iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 10 **Issue:** III **Month of publication:** March 2022

DOI: https://doi.org/10.22214/ijraset.2022.41055

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 10 Issue III Mar 2022- Available at www.ijraset.com

Endophytes Associated with Macrophyte *Ceratophyllum Demersum*

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Abstract: ***Endophytes are microorganisms living inside plants which have various types of symbiotic relations and non-symbiotic relations with host plant. The endophytes reside inside a plant without any signs or symptoms in living tissues of their host. Plants without any endophytes are rare, bacteria and fungi have been seen residing inside plants. Endophytes inside fossilized tissues and stems of plants indicates this relationship started during the evolution or formation of higher plants. The Bacterial and fungal endophytes reach the plant tissues from the rhizosphere, phyllosphere or seed of the plant. Endophytes of Ceratophyllum demersum, were isolated by two methods. Endophytic bacteria and fungi were found to reside in the leaves of macrophyte Ceratophyllum demersum. Four fungal isolates and three bacterial isolates were obtained. Results indicates that fungal endophytes were predominant than bacterial endophytes in Ceratophyllum demersum.***
Keywords: Ceratophyllum demersum, Macrophytes, Endophytic Bacteria, Endophytic Fungi.

I. INTRODUCTION

Endophytes are symbiotes which lives in internal tissues and organs of plants. Such relationships are mostly beneficial to both the host and endophytic organisms (Azevedo et al. 2000). They produce a wide range of compounds useful for plants for their growth, protection to environmental conditions, and sustainability and host plant give a good dwelling place for endophytes (Sturz and Atheron,1996); (Duijff et al., 1997) ; (Krishnamurthy and Gnanamanickam, 1997). *Ceratophyllum demersum* or hornwort is a dicotyledonous, submerged aquatic angiosperm belonging to the family Ceratophyllaceae (Thomas Abu,2017). Aquatic plants are very important in regulating various aspects of water such as maintaining water quality, managing nutrient load, absorbing nutrient mineral ions and also reducing sediment resuspension, such plants have symbiotic endophytes helping them in ways by overcoming abiotic stress by releasing bioactive secondary metabolites(G. A. Strobel, et al.2005). Relation between endophytes and hosts are complex and related to biotic and abiotic challenges, phytochemicals present in the host, these endophytes are influential for the growth of plants and have biological effects on their host (Bultman et al,1997).Endophytes are seen in different plant tissues such as stem, leaves, roots, xylem (Carroll GC.1988).Bacterial and fungal endophytes are most common fungal endophytes are present in all divisions of plants from herbs, shrubs, mosses and ferns to monocotyledons(O. Petrini et al.1986) .Endophytic bacteria colonize intercellular spaces than the intracellular spaces of host, they colonize in vascular tissues (A. Sessitsch et al.2002). Endophytes can help the plants to overcome pesticides and other environmental stress by producing secondary metabolites, those secondary metabolites could be used effectively as medicine, pesticides, for industrial applications. Plants which have phytoremediation capability has endophytes which help as bioremediating agents and could enhance plants capabilities and also growth of the plant, helps in phosphate solubilization and nitrogen fixation, producing plant growth promoting compounds. There is considerable increase of interest to develop such compounds by biotechnology (Robert P. Ryan et al.January 2008). Bioactive compounds, such as camptothecin, diosgenin, hypericin, paclitaxel, podophyllotoxin, and vinblastine, which have importance in agricultural and pharmaceutical fields are produced by endophytic fungi present in many plants. (Joseph and Priya, 2011; Zhao et al., 2011a). Secondary metabolites are excellent source of drugs for antiarthritic, antimicrobial, anticancer, antidiabetic, anti-insect and immunosuppressant activities (Jalgaonwala et al., 2011; Godstime et al., 2014). Medicinal plants and their endophytes are an important source of precious bioactive compounds and secondary metabolites that contribute to more than 80% of the natural drugs (Singh and Dubey, 2015). Study was carried out to isolate endophytic bacteria and fungi from perennial aquatic plant *Ceratophyllum demersum.*

II. MATERIALS AND METHODS

A. Sample collection

Plants were identified and procured from concrete fish pond at Chittur Palakkad, Kerala. It is cut and transferred to sterile plastic bags using gloves and transferred to college laboratory. 30 centimeters of these macrophytes were introduced in to 50 litre open mouthed plastic bucket with water after washing with deionized water. Plant was allowed to grow in shade for 10-15 days.

B. Isolation of endophytic Bacteria

1) *Method 1:* 6g of plant was collected and washed thoroughly with tap water and then with distilled water and placed in sterile petri dish. The sample was surface sterilized with distilled water for 5 minutes, then with a solution containing active chloride (w/v 4% added as NaOCl solution) for 3 minutes and 70% ethanol for 1 minute. It was then rinsed with sterile distilled water 6-8 times. Plant slurries were prepared using mortar and pestle, plant as a whole was taken to make plant slurry which was then diluted with 10 ml of distilled water. The final diluted slurry (100 µl) was spread onto Luria-Bertani (LB) agar plates total of 3 plates with one kept as control with distilled water. (Bhore et al., 2010, Guo et al., 2010, Luo et al., 2011) and incubated at 28°C for 5 to 7 days.
2) *Method 2:* Surface sterilized stem and leaf were aseptically placed on LB agar and then incubated at 28°C for 5 to 7 days (Bhore et al., 2010, Guo et al., 2010, Luo et al., 2011). The bacterial colonies obtained from these plates were selected and subcultures were prepared for purification. Colony morphology and microscopic examination was done for each bacterial isolate.

C. Isolation of Fungi

1) *Method 2:* The endophytic fungi were isolated by taking 5 to 6g of the sample, surface sterilized with distilled water for 5 minutes , then with a solution containing active chloride (w/v 4% added as NaOCl solution) for 3 minutes and 70% ethanol for 1 minute and then rinsed with sterile distilled water 6-8 times again. After washing the plant, plant slurries were prepared using mortar and pestle. Plant as a whole was taken to make plant slurry which was then diluted with 10 ml distilled water. The final diluted slurry (100 µl) was plated onto SDA a fungal media used for isolation of endophytic fungi. Media was supplemented with an antibiotic streptomycin(6mg/200ml) to inhibit any bacterial growth in the fungal media and then incubated at room temperature for 5 to 7 days.
2) *Method 2:* Surface sterilized leaf and stem were aseptically placed in the SDA media and then incubated at room temperature for 5 to 7 days. After incubation each plate were assessed for colony morphology and pure cultures were grown on PDA agar plates, after purification colony morphology and microscopic examination using LPCB technique was done for each fungal isolate.

III. RESULTS

In the LB agar with plant slurry, only 2 bacterial colonies were observed, both having 2 different colony morphology. One mucoid white colony and another white powdery irregular colony. 3 colonies were observed around the leaf placed on LB agar media. With 2 different colony morphology one white colony and another powdery raised white colony.

In SDA plate with plant slurry,2 colonies were observed. One white cottony colony which on further incubation turned pink and another white raised colony with green color in middle. Out of 4 colony, 3 were having different colony morphology. Cottony white colonies around the leaf were seen propagating. Green colonies starting from the stem placed on SDA media and white small button colonies are obtained.

From the above results, we have confirmed the presence of endophytic microorganisms in macrophyte *Ceratophyllum demersum.* Both bacteria and fungi were present which is confirmed with the colony morphology. In our study,we isolated 2 different endophytic bacteria and 3 different endophytic fungi from *Ceratophyllum demersum.* Fungi was found to be predominant and there might be an association between the bacteria fungi and plant which will be involved in symbiotic association.

Table:1 Details of Colony Morphology of Endophytic Bacteria and Fungi Isolated from *Ceratophylum demersum*

Sl..No	Organism	Isolate no.	Colony Morphology	Number of Colonies with different morphology
1	Bacteria	CSB1	Mucoid white colonies	2
2	Bacteria	CSB2	White powdery irregular colonies	
3	Bacteria	CLB1	White colonies	
4	Bacteria	CLB2	Powdery raised white colonies	
5	Bacteria	CLB3	Mucoid white colonies	
6	Fungi	CSF1	White cottony colonies on further incubation turned pink	3
7	Fungi	CSF2	White raised colonies with green color in the center	
8	Fungi	CLF1	White cottony colonies on further incubation turned pink	
9	Fungi	CLF2	White raised colonies with green color in the center	
10	Fungi	CLF3	small button like white colony	
11	Fungi	CLF4	white small colony	

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
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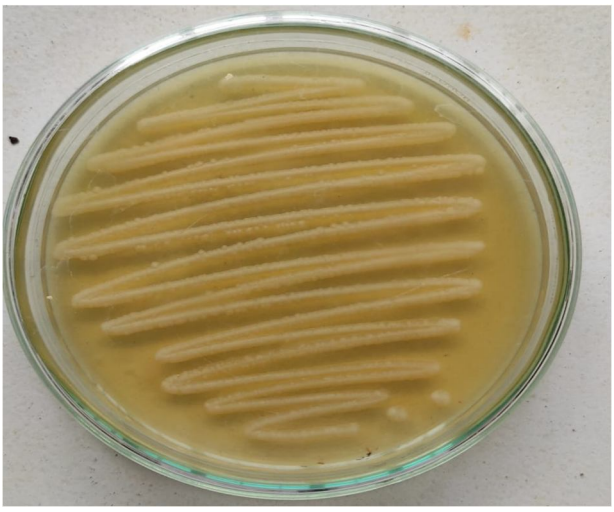

Fig. 1 Bacterial isolate from *Ceratophyllum demersum*

Fig. 1 Fungal isolate from *Ceratophyllum demersum.*

IV. DISCUSSION

Endophytes are organisms vastly present inside almost all variety of plants and have a symbiotic relation with the host. Endophytes perform many roles in host (Zhang, et al). Endophytes makes the plant healthier by producing phytohormones such as indole-3-acetic acid (IAA), cytokines, and other plant growth-promoting substances. Endophytes can enhance the host's absorption of nutritional elements such as nitrogen (V. M. Reis et al,2000) (P. C. Lyons,1990) and phosphorous (L.D.Guo et al,2000) (L. Gasoni et al,1997). Bioactive secondary metabolites and other chemicals produced by endophytes protect the plant from herbivores and pathogens. Secondary metabolites such as alkaloids have the ability to poison herbivorous hence keeping such herbivores away (H. H. Wilkinson et al,2000). Another role of endophytes is ecological where the plants ecology is modified and shaped (R. J. Ganley et al,2004). Endophytes helps in thermotolerance capacity of plant in geothermal soils (R. J. Ganley, et al.,2002), reshaping biodiverse community and such interactions have great importance in shaping plant biodiversity. Some endophytes of aquatic plants have the ability to degrade pesticides from water and also phosphate solubilization. Studies have been conducted to understand the role of bacterial and fungal endophytes of aquatic plants for bioremediation. Very little studies are conducted using the combination of aquatic plants as phyto remediating agents along with bioremediating endophytes (Ryan et al., 2008, McGuinness & Dowling 2009, Weyens et al. 2009). *Ceratophyllum demersum*, a submerged aquatic plant is associated with both fungal as well as bacterial endophytes. Detailed studies are required to understand the relationship between the different endophytes and host plant and its potential to be used as bioremediating agents and understanding further about aquatic plants and its endophytes.

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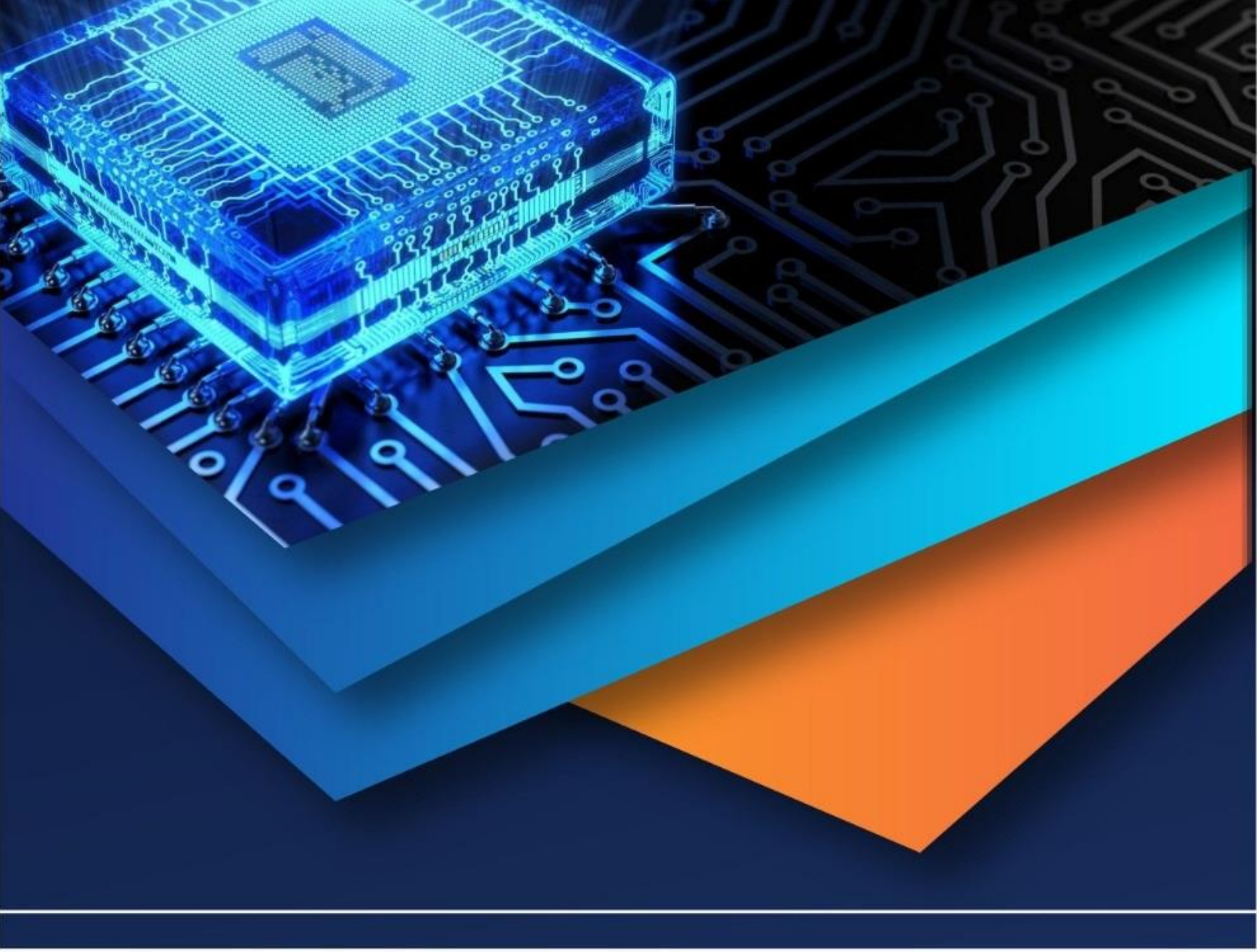

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