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Biosurfactant Bacteria from Producing Petrol Pump Soil Sample

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Abstract: *Amphiphilic substances known as biosurfactants are generated extracellularly or discharged extracellularly by microorganisms on cell surfaces. They contain hydrophilic and hydrophobic moieties, which lessen surface and interface interfacial tension between molecules. The current study concentrated on isolating bacteria that produce biosurfactants from soil samples taken from gas station pumps and evaluating their potential using a variety of standard techniques. The potential of isolates to produce biosurfactant was tested using oil displacement methods (biosurfactant activity). Additionally, studies utilising diesel and engine oil as a source of hydrocarbons were conducted. It was discovered that the soil-isolated bacteria Micrococcus ap. and Kocuria rosea had the ability to produce biosurfactants. The biosurfactant activity of the Kocuria rosea A7 biosurfactants was equal to diesel and engine.*

Keywords: Biosurfactants, bacteria, isolation, Micrococus sp., Kocuria sp

I. INTRODUCTION

The solubilization of microbial biofilms is one of the known uses for microbial biosurfactants, which are surface active substances with emulsifying capabilities produced by fungus, yeast, and bacteria. Due to their surface activity, biosurfactants may be involved in disease; however, for security and regulatory reasons, production strains should be nonpathogenic. Bacteria are found everywhere in nature, which allows them to live in any environment by creating biofilms on any suitable substrate. Both inside the human body and in the environment, biofilms can house infectious human agents. Both hydrophobic and hydrophilic domains are present. One of the most significant species in the human gastrointestinal tract may be *E. coli*, which may both form in vitro and in vivo biofilms. Surfactants, which stand for "surface-active agents," are essentially chemical substances that reduce a liquid's surface tension as well as the tension at the interfaces between liquids and solids. These surfactants are known as biosurfactants because they are produced by a wide range of microorganisms, including yeasts, bacteria, and filamentous fungi (1). In addition to acting as detergents, wetting agents, emulsifiers, foaming agents, and dispersants, biosurfactants also exhibit a variety of other qualities. These are often organic substances that have both hydrophobic and hydrophilic properties and are classified as amphiphilic. The hydrophobic (non-polar) component of the biosurfactant, which may contain a long chain of fatty acids, hydroxyl fatty acids, or -alkyl-hydroxy fatty acids, is insoluble in water. A carbohydrate, an amino acid, or another polar hydrophilic end (2,3).

The two types of biosurfactants—low molecular weight and high molecular weight molecules—can be distinguished by the first's lower surface and interfacial tensions and the latter's tighter adhesion

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to surfaces (4,9,10). Biosurfactant-producing microorganisms contribute to the rapid bioremediation of hydrocarbon-contaminated sites by increasing the contact between pollutant and microorganisms in the presence of the biosurfactant. This increases the bioavailability of hydrocarbons. The goal of the current investigation was to determine whether bacteria isolated from contaminated soil and uncontaminated water samples had the ability to produce biosurfactants.

Biotechnology for industrial and medical uses heavily relies on biosurfactants. The adsorption of biosurfactants changes the surface hydrophobicity, interfering with the microbiological process.

The assemblance of biosurfactants by probiotic bacteria in vivo acts as a defense weapon against most troublesome pathogenic strains in the urogenital and gastrointestinal tracts and on medical devices.

Biosurfactants are surface-active compounds with emulsifying activities. These compounds are described as amphiphilic, due to which they can interact at the interface between aqueous and non-aqueous systems (6,7,8). There are six classes of biosurfactants: glycolipids, lipopeptides or lipoproteins, neutral lipids, phospholipids, substituted fatty acids and lipopolysaccharides (4,5, 11, 12)

II. METHODOLOGY

2.1 Sample Collection

Two contaminated soil samples were taken, one from a location 1 metres away and the other from one that was around 5 metres away from an Indian Oil gas station. The samples were collected in vials, transferred right away to the lab, and kept there at 4°C until additional examination.

2.2 Media Composition

Using nutrient broth and agar media, the bacteria that produce biosurfactants was isolated. The liquid medium's makeup was as follows (gram per litre of distilled water) Peptone, meat extract, sodium chloride, and a solid medium without agar were created by adding 1.5% agar and adjusting the pH to 7.2 0.2 before being autoclaved at 121 °C for 15 minutes at 15 lbs of pressure.

2.3 Inocula Preparation

Samples were serially diluted, plated using the spread plate technique on nutritional agar medium, and incubated for 24 hours at 37°C under aerobic conditions. Cotton buds were utilised to provide diesel as a hydrocarbon source to each petri dish, and a control with no diesel was also kept. Hydrocarbon sources were added to the medium in the form of vapours. These 24 hour-old colonies were inoculated in a 250 ml Erlenmeyer flask containing 1% water-soluble fraction 6 of diesel at 200 rpm for 7 days at 30 °C with 100 ml of nutrition broth medium. 1 millilitre of the inoculum was transferred to 99 ml of nutritional broth containing 1% diesel after one week of incubation, where it was incubated for two days. The process was continued by transferring 1 ml of inoculum to a 99 ml broth kept for one day (10, 12).

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2.4 Oil displacement Test (Biosurfactant Activity Test)

The diameter of the clear zone can be measured via oil displacement, which takes place after introducing a surfactant-containing solution to an oil-water interphase. The effectiveness of a particular biosurfactant in reducing surface tension can be determined by measuring diameter. In this experiment, a petri dish with a diameter of 90 mm was filled with 15 ml of distilled water. Following the injection of 20 l of cell culture supernatant to the oil surface, 100 l of diesel was poured to the water surface. After 30 seconds (7,13,14), the diameter and clean halo were observed and measured.

2.5 Identification of the Strain

Bergey's manual of determinative bacteriology, the IMViC test kit from Hi-media, and Viteck-2 Software were used to identify and characterise the isolates (Jagatap Pathology, Barshi).

III. RESULT AND DISCUSSION

11 isolates were discovered from a total of 5 sample isolates. This study only characterised three strains (A3, A7, and A10) for the generation of biosurfactants. Different staining, culture morphology, and biochemical traits of Micrococcus sp., Kokurea rosea sp., and A3, A7, and A10, respectively, were used to identify them. The distinctive traits of the three strains discovered using Vitek-2 Software are displayed in Table 1.

Table 1: Identification of bacteria by VITEK-2 Software

Well	Test	Mnemonic	Amount /Well (mg)	A3 19515	A7 19515	A10 19515
2	Amygdalin	AMY	0.1875	–	–	+
4	Phosphatidylinositol Phospholipase C	PIPLC	0.015	–	–	+
5	Xylose	dXYL	0.3	–	–	–
8	Arginine Hihydrolase 1	ADH1	0.111	–	–	–
9	Beta Galactosidase	BGAL	0.036	–	–	–
11	Alpha Glucosidase	AGLU	0.036	–	–	–
13	Ala-Phe-Pro Arylamidase	APPA	0.0384	–	–	–
14	Cyclodextrin	CDEX	0.3	–	–	–
15	L Aspartate Arylamidase	AspA	0.024	–	–	–
16	Beta Galactopyranosidase	BGAR	0.00204	–	–	–
17	Alpha-Mannosidase	AMAN	0.036	–	–	–
19	Phosphatase	PHOS	0.0504	–	–	–
20	Leucine Arylamidase	LeuA	0.0234	+	+	+
23	L-Proline Arylamidase	ProA	0.0234	+	–	–
24	Beta Glucuronidase	BGURr	0.0018	–	–	–
25	Alpha-Galactosidase	AGAL	0.036	–	–	–

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Well	Test	Mnemonic	Amount /Well (mg)	A3 19515	A7 19515	A10 19515
26	L-Pyrrolidonyl- Arylamidase	PyrA	0.018	–	–	–
27	Beta-Glucuronidase	BGUR	0.0378	–	–	–
28	Alanine Arylamidase	AlaA	0.0216	+	+	+
29	Tyrosine Arylamidase	TyrA	0.0276	+	+	+
30	D-Sorbitol	dSOR	0.1875	–	–	–
31	Urease	URE	0.15	–	–	–
32	Polymyxin B Resistance	POLYB	0.00093	–	–	–
37	D-Galactose	dGAL	0.3	–	–	–
38	D-Ribose	dRIB	0.3	–	–	–
39	L-Lactate alkalinization	lLATk	0.15	+	–	–
42	Lactose	LAC	0.96	–	–	–
44	N-Acetyl-D-Glucosamine	NAG	0.3	–	–	–
45	D-Maltose	dMAL	0.3	–	–	–
46	Bactracin Resistance	BACI	0.0006	–	–	–
47	Novobiocin Resistance	NOVO	0.000075	–	–	–
50	Growth in 6.5 % NaCl	NC6.5	1.68	–	–	–
52	D-Mannitol	dMAN	0.1875	–	–	(–)
53	D-Mannose	dMNE	0.3	–	–	–
54	Methyl-B-D-Glucopyranoside	MBdG	0.3	–	–	–
56	Pullulan	PUL	0.3	–	–	–
57	D-Raffinose	dRAF	0.3	–	–	–
58	O/129 Resistance (comp. vibrio.)	O129R	0.0084	–	–	–
59	Salicin	SAL	0.3	–	–	–
60	Saccharose / Sucrose	SAC	0.3	–	–	(–)
42	D-Trehalose	dTRE	0.3	–	–	–
63	Arginine Dihydrolase 2	ADH2s	0.27	+	–	–
64	Optochin Resistance	OPTO	0.000399	–	_	–

Sr. No.	Acc No.	Organism identified by Vitek-2	Organisms identified by PubWin
1.	**A3 19515**	*Unidentified*	*Micrococcus spp*
2.	**A7 19515**	*Kocuria rosea*	--
3.	**A10**	*Kocuria rosea*	--

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	19515		

The strain A7 showed more emulsifying ability comparing to A3 and A10 when both of the hydrocarbons i.e. diesel and engine oil.

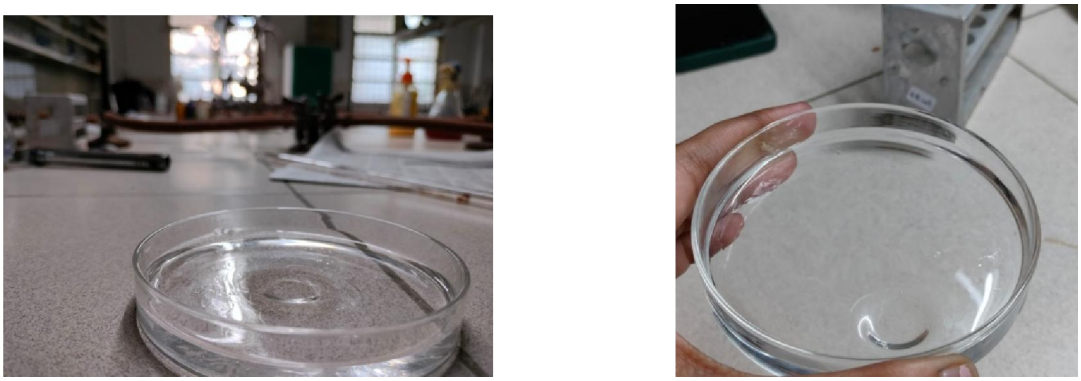

Figure: Oil displacement test

IV. CONCLUSION

It is discovered that the bacteria *Micrococcus sp.* A3, *Kocuria rosea* (A7, A10), create large amounts of biosurfactants. Since *Kocuria rosea* A7 was found on a petrol pumo soil contamination site, it was predicted that it would create biosurfactants. The difference in biosurfactant activity between the experimental and control groups suggested that *Kocuria rosea* may have caused the biosurfactant-producing activity in the presence of the pollutant. To artificially create ecologically friendly surfactants, the relevant gene isolation and subsequent PCR amplification may prove helpful. These two strains may be useful in upcoming research on biosurfactants and bioremediation techniques. The ability of *B. subtilis* and *B. cereus* to produce biosurfactants that aid in the degradation of oil and other hydrocarbon pollutants to the environment may be inferred from the results of this study.

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