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Study of Hydrocarbon degrading ability of a bacterial endophyte, *Psuedomonas* from *Ficus racemosa L.*

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Abstract

A bacterial endophytic strain was isolated from the medicinal tree *Ficus racemosa L* and tested for its bioactive properties. The endophytic strain was found to possess the ability to degrade octane. The strain showed dense growth in a sterile growth media containing octane (petrol) as the sole source of carbon. The strain was further tested to determine the minimum inhibitory concentration of petrol to the organism. The location of the gene responsible for hydrocarbon utilization was determined by plasmid curing technique.

Keywords: Bacteria, Endophytes, Hydrocarbon utilization, *Ficus racemosa L*

Introduction

'Endophytes' are the micro organisms residing in association with plants without causing any apparent symptoms or damage to the host plants. Various bacterial and fungal endophytes have been found to inhabit the inner tissues of many plants. In fact, they have been found in virtually all the plants screened for endophytes. Considering the number of plant species known to man, quite a large number of plants remain uninvestigated and hence an important avenue of research remains unexplored. Endophytes are often found to be living in a wide range of associations with the host plant like mutualistic, commensalistic or rarely parasitic. Most of the time endophytes produce a compound/s that possesses properties beneficial to the host plant.

The plant under current study is *Ficus racemosa L.* It is also known as *Ficus glomerata L.*, Indian fig tree, Cluster fig tree, Gular tree and Udumbara. This tree belongs to the family Moraceae. The trees in genus Ficus are known to have immense medicinal value. *Ficus racemosa L.* is considered sacred and has golden coloured exudates and black bark. This tree is native to Australia, South East Asia and the Indian subcontinent. It grows all over India in many forests and hills. It is frequently found around the water streams and is also cultivated. It is one of the herbs mentioned in all ancient scriptures of Ayurveda. (Shiksharthi, et. al. 2011)

Oil spills are a major environmental problem nowadays. Widespread contamination has been reported in both marine and subsurface environments due to accidents involving motorized vehicles (ships, cars, trucks, etc.), leaks and spills from underground storage tanks, pipelines and illegal disposals. This mode of pollution has to be controlled

because of its deleterious effect on the ecosystems and involves immeasurable damage to the flora and fauna. The oil coats the surfaces of living organisms thus affecting their viability. The thick layer of oil formed on the water restricts the sunlight and oxygen penetrating water and hence, affects aquatic organisms. Bioremediation of such environments using micro organisms can potentially offer a cost effective solutions. Bioaugmentation of oil polluted sites using microorganisms has been reported earlier. (Mukherji et al., 1998)

Current evidence suggests that in aquatic and terrestrial environments microorganisms are the chief agents for the biodegradation and bioremediation of pollutants, including petroleum hydrocarbons (Alexander et al., 1982; Swanell and Head, 1994). Hydrocarbon-degrading bacteria, yeast and fungi are widely distributed in marine, fresh water and soil habitats. Bacteria and yeast appear to be the dominant degraders in aquatic ecosystems while fungi and bacteria are the main degraders in soil environments. (Balba et. al., 1998)

Endophytes were isolated from the young leaves of the plant *Ficus racemosa L.*, according to the method described by Vichare et al. (2016). These endophytes were further characterized by performing biochemical tests and were found to be from genus *Pseudomonas*. These endophytes were further screened for their properties.

Materials and methods

Determination of oil degradation ability of the endophytic isolates

The endophytic strains were isolated from three trees, namely, *Ficus racemosa L., Aegle marmelos L.* and *Cymbopogon citratus L.* (Vichare et. al., 2016) One isolate each from *Ficus racemosa L.* and *Aegle marmelos L.* (Isolate 1 and 2 respectively) and two isolates from *Cymbopogon citratus L.* (isolates 3and 4) were tested for oil degradation.

The endophytic isolates were tested for degradation of petrol (octane) by providing it as the sole source of carbon. A fresh culture (18 hour old) was inoculated in M9 minimal medium with petrol as the sole carbon source and incubated on a rotary shaker (60 rpm) at 37°C for 48 to 72 hours.

The initial concentration used for detection of petrol degrading ability was 50µl. The optimum and inhibiting concentrations of petrol were determined by inoculation the organism in the media tubes containing various of concentrations of petrol ranging from 50 µl to 3000 µl. The experiment was carried out in duplicates. The organisms were also inoculated in media tubes without any petrol which served as a control.

Plasmid curing

Plasmid curing was done to locate the gene responsible for degradation of petrol. Plasmid curing procedure was performed according to the method described by Vazquez- Cruz C et al. (1992). Broth cultures (18 hour old) were made in Luria Bertini (LB) medium at pH 6.8. Aliquots of 0.1 ml0 (10^8 cfu/ml) were inoculated into flasks with LB medium supplemented with 8µg/m0l acridine orange and incubated at 32°C. The resulting growth was tested for hydrocarbon utilisation.

Results and discussion

Only one isolate (isolate number 1) out of the four showed oil degradation as seen in figure 1. There was no growth in the media tubes with the remaining 3 isolates. The media tubes without petrol also showed no growth for all 4 isolates. This proves the oil degradation ability of the endophytic isolate 1.

Further testing revealed the inhibiting petrol concentration for the organism. The organism showed growth in tubes with petrol concentrations of 1%, 2%, 5%, 10%, 20% and mild growth in 30%. There was no growth seen in tubes with petrol concentration of 40%, 50% and 60%. This

suggests that 30% is the maximum concentration of octane tolerated by the organism.

The plasmid curing procedure revealed that the bacterium loses its hydrocarbon (petrol) degrading ability post curing procedure. This indicates that the gene responsible for hydrocarbon utilization is borne on a transmittable plasmid.

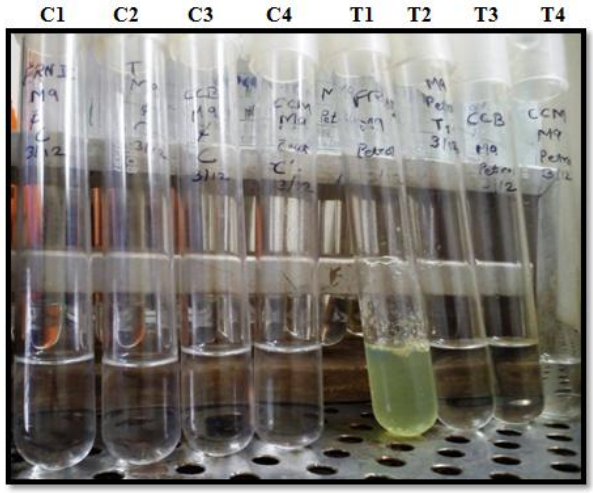

Figure 1: Control tubes: Tubes labeled C1, C2, C3, & C4 contain no source of carbon and show no growth of the test endophytic strains. Test tubes: Tubes labeled T1, T2, T3 & T4 contain petrol (octane) as a sole source of carbon. Only T1 shows dense growth confirming hydrocarbon utilization.

Conclusion

The endophytic bacteria isolated from *Ficus racemosa L.* were successfully tested for hydrocarbon (petrol) degradation by incorporating petrol as the sole source of carbon in the growth media. This study has uncovered yet another interesting prospect of an endophyte. The study can be further developed into an application of tremendous ecological importance as it may be helpful in managing aquatic pollution due to oil spillage in water bodies. This could possibly indicate a strong ecological relationship between endophytes and the host plant. Further one may examine whether this is a more widespread phenomenon.

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