

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

JYOTI SHARMA



**DEPARTMENT OF BIOCHEMICAL ENGINEERING AND
BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI
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by

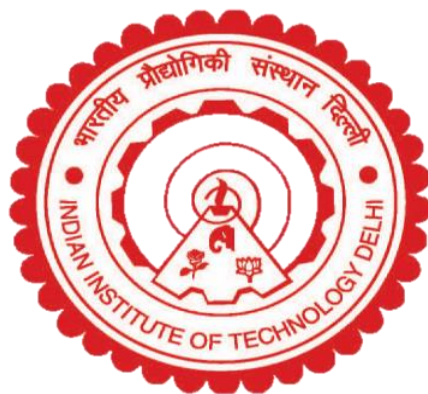
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Submitted

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

to the



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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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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 सर्फैक्टेंट 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 सर्फैक्टेंट 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 अभिलक्षित 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। कूड 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। फ्रैंकोनीबैक्टर 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 ओवरएक्सप्रेसड 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 फ्रैंकोनीबैक्टर एसपी IITDAS19 नाम दिया गया है। माइक्रोबियल संवर्धित तेल रिकवरी के लिए बायोसर्फैक्टेंट क्षमता की भी पुष्टि की गई। यह अध्ययन फ्रैंकोनीबैक्टर 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।

CONTENTS

Certificate	i
Acknowledgements	ii
Abstract	v
Contents	ix
List of figures	xvi
List of Tables	xxii
Abbreviations and Symbols	xxiv
Chapter 1 Introduction and Objectives	1
Chapter 2 Review of Literature	
2.1 Surfactants	4
2.1.1 Biosurfactants	6
2.2 Classification of biosurfactants	8
2.2.1 Classification based on molecular weight	14
2.2.2 Classification based on chemical composition	14
2.2.2.1 Glycolipids	19
2.2.2.2 Lipopeptides and lipoproteins	18
2.2.2.3 Fatty acids, phospholipids, and neutral lipids	20
2.2.2.4 Polymeric biosurfactants	21
2.2.2.5 Particulate biosurfactants	22
2.3 Natural role of biosurfactants	23
2.4 Environmental factors affecting the biosurfactant production	24
2.5 Advantages of biosurfactants over chemical surfactants	27

2.6	Applications of Biosurfactants	31
2.7	Isolation of biosurfactant producing microorganisms and analysis of the produced biosurfactant	40
2.7.1	Some of the commonly used screening methods	41
2.7.2	Purification of biosurfactants	45
2.7.3	Characterization of biosurfactants	47
2.8	Glycolipids and its biosynthesis pathways	50
2.9	Rhamnolipids	52
2.9.1	Rhamnolipid producing strains	53
2.9.2	Rhamnolipid biosynthesis and genetic regulation	55
2.10	Commercial production of biosurfactants	57
2.11	Process optimization studies for the biosurfactant production	61
2.11.1	Optimization of medium composition	61
2.11.2	Cultivation strategies and aeration systems	64
2.11.3	Downstream processing	65
2.11.4	Genetic engineering for increased yield of biosurfactant	66
2.11.4.1	Improved yield through random mutagenesis	67
2.11.4.2	Metabolic engineering for enhanced production	67
2.12	Need of biosurfactants	73

Chapter 3 Materials and Methods

3.1	Bacterial strains, primers and plasmids used in the present study	75
3.2	Isolation and screening of biosurfactant producing bacteria	78
3.2.1	Sample collection and isolation	78

3.2.2	Screening assay for biosurfactant production	79
3.2.2.1	Emulsification index assay	79
3.2.2.2	Oil spreading assay	80
3.2.2.3	Drop-collapse assay	80
3.3	Identification of the strain	80
3.3.1	Colony morphology and Gram staining	81
3.3.2	16S rRNA sequencing	81
3.3.3	Scanning Electron Microscopy	82
3.4	Blue agar plate assay	82
3.5	Determination of biomass and biosurfactant concentration	84
3.6	Determination of contact angle	84
3.7	Determination of surface tension	85
3.8	Critical micelle concentration of the produced biosurfactant by IITDAS19	85
3.9	Emulsification of different hydrocarbons by the produced biosurfactant	86
3.10	Effect of extreme environmental conditions on emulsion stability	86
3.11	Purification and characterization of biosurfactant	87
3.11.1	Extraction of biosurfactant	87
3.11.2	Purification of biosurfactant	88
3.11.3	Characterization of biosurfactant	89
3.11.3.1	Thin layer chromatography	89
3.11.3.2	Characterization of the spots observed on the TLC plate	90
3.11.3.3	Fourier-transform infrared spectroscopy	90

3.11.3.4	Liquid chromatography-mass spectrometry	91
3.11.3.5	Gas chromatography-mass spectrometry	91
3.12	Characterization of genes for biosurfactant biosynthesis	92
3.12.1	Genomic DNA isolation	92
3.12.2	Whole genome sequencing	93
3.12.3	Plasmid isolation from <i>E. coli</i> (manual method)	94
3.12.4	Plasmid isolation from <i>E. coli</i> (kit method)	96
3.12.5	Preparation of competent <i>E. coli</i> cells (Chemical method)	96
3.12.6	Transformation in chemically prepared <i>E. coli</i> competent cells	97
3.12.7	Agarose Gel Electrophoresis	98
3.12.8	Restriction digestion with specific enzymes	98
3.12.9	Polymerase chain reaction	99
3.12.10	Gel extraction	101
3.12.11	Ligation	101
3.12.12	Preparation of electrocompetent cells of IITDAS19	103
3.12.13	Electroporation of plasmid into the electrocompetent cells of IITDAS19	103
3.12.14	Construction of plasmid pJS1 and pJS2 (cloning of probable <i>AT</i> genes)	104
3.12.15	Expression of genes for biosurfactant biosynthesis in a heterologous host	104
3.12.16	SDS-PAGE for the protein analysis	105
3.12.16.1	Sample preparation for the SDS- PAGE	107
3.12.17	Expression of genes for biosurfactant biosynthesis in native host	108

3.12.18	RNA Isolation	108
3.12.19	cDNA synthesis	119
3.12.20	Quantitative Reverse Transcription Polymerase Chain Reaction	110
3.12.21	Expression of probable <i>AT</i> genes with respect to growth and biosurfactant production in IITDAS19	112
3.12.22	Growth kinetics of recombinants and enhanced production of biosurfactants	112
3.12.23	Replication of different plasmids in IITDAS19	113
3.12.24	Yield of biosurfactant and biomass from the recombinant bacteria	113
3.12.25	Preparation of electrocompetent cells for recombineering	113
3.12.26	Genomic deletion of <i>AT1_Y/AT2_Y</i> gene	114
3.13	Application of the produced biosurfactant	114
3.13.1	Soil washing	115
3.13.2	Sand packed column method	115

Chapter 4 Results and Discussion

4.1	Isolation and identification of a biosurfactant producing bacterium <i>Franconibacter</i> sp. IITDAS19	118
4.1.1	Isolation of biosurfactant producing bacteria from oil-contaminated soil	118
4.1.2	Emulsification assay for screening of the isolated colonies	122
4.1.3	Drop collapse assay for screening of isolates	127
4.1.4	Oil displacement assay for screening of the isolates	128
4.1.5	Identification of the isolates	131
4.1.6	Selection of IITDAS19 for further study	134
4.1.7	Emulsification index assay with different types of oils	136

4.1.8	Blue agar assay	138
4.1.9	Stability of emulsion under different environmental conditions	139
4.1.10	Contact angle measurement	143
4.1.11	Surface tension measurement	144
4.2	Purification and characterization of produced biosurfactant	146
4.2.1	Biosurfactant extraction	146
4.2.2	Purification of the crude biosurfactant	146
4.2.3	Critical micelle concentration	149
4.2.4	Characterization of the purified biosurfactant	151
4.2.4.1	Thin layer chromatography	151
4.2.4.2	FTIR analysis of the produced biosurfactant	153
4.2.4.3	GC-MS analysis of the purified biosurfactant	154
4.2.4.4	LC-MS analysis of the pure biosurfactant	155
4.3	Characterization of genes for biosurfactant biosynthesis	158
4.3.1	Whole genome sequencing of IITDAS19	158
4.3.2	Growth monitoring of IITDAS19 with biosurfactant production	163
4.3.3	Gene expression of probable <i>AT</i> genes at various OD ₆₀₀	166
4.3.4	The organization of <i>AT1_Y/AT2_Y</i> genes in the genome of IITDAS19	169
4.3.5	Cloning of <i>AT</i> genes	170
4.3.6	Expression analysis of pJS1 and pJS2	174
4.3.7	Growth kinetics and biosurfactant production in recombinant bacteria (<i>E. coli</i>)	175
4.3.8	Biosurfactant production in the native host (IITDAS19)	178

4.3.9	Growth kinetics and biosurfactant production in the recombinant IITDAS19	178
4.3.10	Biosurfactant concentration and biomass of the recombinant bacteria	182
4.3.11	Recombineering in <i>Franconibacter</i> sp.	184
4.3.11.1	Optimization of various parameters for electroporation in <i>Franconibacter</i> sp.	184
4.3.12	Replication of various plasmid replicons in <i>Franconibacter</i> sp.	187
4.4	Application of the produced biosurfactant in enhanced oil recovery	190
4.4.1	Soil washing method	190
4.4.2	Sand packed column method	191

Chapter 5 Conclusions, Major findings and Future prospects

Conclusions	194
Major findings	196
Future prospects	198
References	199
Appendix	240
Curriculum vitae	254

LIST OF FIGURES

Figure No.	Title	Page No.
2.1	Structure of a surfactant monomer and different aggregate forms of micellar structure in polar and non-polar solvents	6
2.2	Structure of rhamnolipids	15
2.3	Structure of the common trehalolipids reported in literature along with the side chains	17
2.4	Structure of sophorolipids	18
2.5	Structure of surfactin	19
2.6	Structure of Lichenysin	20
2.7	Structure of Corynomycolic acid	21
2.8	Structure of Emulsan	22
2.9	Schematic diagram of Surface tension measurement by Du-Nouy-Ring method using tensiometer	42
2.10	Fitting of Young's equation for the calculation of contact angle (θ_Y)	44
2.11	Synthesis of the precursors for the glycolipids (a) when different carbohydrate substrates are used with enzymes (i) phosphofructokinase (ii) pyruvate kinase; and (iii) isocitrate dehydrogenase are responsible for the flow of carbon, (b) when hydrocarbon substrates are used with enzymes (iv) isocitrate lyase; (v) malate synthase; (vi) phosphoenolpyruvate carboxykinase; and (vii) fructose-1-phosphatase are responsible for the carbon flow	52
2.12	Biosynthesis and genetic regulation of rhamnolipids	56

4.1	Different colonies obtained after incubation at different temperatures A. colonies obtained at 30 °C, B. colonies obtained at 37 °C, C. colonies obtained at 50 °C	118
4.2	Pure colonies obtained at 37 °C, named as AS1, AS2, AS3, AS4, AS5, AS6, AS7, AS8	119
4.3	Pure colonies obtained at 30 °C, named as AS9, AS10, AS11, AS12, AS13, AS19	120
4.4	Colonies obtained at 50 °C, named as AS14, AS15, AS16, AS17	121
4.5	EI% from the colonies obtained at 37 °C, only AS 2, AS 5, AS 8 have EI% above 35%	121
4.6	Emulsification index assay from the colonies obtained at 30 °C. Only AS 9, AS 11, AS 19 have EI% above 35%	123
4.7	Emulsification assay performed with the colonies obtained at 50 °C	124
4.8	EI assay of the selected isolates with aromatic hydrocarbon (Toluene)	125
4.9	EI assay of the selected isolates with aliphatic hydrocarbon (Heptane)	126
4.10	Drop collapse assay of all the isolates A. with used motor oil Isolates with the positive drop collapse assay results are in circles. Tween 20 was used as a positive control and distilled water was used as a negative control	128
4.11	Oil displacement assay of all the isolates with used motor oil. Isolates with the highest positive oil displacement assay results are in circles. Tween 20 was used as a positive control and distilled water was used as a negative control	129
4.12	A. and B. colony and cell morphology of AS5 C. amplification of 16S rRNA	131
4.13	A. and B. colony and cell morphology of AS8 C. amplification of 16S rRNA	132
4.14	A. and B. colony and cell morphology of AS9 C. amplification of 16S rRNA	133

4.15	A. and B. colony and cell morphology of AS11 C. amplification of 16S rRNA	133
4.16	A. and B. colony and cell morphology of AS19 C. amplification of 16S rRNA	134
4.17	Cell morphology of IITDAS19 visualized through scanning electron microscopy with different magnification (left image is 6000 times magnified and right image is 9000 times magnified)	135
4.18	Positive result in all qualitative assays A. Drop collapse assay with used motor oil B. Drop collapse assay with mustard oil C. Oil displacement assay indicates the presence of biosurfactant in cell free supernatant	136
4.19	Emulsification index assay with different oil A. by using cell free supernatant of IITDAS19 B. using chemical surfactant i.e., 5% SDS solution	147
4.20	Halo formation by the cell free supernatant of IITDAS19 around the well in blue agar assay	139
4.21	Effect of different A. Temperatures (Degrees) B. pH C. Salt concentration (%) on biosurfactant activity	142
4.22	Biosurfactant drop on the parafilm captured by smartphone. The image was cropped and thresholded to get a black and white image. Angles were fitted using drop analysis LB-ADSA to calculate the contact angle automatically	144
4.23	Drop hanging from the needle is shown in the above image. The drop area is then cropped and automatically analyzed with the help of drop analysis plugin in Fiji (ImageJ)	145
4.24	A. Oil displacement assay with purified biosurfactant B. Surface tension measurement through tensiometer	149
4.25	The CMC value was found to be 80 mg/l. The surface tension of the water was measured through tensiometer, which reduced from 71 mN/m to 31 mN/m	150

4.26	Characterization of Biosurfactant by Thin Layer Chromatography, ninhydrin test (left), carbohydrate test (center) and lipid presence test (right)	152
4.27	Characterization of the pink spot observed in Ninhydrin test. A. FTIR and B. GC-MS was performed to determine any protein presence in the sample	153
4.28	FTIR spectrum of the purified biosurfactant produced by IITAS19 showing absorption peaks at various wavenumber (cm^{-1}). FTIR results reveal the presence of the long fatty acid chains and sugar molecules as aldehyde, ketone or carboxylic groups, C-H and O-H stretches	154
4.29	GC-MS analysis of the biosurfactant confirms the presence of fatty acid chains and sugar molecules at different retention times, which suggests that the biosurfactant is a glycolipid	155
4.30	The LC-MS spectrum in the positive mode revealed two molecules ($M+\text{Na}^+$) ions at 964.9 m/z represents the presence of glycolipid and 851.45 m/z represents the presence of glycolipid, these both form the complex ion. Figure a) and b) shows the fractional fragmentation of both the peaks	157
4.31	Isolated Genomic DNA of IITDAS19 checked on 1% agarose gel	159
4.32	Phylogenetic tree of IITDAS19 showing closely related species. The distances between the microorganisms were calculated using GBDP (Genome Blast Distance Phylogeny)	160
4.33	Map of the assembled IITDAS19 genome showing clustering of genes for major metabolic pathways	161
4.34	A. Growth curve with biosurfactant production. Doubling time of IITDAS19 is around 6 hours and B. the biosurfactant production is increasing with the increasing OD_{600}	165
4.35	1.5% agarose gel RNA isolated from the IITDAS19 cells of different OD_{600} . Lane 1: sample of $\text{OD}_{600}= 1.2$, Lane 2: sample of $\text{OD}_{600}= 3.3$,	166

	Lane 3: sample of OD ₆₀₀ = 3.9, sample of OD ₆₀₀ = 4.5, Lane M: 1 kb DNA ladder	
4.36	Gene expression of probable <i>AT</i> genes at various OD ₆₀₀ . At an OD ₆₀₀ = 4.5 (D), approximately 1-million-fold change in the expression of the <i>AT1_Y</i> and <i>AT2_Y</i> as compared to the other probable <i>AT</i> genes was observed	168
4.37	Genome map showing the organization of selected <i>AT1_Y</i> and <i>AT2_Y</i> genes	169
4.38	Agarose gel electrophoresis showing vector and insert preparation A. amplification of <i>AT1_Y/AT2_Y</i> genes Lane M: showing 1 kb DNA ladder, Lane 1: showing amplification of <i>AT2_Y</i> gene (1001 bp) Lane 2: showing amplification of <i>AT1_Y</i> gene (914 bp). B. isolation of pDJ2 plasmid, Lane M: showing 1 kb DNA ladder, Lane 1: showing isolated pDJ2 plasmid. C. Digestion of plasmid with <i>Nde</i> I and <i>Xho</i> I, Lane M: showing 1 kb DNA ladder, Lane 1: showing digested plasmid with the release of 200 bp. D. Vector preparation after gel extraction, Lane M: showing 1 kb DNA ladder, Lane 1: showing the purified vector. E. Preparation of insert, Lane M: showing 1 kb DNA ladder, Lane 1: purified gel eluted <i>AT2_Y</i> insert and Lane 2: purified <i>AT1_Y</i> insert	171
4.39	Cloning strategy diagram for cloning of <i>AT1_Y</i> and <i>AT2_Y</i> in pDJ2 to construct pJS1 and pJS2	172
4.40	Screening of the transformants. A. Screening of the pJS1 clones by colony PCR using <i>ptac</i> internal forward primer and reverse primer of <i>AT1_Y</i> , Lane M: 1 kb DNA ladder, Lane 1 and 2: colony PCR of <i>E. coli</i> transformed with pJS1 with the expected amplicon of 1538 bp. B. Isolation of pJS1 plasmid and its digestion with <i>Nde</i> I and <i>Xho</i> I, Lane M: 1 kb DNA ladder, Lane 1: digested pJS1 with the expected release of 914 bp and Lane 2: showing the isolated plasmid of pJS1. C. Screening of the pJS2 clones by colony PCR using <i>ptac</i> internal forward primer and reverse primer of <i>AT2_Y</i> , Lane M: 1 kb DNA ladder, Lane 1 and 2: positive result of pJS2 by colony PCR with the	173

	expected outcome of 1624 bp. D. Digestion of pJS2 with <i>Nde</i> I and <i>Xho</i> I, Lane M: 1 kb DNA ladder, Lane 1: digested pJS2 with the expected release of 1001 bp.	
4.41	The SDS-PAGE image showing the expression of AT1_Y (in A.) and AT2_Y (in B.) in <i>E. coli</i> after IPTG induction of 3 hours, 5 hours and overnight	175
4.42	A. Growth curve of wild type <i>E. coli</i> , recombinant <i>E. coli</i> with AT1_Y and recombinant <i>E. coli</i> with AT2_Y. B, C & D. Growth dependent biosurfactant production from the wild type <i>E. coli</i> , recombinant <i>E. coli</i> with AT1_Y and recombinant <i>E. coli</i> with AT2_Y at different OD ₆₀₀	177
4.43	Agarose gel electrophoresis showing the isolated plasmids from the positive transformed colonies in the native host (IITDAS19). Lane 1,2,3 show the plasmid pJS1 isolated from the recombinant IITDAS19, Lane 4,5,6 show the plasmid pJS2 isolated from the recombinant IITDAS19, Lane M:1 kb DNA ladder	178
4.44	A. Growth curve of wild type IITDAS19, recombinant IITDAS19 with AT1_Y and recombinant IITDAS19 with AT2_Y. B, C and D Growth dependent biosurfactant production from the wild type IITDAS19, recombinant IITDAS19 harboring AT1_Y and recombinant IITDAS19 containing AT2_Y, respectively, at different OD ₆₀₀ and their respective EI% are mentioned below the image	181
4.45	Agarose gel electrophoresis of the isolated plasmids from <i>E. coli</i> DH5 α , A. Lane M: 1 kb DNA ladder, Lane 1: plasmid pSC101. B. Lane M: 1 kb DNA ladder, Lane 1: isolated pBBR1. C. Lane M: 1 kb DNA ladder, Lane 1: isolated RK2	188
4.46	A. Agarose gel electrophoresis of the isolated plasmids Lane M: 1 kb DNA ladder, Lane 1: plasmid pSIM7, Lane 2: plasmid pSIM9 isolated from the transformed IITDAS19 colonies. B.-streaked transformants on chloramphenicol (10mg/ml stock solution) plates left: containing pSIM9 in IITDAS19, right: containing pSIM7 in IITDAS19	189

LIST OF TABLES

Table No.	Title	Page No.
2.1	Types of chemical surfactants	8
2.2	Classification of biosurfactants and their producing organisms	9
2.3	Rhamnolipid producing strains with their yield (g/l)	54
2.4	Major biosurfactant producing companies and their application areas	59
2.5	Various low-cost waste substrates used for biosurfactant production	63
2.6	Improved production of rhamnolipids in the host microorganisms with the final yield of biosurfactant produced	70
3.1	Bacterial strains used in the present study	75
3.2	Plasmids used in the present study	75
3.3	Primers used in the present study	76
3.4	Composition of Mineral salt agar medium	83
3.5	Composition of Trace elements solution	83
3.6	Ingredients of a PCR reaction with the quantity	99
3.7	PCR reaction conditions	100
3.8	Components added in 10 µl ligation mixture reaction	102
3.9	SDS- PAGE 12% separating gel composition	105
3.10	SDS- PAGE 5% stacking gel composition	106
3.11	Components for the cDNA synthesis reaction mixture	109
3.12	Components of qRT-PCR reaction mixture	111

3.13	Conditions for the qRT-PCR reaction	111
4.1	EI% from the colonies obtained at 37 °C	123
4.2	EI% from the colonies obtained at 30 °C	124
4.3	EI% of the selected isolates with aromatic hydrocarbon (Toluene)	125
4.4	EI% of the selected isolates with aliphatic hydrocarbon (Heptane)	126
4.5	Summary of results obtained using the various biosurfactant screening methods	130
4.6	Assembly statistics for IITDAS19 genome	159
4.7	Similarity percentage of shortlisted 9 <i>AT</i> genes from the whole genomic data of IITDAS19 with the already known <i>AT</i> genes [C1 (<i>rhIB</i>) and C2 (<i>rhIA</i>) (yellow highlighted)], made by using T-Coffee multiple sequence alignment tool	162
4.8	Selection of probable biosurfactant synthesis genes from the whole genome data	162
4.9	Biosurfactant concentration (g/l) with respect to biomass (g/l), produced by wild type and recombinant <i>Franconibacter</i> sp. IITDAS19	182
4.10	Optimization of the various parameters for electroporation in <i>Franconibacter</i> sp.	185
4.11	Parameters used in sand-packed column method for Enhanced oil recovery	192

ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
%	Percentage
α	Alpha
β	Beta
H ₂ O	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
<i>AT</i>	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