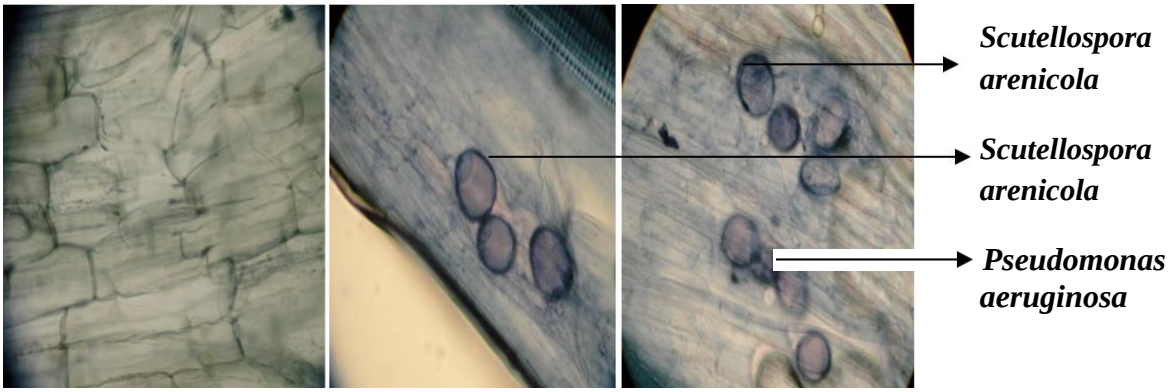
The statistical data of symbiotic association of spores with mycorrhiza in host plant were tabulated using bar diagram.

RESULTS AND DISCUSSION

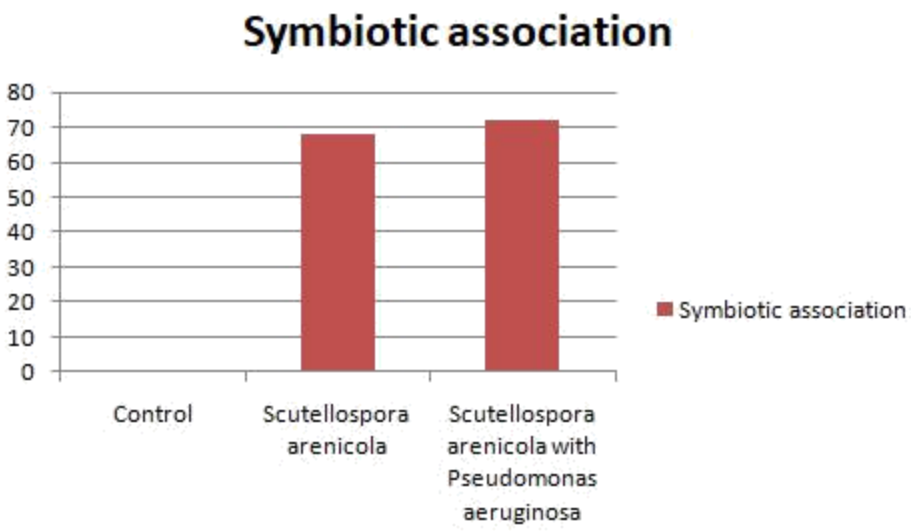
The BLO and the spores in rhizosphere soil was isolated by the wet sieving and decantation method and inoculated into the nutrient broth. The mycorrhizal colonization was identified by trap culture. The root sample from the trap culture was cut and stained [16] with tryphan blue [15]. Thus, the spores was identified as *Scutellospora arenicola* (fig-1) based upon the morphology [17], BLO was identified as *Pseudomonas* species based on colony, physical characterization, and biochemical test (Table-1) were performed [18]. The symbiotic association of the *Scutellospora arenicola* associated with *Allium cepa* shows an average of 68% while the *Scutellospora arenicola* with *Pseudomonas aeruginosa* that associated with *Allium cepa* shows a higher percentage of colonization of 72% (fig-2,3), [19],[20]. The *Pseudomonas sp.* was identified at molecular level through universal primer16S rRNA sequencing, 1500 length base pair (Fig-4),[21]. The NCBI sequence number is MN611376. The BLAST and phylogenetic tree confirmed the species as *Pseudomonas aeruginosa.* Comparatively, *Scutellospora arenicola* along with *Pseudomonas aeruginosa* shows a greater percentage of colonization than *Scutellospora arenicola* associated with *Allium cepa*. Thus, it helps in the fertility for the plant to grow.



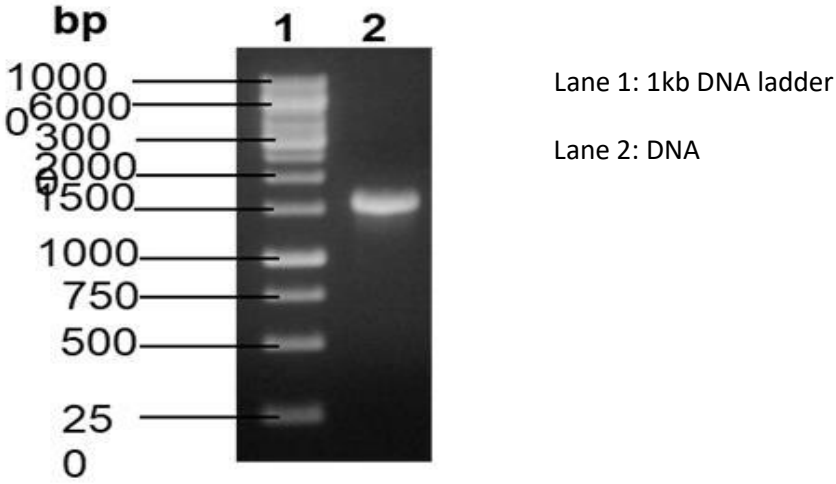
(Fig-1: *Scutellospora aernicola*)



(Fig-2 : symbiotic association of *Scutellospora arenicola* with *Pseudomonas aeruginosa* in *Allium cepa*)



(Fig-3:Bar diagram of association of spores with bacteria in *Allium cepa* as a host plant)



(Fig-4: PCR amplification of the 16S rRNA of the gene region)

TEST	RESULT
IMViC	
Indole	Negative
MR	Negative
VP	Negative
Citrate	Positive
TSI	

Butt	Negative
Slant	Negative
H_2S production	Negative
Gas production	Negative
Carbohydrate	
Glucose	Negative
Lactose	Negative
Maltose	Negative
Mannitol	Positive
Sucrose	Negative
Oxidase	Positive
Catalase	Positive

(Table-1: List of +, - of IMViC, TSI and Carbohydrates)

CONCLUSION

The rhizosphere soil that contains numerous organism which help in nourishment of plant. The *Pseudomonas aeruginosa* (BLO) along with *Scutellospora arenicola* in rhizosphere soil shows a greater symbiotic association in *Allium cepa* other than *Pseudomonas aeruginosa* (BLO) associated with host plant *Allium cepa*. *Scutellospora arenicola* enrich the phosphate in the soil and along with *Pseudomonas aeruginosa* (BLO), they help us to promote plant growth, promote the symbiotic association, helps in the nutrient enrichment and it prevents from soil erosion, draught resistance and biofertilizer. Mycorrhiza will be the best remedy for wasteland management. *Scutellospora arenicola* with *Pseudomonas aeruginosa* (BLO) and without *Pseudomonas aeruginosa* (BLO) has been studied and the symbiotic association was compared. In further field, the *Scutellospora arenicola* with *Pseudomonas aeruginosa* (BLO) helps in the agriculture by preventing the plants from pathogens, stress tolerance and increasing in the fertility of the soil.

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