MOLECULAR CHARACTERIZATION OF A BIOSURFACTANT FROM *FRANCONIBACTER* SP. AND ITS APPLICATION IN OIL RECOVERY

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JULY 2023

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by

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Submitted

In fulfilment of the requirements of the degree of Doctor of Philosophy

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2023

CERTIFICATE

This is to certify that the thesis titled "**Molecular characterization of a biosurfactant from *Franconibacter* sp. and its application in oil recovery**" being submitted by **Ms. Jyoti Sharma** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi.

The results presented in this thesis have not been submitted in part or full to any other University or Institute for the award of any other degree or diploma.

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ACKNOWLEDGMENTS

Undertaking this PhD has been a truly life-changing experience for me and I owe a great debt of gratitude to the many people who provided me with the guidance and encouragement I needed to succeed.

First and foremost, I am deeply grateful for the continuous support, insight and patience of my supervisors, Prof. Preeti Srivastava and Prof. D. Sundar. Without their constant trust, valuable suggestions and guidance, this thesis would not have been completed. I count myself fortunate to have you both as my supervisors. You both played a huge role in my development as a research scholar. Words can only inadequately express my gratitude to my supervisor Prof. Preeti Srivastava. Her encouragement, constant support, perceptive guidance, spectacular ideas, and suggestions from the beginning to the end enabled me to develop an understanding of the subject. Her scholarly ideas, immense interest in science, constant help and friendly behavior have been a source of inspiration for me. Her suggestions will remain with me as an inexhaustible source of scientific learning throughout my life. I would like to say a very big thank you to my supervisor Prof. D. Sundar. He has been a source of support and encouragement throughout these years, and his input into my thesis has been invaluable. His insightful comments have helped me to enhance my experimentation and presentation skills. His helpful nature towards my work plays a major role in completing this thesis work.

I would like to convey my sincere gratitude to my SRC members: Prof. Shilpi Sharma, Prof. Ashok Kumar Patel and Prof. Lucinda Elizabeth Doyle for always giving me their valuable suggestions and for always saying inspiring words to encourage me. I would

also like to thank other faculty members of DBEB for their support and help by allowing me use their respective lab resources.

My heartful thanks to my friends in IIT Delhi. Khushboo, Vidhu, Sanjay, Manisha and Nudrat for always being there and handling me in my good and bad times and making my life easier in IIT Delhi. I would like to thank my past lab juniors Shefali, Arti, Sneha, Jagat, Gaurav for always listening to me. You all are like my younger brothers and sisters. A very special thank you to my current RNA-1 lab colleagues, without whom I could never have finished this thesis work by the deadline. I have no doubt that my lab mates are the best colleagues in the world because they all are so encouraging and helpful. Thanks to Jananee and Arif for always hearing about and resolving my scientific issues. Aditi and Shabnam for putting me at ease in the lab, assisting me in studying, and clearing up any questions I had regarding molecular biology. Nidhi, thanks for being friendly and helpful. You're like my little sister. Thanks to Ashmeeta for sustaining my interest in makeup and for engrossing me in her storytelling. Thanks to my juniors Sourabh, Deeksha, Shreyoshi, and Poorvika are being so adorable and kind to me. I always miss you all for our chai and food sessions. Amit bhaiya for keeping the lab clean and making chai for me. I would especially like to thank Yogesh for all of his help in this thesis. Without his assistance, it would have been impossible to complete the computational work for this project. Every time I had a question about computational work, he assisted and explained it to me very nicely.

I would also like to thank Prof. Rajesh Khanna for allowing me use the tensiometer in his lab, AIRF facility from JNU for processing my samples of GC-MS, LC-MS in a single day. I would further like to express my thanks to UG lab assistants; Sanjay bhaiya, Ashish

bhaiya, Sumit sir, Anish sir for always allowing me use UG lab instruments, whenever I need them to use for my experiments.

Finally, I want to thank my parents for always supporting me and having faith in me. Without your love and encouragement, I would never have considered pursuing a doctorate program. My grandparents (my Nanaji, Naniji and Dadaji), I lost them physically during this journey but you all and your blessings will always remain in my heart. I still feel you're all still with me, and I can imagine your proud faces looking at me from heaven. I want to express my love to Dadiji for always encouraging me to study as much as I want and giving me blessing every day. I am quite fortunate to have such wonderful in-laws; they greatly aided me in finishing this voyage. They constantly inspired me to maintain my calm and put in my best effort. My brother for always listening my professional and personal problems, and taking stress out of me. A very big thank you and love to my husband Akshay for always being so supportive. He used to listen my everyday story of passed and failed experiments. Even though he has no prior experience with biology background but, he still pays attention and makes an effort to learn molecular biology with me. His support for always taking tension and stress out of me. He consistently gave me a confidence boost and made an effort to provide me breaks from the strain of my lab work. Lastly, I really want to thank my baby in my womb, for never troubling me in my pregnancy journey and letting me to do my lab and thesis work peacefully.

Finally, I would bow my head and thanks to God, for always being my strength and guiding light in my life.

JYOTI SHARMA

ABSTRACT

Surfactants of biological-origin are called biosurfactants. In comparison to synthetic surfactants, biosurfactants are biodegradable, less-toxic to the environment and are functional under extreme environmental conditions (temperatures, pH and salt concentrations). They are structurally more diverse as compared to the synthetic ones. All these properties make them a potential alternative to synthetic surfactants for various applications in petroleum, food, pharmaceuticals, cosmetic, agriculture and bioremediation etc. Despite having better physico-chemical properties and structural diversity, biosurfactant production suffers from limitations such as low productivity, high downstream production cost and lack of knowledge about the metabolic pathways for their production at larger scale for commercialization.

Although, several microorganisms have been reported for biosurfactant production, the latter has been characterized from only a few of them. Furthermore, very few biosurfactant synthesizing microbes have been genetically characterized. This thesis aimed at the production of biosurfactant from a potent bacterium obtained from the oil contaminated soil, its molecular characterization and its utilization in secondary oil recovery.

In this study, a Gram-negative, rod-shaped bacterium was isolated from the oil contaminated soil and its biosurfactant production ability was determined. The isolated bacterium was identified as *Franconibacter* sp. IITDAS19 through 16S rRNA sequencing. The produced biosurfactant was isolated, purified and characterized by TLC, FTIR, GC-MS and LC-MS. It was identified as a glycolipid. It was found to be very stable at a wide

range of temperatures, pH and salt concentrations. It could reduce the surface tension of water from 71 mN/m to 31 mN/m. It also showed very high efficacy towards both aliphatic and aromatic hydrocarbons. The concentration of the crude biosurfactant was found to be 4.1 ± 0.5 g/l. The emulsion produced from the biosurfactant was found to be stable for more than 2 months. All these properties make the isolated biosurfactant useful for industrial scale applications. Whole genome sequencing of *Franconibacter* sp. IITDAS19 was also conducted to find out the genes responsible for the biosurfactant production. The genes were overexpressed in a heterologous host and in the native host for enhanced production of the biosurfactant. The produced biosurfactant showed about 63% recovery of residual oil in a sand pack column, which establishes its potential value for microbial enhanced oil recovery.

Thus, this study has led to the production and characterization of a potent biosurfactant from the oil contaminated soil isolate, named as *Franconibacter* sp. IITDAS19. Biosurfactant potential for microbial enhanced oil recovery was also confirmed. This study also provides understanding of the genetic basis of biosurfactant biosynthesis in *Franconibacter* sp. IITDAS19. This can be utilized for metabolic engineering for enhanced biosurfactant production in future.

सार

जैविक-मूल के सर्फैक्टेंट्स को बायोसर्फैक्टेंट्स कहा जाता है। सिंथेटिक सर्फेक्टेंट की तुलना में, बायोसर्फैक्टेंट बायोडिग्रेडेबल, पर्यावरण के लिए कम विषैले होते हैं और अत्यधिक पर्यावरणीय परिस्थितियों (तापमान, पीएच) के तहत कार्यात्मक होते हैं। सिंथेटिक की तुलना में वे संरचनात्मक रूप से अधिक विविध हैं। ये सभी गुण उन्हें पेट्रोलियम, भोजन, फार्मास्यूटिकल्स, कॉस्मेटिक, कृषि और बायोरेमेडिएशन आदि में विभिन्न अनुप्रयोगों के लिए सिंथेटिक सर्फेक्टेंट का एक संभावित विकल्प बनाते हैं। बेहतर भौतिक-रासायनिक गुणों और संरचनात्मक विविधता के बावजूद, बायोसर्फैक्टेंट उत्पादन कम उत्पादकता, उच्च जैसी सीमाओं से ग्रस्त है। डाउनस्ट्रीम उत्पादन लागत और व्यावसायीकरण के लिए बड़े पैमाने पर उनके उत्पादन के लिए चयापचय मार्गों के बारे में ज्ञान की कमी।

हालाँकि, बायोसर्फैक्टेंट उत्पादन के लिए कई सूक्ष्मजीवों की सूचना दी गई है, बाद वाले को उनमें से कुछ की विशेषता बताई गई है। इसके अलावा, केवल कुछ बायोसर्फैक्टेंट उत्पादक बैक्टीरिया के आनुवंशिक लक्षण वर्णन किए गए हैं। इस थीसिस का उद्देश्य तेल दूषित मिट्टी से पृथक एक शक्तिशाली जीवाणु से बायोसर्फैक्टेंट का उत्पादन, इसके आणविक लक्षण वर्णन और द्वितीयक तेल पुनर्प्राप्ति में इसका अनुप्रयोग है।

इस अध्ययन में, एक ग्राम-नकारात्मक, छड़ के आकार के जीवाणु को तेल दूषित मिट्टी से अलग किया गया था और इसकी बायोसर्फैक्टेंट उत्पादन क्षमता निर्धारित की गई थी। अलग किए गए जीवाणु की पहचान 16S rRNA अनुक्रमण के माध्यम से फ्रेंकोनीबैक्टर एसपी IITDAS19 के रूप में की गई। उत्पादित बायोसर्फैक्टेंट को टीएलसी, एफटीआईआर, जीसी-एमएस और एलसी-एमएस द्वारा पृथक, शुद्ध और अभिलक्षणित किया गया था। इसकी पहचान ग्लाइकोलिपिड के रूप में की गई थी। यह तापमान, पीएच और नमक सांद्रता की

विस्तृत श्रृंखला में बहुत स्थिर पाया गया। यह पानी के सतही तनाव को 71 mN/m से घटाकर 31 mN/m कर सकता है। इसने स्निग्ध और सुगंधित हाइड्रोकार्बन दोनों के प्रति बहुत अधिक प्रभावकारिता दिखाई। क्रूड बायोसर्फैक्टेंट की सांद्रता 4.1 ± 0.5 ग्राम/लीटर पाई गई। बायोसर्फैक्टेंट से उत्पन्न इमल्शन 2 महीने से अधिक समय तक स्थिर पाया गया। ये सभी गुण पृथक बायोसर्फैक्टेंट को औद्योगिक पैमाने के अनुप्रयोगों के लिए उपयोगी बनाते हैं। फ्रेंकोनीबैक्टर एसपी IITDAS19 के पूरे जीनोम अनुक्रमण को बायोसर्फैक्टेंट उत्पादन के लिए जिम्मेदार जीन का पता लगाने के लिए भी आयोजित किया गया था। बायोसर्फैक्टेंट के वर्धित उत्पादन के लिए हेटेरोलॉगस होस्ट और मूल होस्ट में जीनों को ओवरएक्सप्रेस्ड किया गया था। उत्पादित बायोसर्फैक्टेंट ने सैंड पैक कॉलम में अवशिष्ट तेल की लगभग 63% रिकवरी दिखाई, जो माइक्रोबियल संवर्धित तेल रिकवरी के लिए इसके संभावित मूल्य को स्थापित करता है।

इस प्रकार, इस अध्ययन ने तेल दूषित मिट्टी के आइसोलेट से एक शक्तिशाली बायोसर्फैक्टेंट के उत्पादन और लक्षण वर्णन को प्रेरित किया है, जिसे फ्रेंकोनीबैक्टर एसपी IITDAS19 नाम दिया गया है। माइक्रोबियल संवर्धित तेल रिकवरी के लिए बायोसर्फैक्टेंट क्षमता की भी पुष्टि की गई। यह अध्ययन फ्रेंकोनीबैक्टर एसपी IITDAS19 में बायोसर्फैक्टेंट बायोसिंथेसिस के आनुवंशिक आधार की समझ भी प्रदान करता है। इसका उपयोग भविष्य में उन्नत बायोसर्फैक्टेंट उत्पादन के लिए मेटाबोलिक इंजीनियरिंग के लिए किया जा सकता है।

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ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
%	Percentage
α	Alpha
β	Beta
H_2O	Water
Ω	Ohm
λ	Lambda
µg	Microgram
µl	Microlitre
µm	Micrometer
16S rRNA	16 Svedberg ribosomal Ribonucleic Acid
Amp	Ampicillin
APS	Ammonium per sulphate
AOR	Additional Oil Recovery
ANI	Average Nucleotide Identity
AT	Acyl-Transferases
BLAST	Basic Local Alignment Search Tool
BSM	Basal Salt Medium
bp	Base pair
cDNA	Complimentary DNA
CDSs	Coding DNA sequences

CFU	Colony forming unit
CMC	Critical Micelle Concentration
CSTR	Continuous Stirred Tank Reactor
cm^{-1}	Per centimeter
DMF	Dimethylformamide
DNA	Deoxyribose Nucleic Acid
DMSO	Dimethyl sulfoxide
dNTP	Deoxy ribose nucleotide triphosphate
E_{24}	Emulsion Index after 24 hours
EB	Elution buffer
EDTA	Ethylene diamine tetra acetic acid
EtBr	Ethidium bromide
EOR	Enhanced Oil Recovery
FT-IR	Fourier Transform Infrared Spectroscopy
GC	Guanine-cytosine
GFPuv	Green fluorescent protein
gm	Gram
GBDP	Genome Blast Distance Phylogeny
GC-MS	Gas Chromatography coupled to Mass Spectroscopy
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
IOR	Improved Oil Recovery
IPTG	Isopropyl β-D thio galactopyranoside

KanR	Kanamycin resistance
kb	Kilobase
kDa	Kilo Dalton
kV	Kilovolt
LA	Luria Agar
LB	Luria Broth
LB-ADSA	Low Bond Axisymmetric Drop Shape Analysis
LC-MS	Liquid Chromatography coupled to Mass Spectroscopy
m/z	Mass to charge ratio
MEOR	Microbial Enhanced Oil Recovery
mins	Minutes
mg	Milligram
ml	Microliter
mM	Millimolar
mN/m	Milli Newton per meter
MALDI	Matrix assisted laser desorption ionization
MTCC	Microbial Type Culture Collection
NaOH	Sodium Hydroxide
NCBI	National Center of Biotechnology Information
NEB	New England Biolabs
ng	Nanogram
NGS	Next-generation sequencing
Nm	Nanometer

NMR	Nuclear Magnetic Resonance
OD	Optical Density
PAH	Polyaromatic Hydrocarbon
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PGAP	Prokaryotic Genome Annotation Pipeline
qPCR	Quantitative PCR
qRT-PCR	Quantitative Reverse transcriptase PCR
Rpm	Revolutions Per Minute
RNA	Ribonucleic acid
rRNA	Ribosomal Ribonucleic acid
RNase	Ribonuclease
RT	Room temperature
RT-PCR	Real-time PCR
RSM	Response Surface Methodology
SDS	Sodium Dodecyl Sulfate
SRA	Sequence Read Archive
Sec	Second
SSU	Smaller Subunit
TAE	Tris-glacial acetic acid-EDTA
TE	Tris-EDTA
TLC	Thin Layer Chromatography

TEMED	N N N’ N’ tetramethylene diamine
tRNA	Transfer Ribonucleic acid
Tris	Tris (hydroxymethyl) amino methane
UV	Ultraviolet
v/v	Volume per Volume
V	Volts
WGS	Whole Genome Sequencing
w/v	Weight per Volume
X-gal	5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside