STUDIES AND IDENTIFICATION OF OCHROBACTRUM INTERMEDIUM FROM SCUTELLOSPORA NIGRA BY PARTIAL SEQUENCING OF 16S rRNA GENE REGION

Premi. R [1], Jayashree.S *[1], Dr. Amutha K [2], sai shrudhi R [1], lincy D 1, Gajalakshmi. R.

[1] DEPARTMENT OF MICROBIOLOGY
SRI KRISHNA ARTS AND SCIENCE COLLEGE
Kuniyamuthur, Tamil Nadu 641008
[2] Department of Biotechnology, Vels university, Chennai

E.mail ID-*jayashreesis@gmail.com

ABSTRACT
Mycorrhizas are commonly found in rhizosphere soil. Mycorrhizae make plants increasingly safe against plant pathogens and infections. Mycorrhizae invigorate the plant creating hormone like cytokinins *intermedium* and its application in aiding plant growth along with *Scutellospora nigra* spores by holding *Allium cepa* as a host plant. Their growth is monitored and the bacteria attached to the mycorrhizal spores are identified through morphological studies, DNA is isolation, PCR amplification with 16S rRNA primer. From BLAST analysis and phylogenetic tree that bacteria and gibberellins.The plant and its associated organisms are together called as region of mycorrizal association. Bacteria Like Organisms (BLO) are found on the surface of the mycorrhizal spores, Bacteria have been found adhering to the AM hyphae as well as embedded within the AM spore walls. Mycorrhiza Helper Bacteria (MHB) is diverse and belongs to different bacterial phyla including both gram + positive and gram – negative bacteria. This work deals with *Ocrobactrum* is confirmed as *Ochrobactrum intermedium.* The maximum growth rate of trap culture is observed in the pot with BLO.
KEYWORDS : *Ochrobactrum intermedium, Scutellospora nigra,* 16S rRNA

INTRODUCTION:

Mycorrhizae can be portrayed as a harmonious connection between an organism and a plant . Arbuscular mycorrizal fungai (AMF) encourage the host plant to become energetically under distressing condition by interceding a progression of complex correspondence occasions between the plant and the organism prompting the upgraded photosynthetic rate and different gas trade related attributes[1]. Various report portray improved protection from an assortment of stress including dry season, saltiness herbivory, temperature, metal and infection because of contagious advantageous interaction[2]. AMF improve the nature of soil by impacting its structure and surface and subsequently plant wellbeing [3]. The advantageous interaction of AMF with plants had been accounted for 400 million years prior[4]. They can improve the quality of soil and subsequently and support plant advancement in typical just as distressing

conditions [5]. This growth additionally shapes a mantle around the outside of the underlying foundations of plants and expands the surface zone of the roots and goes about as a shield against pathogens.[6]. AMF are utilized as bio-inoculance, and examines energize their utilization as conspicuous bio composts in reasonable yield profitability[7].

BLO are microorganisms like creatures found on the outside of the mycorrhizalspores. Mycorrhiza Helper Bacteria(MHB) are various and have a place with various bacterial phyla including both gram + positive and gram – negative bacteria [8]. Microscopic organisms holding fast to the AMF mycelium may benefit from hyphal exudates as well as utilize the mycelium as a vehicle for colonization of the rhizosphere [9]. It has for quite some time been realized that the nearness free living microbial networks animates mycorriza arrangement and that this impact can likewise be instigated by explicit strains of microscopic organisms disengaged from mycorrizal roots which have been named mycorrhization partner microorganisms (MHB) [10].The MHB is perceived as the dirt compartment possessed nad influenzed by mycorrzal fungai [11]. From investigations of confinement and ID of bacterial species present in mycorrizal fungai and examinations of the bacterial activity on the advantageous interaction,[12]. proposed just because the term Mycorrihization Helper Bacteria (MHB) reffering just to microscopic organisms that advanced the foundation of the root parasite advantageous interaction. Numerous MHB are viewed as now a days as Plant Growth Promoting Rhizobacteria (PGPR, for example, Pseudomonas sp,[13]. The bacterial decent variety related with AMF propaguleswuthin the underlying foundations of the plants developing in incredibly oil hydrocarbon contaminated soil [14].

Parasites and microorganisms discharge S from sulfate esters utilizing sulfatases,however, arrival of S from sulfatases is catalyzed by a bacterial multi-segment mono-oxygenase framework [15].There are three understanding of Bacteria Like Organisms (BLOs) they are impermanent cytobionts that can continually be obtained are last by fungai ; changeless and non prokaryotic trademark certain fungai and organelles of unkmown root and capacity[16].The plant development is profoundly subject to microbes and mycorrhizal growths which encourage the supplements the connection between the growths and their related microscopic organisms might be incredible significance for manageable agribusiness. The parasitic spores and hyphae give locales to bacterial populace. The present studydeals with the bacteria like organisms which are found on the surface of the mycorrhizae are helping in promoting plant growth and are used as natural bio fertilifers. They are identified and isolated through the following techniques,therir morphological characters and molecular

characters are studied for further analysis.Isolation and identification of (BLO) bacteria like organisms from AMF spore. Identification of (BLO) from trap culture with *Allium cepa*. Comparative analysis of AMF fungi with and without bacteria.

MATERIALS AND METHODS

1. **COLLECTION OF SOIL SAMPLES:**
   Plant roots and rhizosphere soil (ie) dark soil, beach front soil tests and casuarina have been gathered from differing areas of different environmental zones of Chennai. For the confinement of AMF spores different systems have been finished.

2. DETECTION OF SPORES:

WET SIEVING AND DECANTING METHOD

This is one of the popular technique when contrast with various procedures. At first the dirt is sieved utilizing the sieves[17].The strainer is of various sizes. At that point 10 gram of the sieved soil is taken and blended in with 100 ml refined water and mixed with a glass pole ,at that point permitted to settle down. Following 10 minutes the dirt have settle down and the supernatant and the debries were gathered by sifting the supernatant utilizing watsman channel paper. Utilizing the analyzing magnifying lens, from that debries the spores were picked utilizing pipette or needle.

3.TRAP CULTURE:

Trap culture one of the bygone procedure followed, and is set up by blending the sterile[18]. The dirt of various examples. In 3 distinct pots the sterile soil is included ,An is control which contain just the dirt .In B the dirt is blended in with the mycorrhizal spores and in C the dirt is blended in with both the bacterial culture and the mycorrhizal spores. Following 15 days the dirt from the root locale is investigated for mycorrizalassociastion.[19].

4.PERCENTAGE APPRAISAL OF AMF WITH HOST PLANT

The percentage appraisal of scutellospora nigra with the host plant is 72% .

$$\frac{\text{Mycorrhiza associated with cell} * 100}{\text{Absolute number of cells saw through magnifying lens}}$$

5.DETACHMENT OF BLO FROM SPORES OF *OCHROBACTRUM INTERMEDIUM:*

1.4 g of nutrient agar is blended in with 50 ml of refined water. At that point it is saved for autoclave at 121°C .

After the completion of autoclaving , the agar was changed to petriplates and they were kept at room temperature under Laminar chamber. The spores were put into it and kept at room temperature over night. The microscopic organisms were grown in colonies.

6. Recognization of *Ochrobactrum intermedium*

Recognization of BLO from spores of *Scutellospora nigra*

Initially the Gram staining was performed inorder to find whether it is positive or negative, followed by that the bio chemical testes like IMVIC,TSI and CARBOHYDRATE testes are done to know their characters. Through that results the bacteria is assumed. Then the molecular techniques like genomic DNA separation, agarose gel electrophoresis,PCR,sequencing was done.

Here the DNA is sequenced to find the molecular qualities . Agarose gels are made with between 0.7% (gives great goals of enormous 5–10 kb DNA pieces) and 2% (great goals for little 0.2–1 kb sections). The gel arrangement gives wells to stacking DNA in to it. The stacked DNA particles move towards the decidedly charged cathode (anode) and get isolated along the length of the gel. Ethidium bromide (EtBr), a chromogen is added to the gel to imagine the isolated DNA under UV transillumination. EtBr intercalates between the bases and shines when UV radiation is gone through the gel. The polymerised chain response was performed utilizing. The forward primer 518 and the reverse primer 800 where used for enhancement.. At that point their item was broke down through chromactogram. Then through phylogenetic tree the bacteria is confirmed as *Ochrobacterium intermedium.*

6. SYMBIOTIC RELATIONSHIP OF MYCORRHIZA WITHIN THE SIGHT OF *OCHROBACTERUM INTERMEDIUM:*

The symbiotic relationship of mycorriza in *Ochrobactrum intermedium* was recognized dependent on trap culture technique. With 3 pots .

Control.

Soil with AMF.

Soil with both AMF and BLO.

7. LEVEL OF OCHROBACTERUM INTERMEDIUM RELATIONSHIP WITH HOST PLANT.

The level of *ochrobactrum intermedium* association with have plant is 78% , when contrasted with relationship of spores the bacterial host shows higher rate.

RESULTS AND DISCUSSION:

From undistrubed rhizosphere soil of casheurenia and from clay soil the virulent mycorrhizal spores were identified and allowed for multiplication of spores in the laboratory by means of

trap culture method with Allium cepa host plant.[18].fig 2.The spores were collected from the soil samples using WET SIEVING AND DECANTING method.[17]. Fig 1. Then they were surface sterilized with 0.2 chloramineT amd same being inoculated in nutrient agar(15%) and were kept overnight at room temperature, the growth of (BLO) Bacteria Like Organisms were observed and colonies were separated in nutrient agar ,then subcultured followed by identification methods such as gram staining,biochemical testes,and molecular identification.[20]. Fig 5,6,7,8, In gram staining the organism was found to be the gram negative organism.then the cofirmatory test (ie) bio chemical testes were performed and the results were obtained. (fig). In genome level ,their genomic DNA was isolated through agarose gel electrophoresis, and for making copies thr polimerized chain reaction was preformed. Then they are sequenced with the forward 518 and reverse primer 800. The NCBI Sequence number is MN611377.Followed by that were continued for BLAST analysis fig 9, with that they were subjected to phylogenic tree.fig 10. From that result the (BLO) is confirmed as *Ochrobactrum intermedium*.From trap culture the highest growth rate is observed in the pot with both AMF fungi and (BLO). This will be the evident that (BLO) promote the plant growth and by symbioyic association with plants also used as a natural bio fertilizer.

Fig: 1 Detection of spores

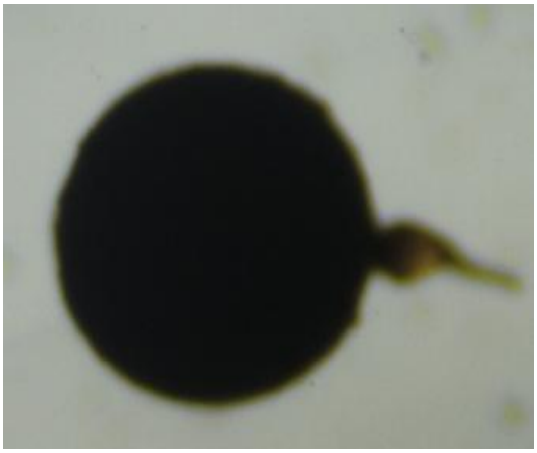


Fig:2 Trap culture

B A
Mycorrhiza Control

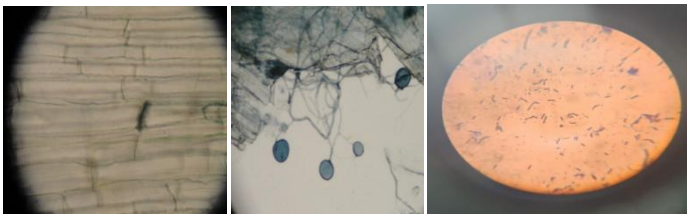
Fig:3.Percentage appraisal of AMF with host

1 2 3

1. AMF with BLO(30)Days
2. AMF 15 Days
3. Control

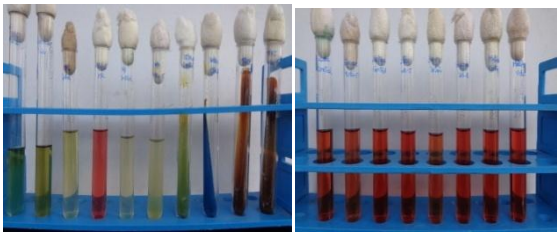
Fig:4 *Scutellospora nigra* association with *Ochrobacteriumintermedium*

1 2 3



1. Control
2. *Ochrobacteriumintermedium*
3. *Scutellospora nigra* with *Ochrobacteriumintermedium*

Fig:5 Biochemical test



Indole	MR	VP	citrate	TSI	Glucose	Lactose	Sucrose	Maltose
-	+	-	+	-	-	-	-	-

Fig: 6 Genomic DNA Isolation

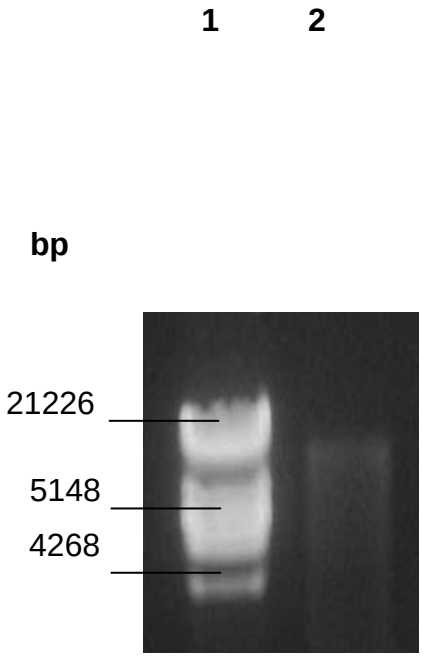


Fig:7 Gel electrophoresis

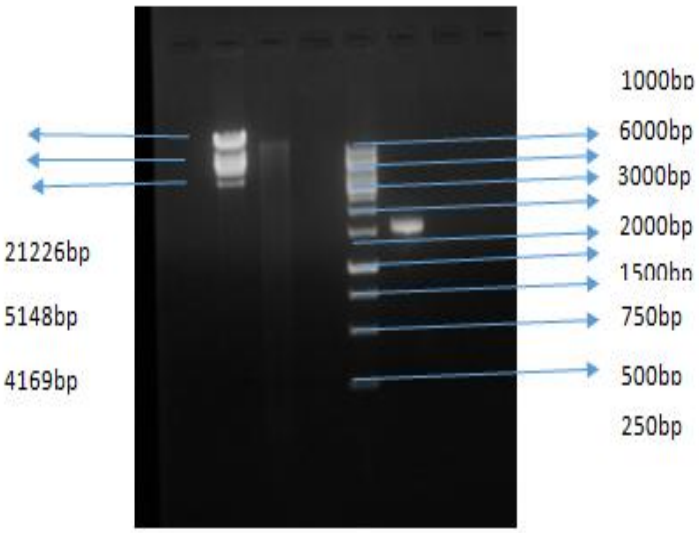


Fig: 8 PCR Amplification

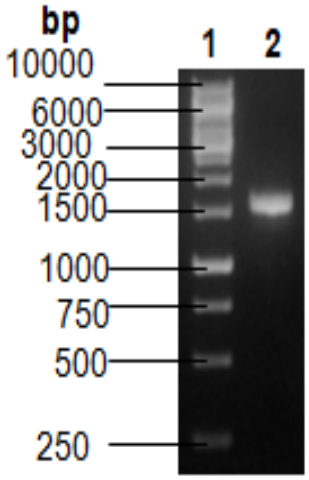


Fig:9 Sequencing

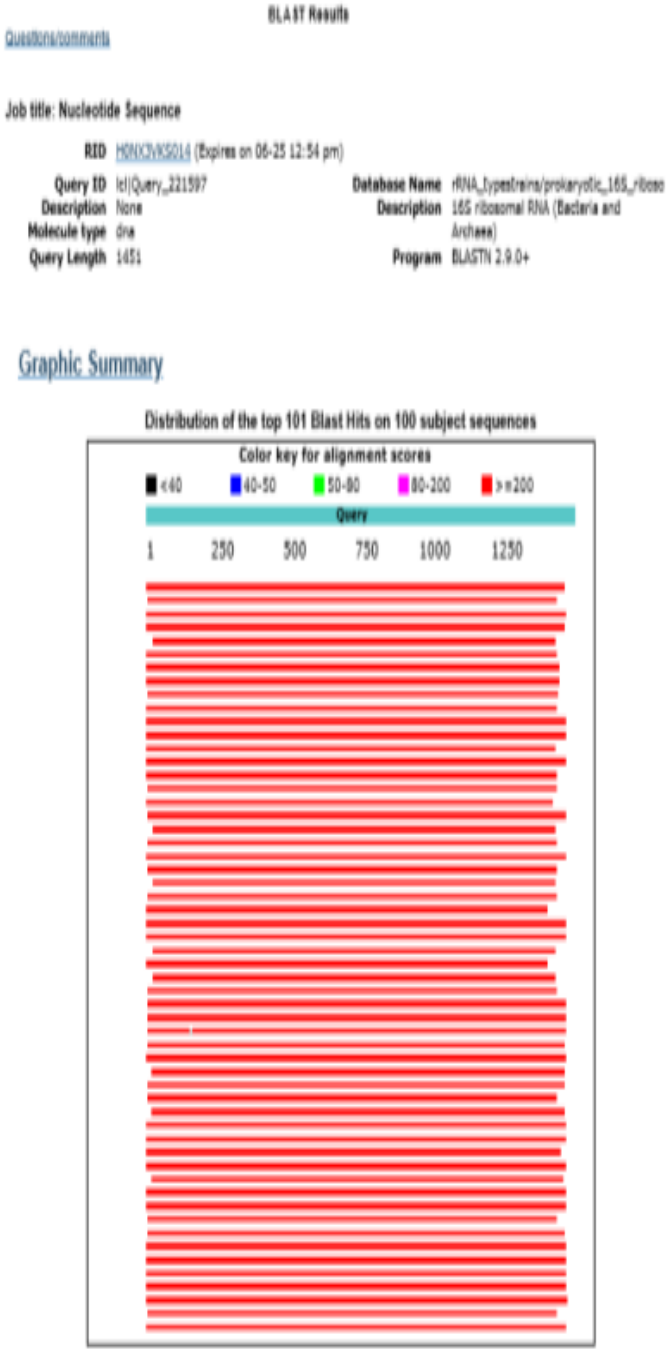


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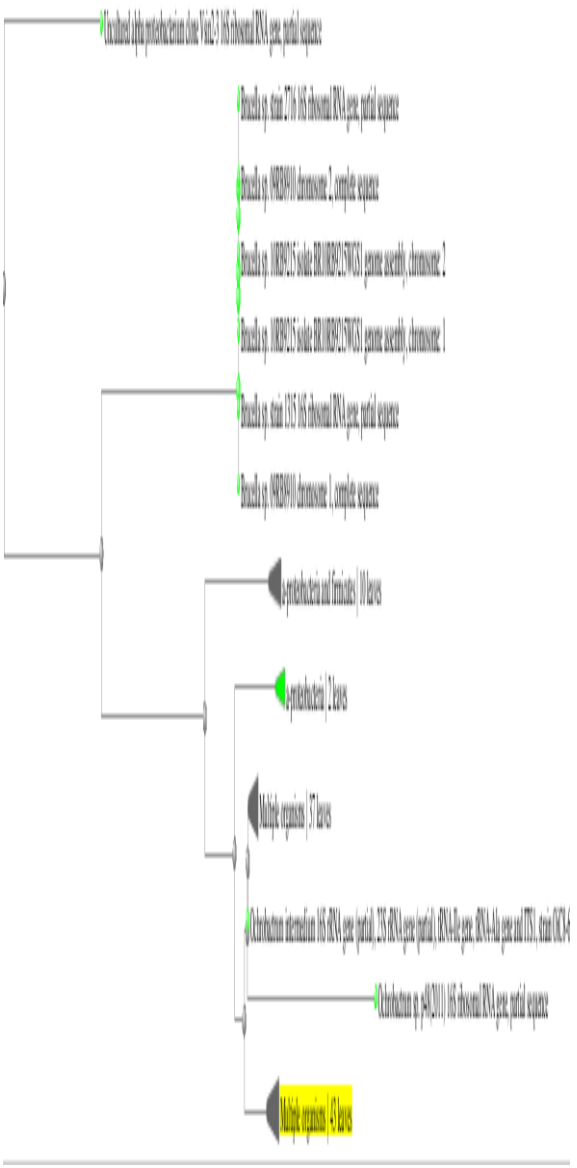
Fig:10 Phylogenetic tree



CONCLUSION:

In this study ,the mychorrhizal spores were collected from the rhizobial soil of casuarina. The soil were sieved and trap cultured with allium cepa as a host , from that the spores were isolated by wet sieving and decanting method . the isolated spores were magnified under the microscope and identified as *Scutellospora nigra*. from that isolated spores their associated bacteria (ie) BLO is seperated . the methods like gram staining , biochemical testes have been done. In genome level , their genomic DNA is isolated and gel electrophorosis is done for their seperation. For making copies of that DNA the PCR amplification is done with the universal primer 16S rRNA. The sequencing is done , the laser produces a chromatogram that shows each nucleotide's fluoresent peak. The BLAST analysis is done and the phylogenetic tree is sketched from that the bacteria is confirmed as *Ochrobactrum intermedium*. This study is continued with different plants as a host.

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