Full Length Research Article

© 2024, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 15; Issue 01 (Jan–Feb 2024); pp 74–77

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Production of Bio Surfactants by Epiphytic Bacteria from Plant Leaf Surface

R. Reshman Manikandan*[1] and T. Srinivasan[2]

[1-2] Department of Microbiology, Hindustan College of Arts and Science, Kelambakkam - 603 103, Tamil Nadu, India

Received: 27 Oct 2023; Revised accepted: 22 Dec 2023; Published online: 10 Jan 2024

Abstract

The presence of hydrocarbon contaminants in the environment can cause significant damage. One environmentally friendly method of reducing such contaminants is through bacterial bioremediation. Although many leaf colonizing bacteria are known to produce surfactants, their potential for remediating hydrocarbon contamination has not been explored. Therefore, the purpose is to investigate whether surfactants produced by leaf colonizing bacteria could improve the degradation of hydrocarbons. Leaves possess a cuticle that consists of lengthy and extremely long chain hydrocarbons. Moreover, cyclic hydrocarbons may also be present. Epiphytic bacteria commonly found on leaves, have the potential to adapt and utilize these cuticle hydrocarbons. To investigate this, we conducted experiments to assess the capacity of various phylogenetically distinct epiphytic bacteria to grow and thrive on diesel and petroleum benzine. we collected sample from 5 different places Consequently, our findings suggest a widespread prevalence of hydrocarbon degradation and surfactant production in epiphytic bacteria. However, it remains uncertain whether epiphytic bacteria employ hydrocarbons derived from the cuticle of living leaves. The application of surfactant producing and hydrocarbon utilizing epiphytic bacteria holds promise as a potential method for hydrocarbon bioremediation.

***Key words*:** Biosurfactant, Epiphytic, Atomized oil assay, Bio-remediation, Hydrocarbon

The phyllosphere refers to the bacterial habitat present on the above ground parts of plants [1]. Leaves make up the majority of the phyllosphere and are coated with a hydrophobic cuticle that is a composite structure of cutin, a polymer made up of crosslinked aliphatics with very long chains, and cuticular waxes, which are soluble aliphatics [2-3]. These waxes may be impregnated within the cutin matrix or overlaying it, known as intracuticular or epicuticular waxes, respectively [4-5]. The primary purpose of intracuticular waxes is to limit non-stomatal water loss, while epicuticular waxes serve to protect the leaf from UV-B radiation and biotic stresses [4-6]. The waxes are primarily made up of long-chain alkanes, primary and secondary alcohols, aldehydes, ketones, and alkyl esters [7].

Leaves host a diverse microbiota that includes bacteria, fungi, and oomycetes, with bacteria being the most abundant at an average of 104 - 105 bacteria per mm^2 [8-9]. Previous studies have found the presence of oil-degrading bacteria on the surface of plant leaves and investigated the diversity of alkane degradation genes in bacterial communities on leaf surfaces [10]. However, it remains uncertain whether bacteria that colonize leaf surfaces gain any fitness benefits by breaking down aliphatic compounds on plant leaves. The hydrocarbons present in the cuticle of leaves may serve as a source of carbon and energy for microorganisms that colonize them, especially bacteria. It has been estimated that there may be up to 1026 bacteria globally residing on plant leaves [11].

In order to facilitate the degradation of hydrocarbons, bacteria have the ability to produce surfactants, which are amphiphilic compounds [12-13]. These surfactants work by reducing surface tension, leading to the accumulation of insoluble compounds at the interface of immiscible fluids. This increases the surface area and enhances the bioavailability and degradation of hydrocarbons. Additionally, surfactants also provide other fitness advantages to bacteria colonizing leaves [14]. Previous studies have highlighted the ability of some endophytic bacteria to degrade oil and its implications for plant-based bioremediation approaches [15-22]. In the present study, we evaluated the ability of 5 bacterial strains, which represent the diversity of the leaf microbiota [23], to degrade common hydrocarbons such as diesel and engine oil. We assessed the prevalence of the ability to degrade hydrocarbons and the production of surfactants.

MATERIALS AND METHODS

Nutrient agar, nutrient broth, Bushnell Haas broth BHB was supplemented with diesel, Engine oil, or glucose, sucrose and/or succinate depending on strain preference.

Sample collection

Plant leaves sample was collected from 5 different places. It is washed with sodium hypochlorite, Methanol and water. with the steril forceps the leaves were placed in the growth media and it was incubated for 24 hours in 37 degree Celsius. The culture is identified and isolated in the selective media.

Hydrocarbon utilisation assay

***Correspondence to:** R. Reshman Manikandan, E-mail: reshmanmani@gmail.com; Tel: +91 7010719943

Citation: Manikandan RR, Srinivasan T. 2023. Production of bio surfactants by epiphytic bacteria from plant leaf surface. *Res. Jr. Agril. Sci.* 15(1): 74-78.

To measure hydrocarbon utilization by epiphytic bacteria, 250 mL conical flasks containing 50 mL of BHB supplemented with 1 % hydrocarbon substrate (diesel or petroleum) were used as only carbon source. For positive controls BHB was supplemented with 1% methanol, or 1% w glucose, sucrose or succinate as sole carbon source. BHB without additional carbon sources as negative control. Media were inoculated with 0.5 mL of 100-times diluted overnight cultures. Cultures were incubated at 30 °C and 200 revolutions per minute (rpm) for up to 23 days. Cell density was measured by doptical density at 600 nm (OD 600nm) every 48 hours using a spectrophotometer. Non-inoculated BHB supplemented with diesel or petroleum benzine served as control for contaminations of the hydrocarbons and if the hydrocarbons alone change the medium's absorbance. All treatments were performed in three biological replicates

Atomized oil assay

The atomized oil assay was performed as previously described. Briefly [24], grown bacterial colonies were harvested from agar plates and suspended in 1 × phosphate buffer saline. The OD600nm was adjusted to 0.5 with PBS and 2 µL of the suspension were pipetted onto NA, LBA or R2A agar plates according to the strains' optimal growth conditions. Plates were incubated at 30° C for up to 5 days, depending on the growth rate of the strains. A fine mist of light paraffin oil was then applied onto the agar plates using an airbrush gun with an air pressure of 100-140 KPa. As a positive control, 2 µL of a 10% v/v Tween-20 solution was pipetted onto LBA. Surfactant-producing bacteria exhibited visible halos where the oil droplets reflected light differently due to the presence of surfactants that changed the hydrophobicity of the agar medium. Halos were visualized by using an indirect light source and photographs of halos were taking using a dark field illumination technique on a photo stage

Drop collapse assay

The drop collapse assay was performed as described previously. Briefly, y [25] 2 µL of Magnatec 10W-40 oil (Castrol) were pipetted into 47 each well of a 96-well plate lid and were allowed to equilibrate for 2 hours to ensure that each well was evenly coated. Bacterial overnight cultures were centrifuged at 2600 × g for 10 minutes. 5 µL of the culture supernatant were pipetted into the centre of an oil filled well. Drops were observed for up to 5 minutes and the time to collapse was determined. Drops that collapsed into the oil, i.e. decreased their contact angle, were positive for surfactant production while drops that remained intact and stayed on top of the oil were negative for surfactant production

RESULTS AND DISCUSSION

Drop collapse assay

Drops that fell into the oil had a smaller contact angle and produced more surfactant.

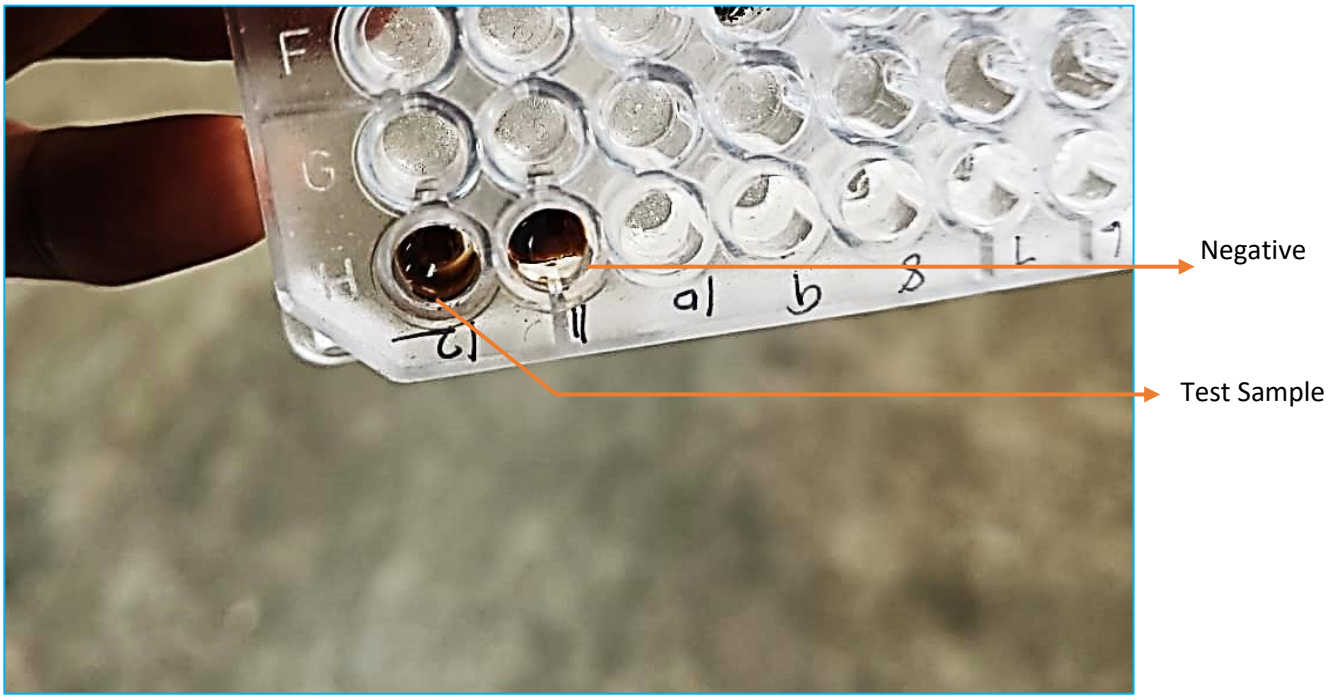

Fig 1 Drop collapse assay

Atomized oil assay

The presence of amphiphilic chemicals was found when bacterial colonies were sprayed with a fine mist of paraffin oil, altering the reflection of oil droplets onto agar plates.

Hydrocarbon utilization assay

Using a spectrophotometer, the optical density at 600 nm (OD 600 nm) was measured every 48 hours to determine cell density. The result indicates the use of hydrocarbon in both engine oil and diesel by raising the OD value.

Previous research has established that plant leaves bacteria capable of breaking down hydrocarbons, as demonstrated by hydrocarbon assays [26]. The findings presented here offer additional support, revealing that the epiphytic microbiota consists predominantly of hydrocarbon-degrading bacteria with the capacity to break down environmental hydrocarbons.

CONCLUSION

Bacteria that can use hydrocarbons and produce surfactants are widespread on the surfaces of leaves, indicating that this trait might be advantageous in the phyllosphere nearly, every species that made surfactants had the ability to degrade

engine oil and fuel. It is yet unknown if these bacteria are able to break down hydrocarbons using the many long chain aliphates that comprise the cuticle and the waxes on the cuticle. the widespread presence of bacteria on leaf surfaces that can produce surfactants and potentially degrade hydrocarbons suggests a fascinating ecological interplay.

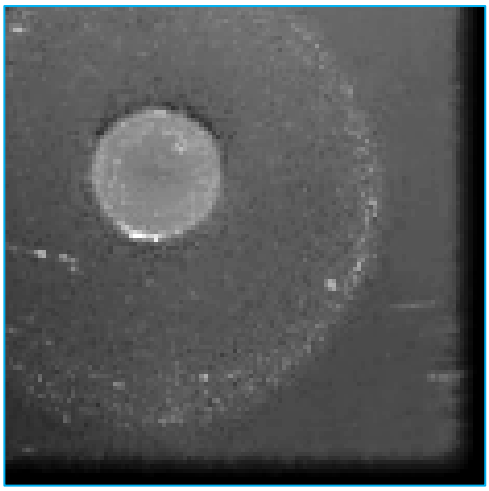

Fig 2 Atomized oil assay

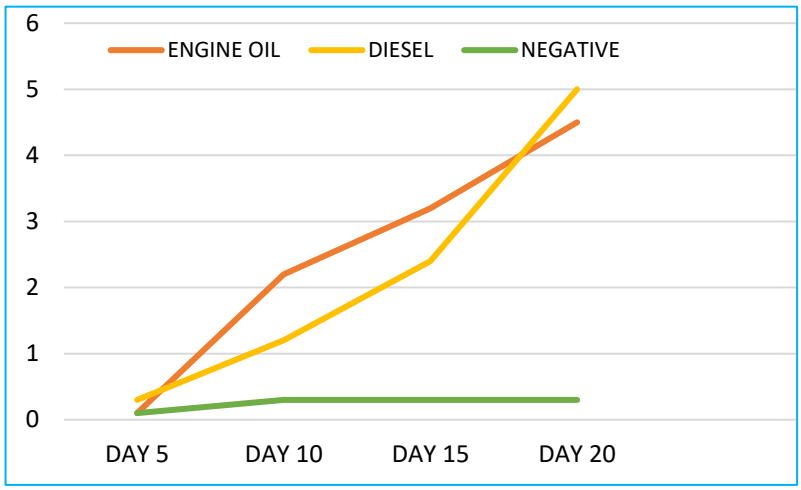

Fig 3 Hydrocarbon utilization assay

LITERATURE CITED

1. Ruinen J. 1956. Occurrence of Beijerinckia species in the Phyllosphere. *Nature* 177: 220. doi: https://doi.org/10.1038/177220a0
2. Kolattukudy PE. 1980. Biopolyester membranes of plants: Cutin and Suberin. *Science* 208: 990-1000. doi: 10.1126/science.208.4447.990
3. Müller C, Riederer M. 2005. Plant surface properties in chemical ecology. *Journal of Chemical Ecology* 31(11): 2621-2651. doi: 10.1007/s10886-005-7617-7
4. Buschhaus C, Jetter R. 2011. Composition differences between epicuticular and intracuticular wax substructures: How do plants seal their epidermal surfaces? *Journal of experimental Botany* 62: 841-853. doi: https://doi.org/10.1093/jxb/erq366
5. Zeisler V, Schreiber L. 2016. Epicuticular wax on cherry laurel (*Prunus laurocerasus*) leaves does not constitute the cuticular transpiration barrier. *Planta* 243: 65-81. doi: https://doi.org/10.1007/s00425-015-2397-y
6. Barthlott W, Neinhuis C. 1997. Purity of the sacred lotus or escape from contamination in biological surfaces. *Planta* 202: 1-8. doi: https://doi.org/10.1007/s004250050096
7. Jetter R, Kunst L, Samuels AL. 2006. Composition of plant cuticular waxes. *In*: (Eds) Riederer M, Müller C. Biology of the Plant Cuticle. Oxford, UK: *Blackwell Publishing Ltd*. 2006: 145-181. doi: 10.3389/fpls.2022.785812
8. Gekenidis MT, Gossin D, Schmelcher M, Schoner U, Remus-Emsermann MNP, Drissner D. 2017. Dynamics of culturable mesophilic bacterial communities of three fresh herbs and their production environment. *Journal of Applied Microbiology* 123: 916-932. doi: 10.1111/jam.13532
9. Remus-Emsermann MNP, Schlechter RO. 2018. Phyllosphere microbiology: At the interface between microbial individuals and the plant host. *New Phytologist* 218: 1327-1333. doi: https://doi.org/10.1111/nph.15054
10. Gandolfi I, Canedoli C, Imperato V, Tagliaferri I, Gkorezis JV, Vangronsveld J, Schioppa EP, Papacchini M, Bestetti G, Franzetti A. 2017. Diversity and hydrocarbon degrading potential of epiphytic microbial communities on *Platanus x acerifolia* leaves in an urban area. *Environmental Pollution* 220: 650-658. doi: 10.1016/j.envpol.2016.10.022
11. Vorholt JA. 2012. Microbial life in the phyllosphere. *Nature Review Microbiology* 10: 828-840. doi: https://doi.org/10.1038/nrmicro2910
12. Oberbremer A, Müller-Hurtig R, Wagner F. 1990. Effect of the addition of microbial surfactants on hydrocarbon degradation in a soil population in a stirred reactor. *Applied Microbiology and Biotechnology* 32: 485-489. doi: https://doi.org/10.1007/BF00903788
13. Bautista FL, Sanz R, Molina CM, Gonzalez N, Sanchez D. 2009. Effect of different nonionic surfactants on the biodegradation of PAHs by diverse aerobic bacteria. *International Biodeterioration and Biodegradation* 63: 913-22. doi: https://doi.org/10.1016/j.ibiod.2009.06.013
14. Burch AY, Zeisler V, Yokota K, Schreiber L, Lindow SE. 2014. The hygroscopic biosurfactant Syringafactin produced by *Pseudomonas syringae* enhances fitness on leaf surfaces during fluctuating humidity. *Environmental Microbiology* 16: 2086-98. doi: 10.1111/1462-2920.12437
15. Phillips LA, Germida JJ, Farrell RE, Greer CW. 2008. Hydrocarbon degradation potential and activity of endophytic bacteria associated with prairie plants. *Soil Biology and Biochemistry* 40: 3054-3064. doi:10.1016/j.soilbio.2008.09.006
16. Gkorezis P, Daghio M, Franzetti A, Van Hamme JD, Sillen W, Vangronveld J. 2016. The interaction between plants and bacteria in the remediation of petroleum hydrocarbons: An environmental perspective. *Frontiers in Microbiology* 7: 1836. doi: https://doi.org/10.3389/fmicb.2016.01836
17. Pawlik M, Canis B, Thijs S, Vangronsveld J, Piotrowska-Seget Z. 2017. Hydrocarbon degradation potential and plant growth-promoting activity of culturable endophytic bacteria of *Lotus corniculatus* and *Oenothera biennis* from a long-term polluted site. *Environmental Science and Pollution Research* 24: 19640-19652. doi: https://doi.org/10.1007/s11356-017-9496-1

18. Ito H, Iizuka H. 1971. Taxonomic studies on a radio-resistant *Pseudomonas*. *Agricultural and Biological Chemistry* 35: 1566-1571.
19. Rivas R, Abril A, Trujillo ME, Velazquez E. 2004. *Sphingomonas phyllosphaerae* sp. nov., from the Phyllosphere of *Acacia caven* in Argentina. *International Journal of Systematic Evolutionary Microbiology* 54: 2147-2150. doi: 10.1099/ijs.0.63102-0
20. Feil H, Feil WS, Chain P, Larimer F, DiBartolo G, Copeland A, Lykidis A, Trong S, Nolan M, Goltsmanet E, Thiel J, Malfatti S, Loper JE, Lapidus A, Detter JC, Land M, Richardson PM, Kyrpides NC, Ivanova N, Lindow SE. 2005. Comparison of the complete genome sequences of *Pseudomonas syringae* pv. syringae B728a and pv. tomato DC3000. *Proceedings of the National Academy of Sciences of the United States of America* 102(31): 11064-11069.
21. Innerebner G, Knief C, Vorholt JA. 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by Sphingomonas strains in a controlled model system. *Applied and Environmental Microbiology* 77: 3202-3210.
22. Remus-Emsermann MNP, Kim EB, Marco ML, Tecon R, Leveau JHJ. 2013. Draft genome sequence of the phyllosphere model bacterium *Pantoea agglomerans* 299R. *Genome Announcement* 1. doi: 10.1128/genomeA.00036-13.
23. Burch AY, Shimada BK, Browne PJ, Lindow SE. 2010. Novel high-throughput detection method to assess bacterial surfactant production. *Applied and Environmental Microbiology* 76: 5363-5372. doi: 10.1128/AEM.00592-10
24. Bod Bodour AA, Miller-Maier RM. 1998. Application of a Modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *Journal of Microbiological Methods* 32: 273-280. doi: 10.1016/S0167-7012(98)00031-1
25. Al-Awadhi H, Al-Mailem D, Dashti N. 2012. The abundant occurrence of hydrocarbon utilizing bacteria in the phyllospheres of cultivated and wild plants in Kuwait. *International Biodeterioration and Biodegradation* 73: 73-79. doi: https://doi.org/10.1016/j.ibiod.2012.05.016
26. Gandolfi I, Canedoli C, Imperato V, Tagliaferri I, Gkorezis P, Vangronsveld J, Padoa E, Papacchini SM, Bestetti G, Franzetti A. 2017. Diversity and hydrocarbon degrading potential of epiphytic microbial communities on platanus x acerifolia leaves in an urban area. *Environmental Pollution* 220(Pt A): 650-658. doi: 10.1016/j.envpol.2016.10.022