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Characterization and biomedical application of Tetradecamethylcycloheptasiloxane, a silicone-type biosurfactant produced by *Streptomyces castaneoglobisporus* AJ9 isolated from solar salt works

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Tetradecamethylcycloheptasiloxane ($C_{14}H_{42}O_7Si_7$), a biosurfactant, was extracted by acid precipitation method from the haloalkaliphilic actinomycetes, *Streptomyces castaneoglobisporus* AJ9 (Gene Bank KC603900.1) and characterized by GC-MS analysis. The purified biosurfactant could effectively degrade dyes such as orange MR, direct violet, cotton red, reactive yellow and nitro green. Antimicrobial screening results showed that biosurfactants could effectively control bacterial pathogens such as *Staphylococcus aureus* and *Vibrio parahaemolyticus* and fungal pathogens such as *Fusarium* sp. and *Penicillium* sp. Even a small diminutive quantity of biosurfactant (100 µg) could totally block the multiplication of White Spot Syndrome Virus (WSSV) in shrimp *in vivo*. The suppression of cancer cells *in vitro* by the biosurfactant was 74 % as confirmed by Response Surface Methodology (RSM) and residual plot analysis. Wheat bran, groundnut oilcake and oilseed cake seem to enhance biosurfactant production.

[**Keywords**: Antimicrobials, Biosurfactants, *Streptomyces castaneoglobisporus*, Tetradecamethylcycloheptasiloxne]

Introduction

The biosurfactants obtained from extremophilic origin have become more important in recent years because of their various medical and industrial applications. Extremophiles are using crucial organisms for yielding novel and valuable bioactive compounds in the current scenario[1]. Halophiles obtained from hypersaline origins offer a wide range of industrial applications including biosurfactants as well[2]. Searching biosurfactants amongst extremophiles seems to be promising as they have selective adaptations to increase stability in adverse environments. Such adaptations can potentially increase their stability in harsh environmental conditions when employed as a tool in applied biotechnology[3]. Due to their exclusively unique halophilic properties, many bacteria and actinomycetes can grow at higher pH and NaCl-containing medium at higher temperature ranges, making contamination-free fermentation processes under non-sterile conditions and in a continual way possible[4].

Biosurfactants are smart compounds because of their skilled novel structure, diversity and versatility and utility for varied industrial, medical and food applications[5-7]. They are the best alternative substitutes against synthetic chemical surfactants because of the versatile characteristics of lesser toxicity, biodegradation and tolerance to high pH and temperature[8]. Henceforth, these biomolecules conclusively emerged as potential agents for the health care and food processing industries. Many medical applications including antimicrobial activities of the different biosurfactant molecules have been reported[6]. Biosurfactants are good therapeutics for multiple diseases including cancer and serve as immunoadjuvants because of their specific surface activity interacting with the cell wall of various organisms. Also, it would act as a carrier for drug molecules in various medical applications[9]. Biosurfactants are reported as effective eco-friendly therapeutic medicine against synthetic chemical drugs and play a very significant role in the field of medicine such as antibacterial, biomaterials for anti-adhesive coatings, immunoadjuvants, transfection agents, inhibitors of fibrin clot formation and probiotic preparations, etc[10].

The production and optimization of biosurfactants have been studied extensively inrecent years by manifold researchers, all concluding that carbon

sources are notable factors to induce production at higher levels[11]. The carbon sources have exerted greater influence to improve the quality and quantity of biosurfactant production[12]. The crucial component hampering the immense need for biosurfactants is those microbes normally yield minute quantities of their molecules. The best example of large yields of sophorolipids inherent within most biosurfactants is so low that recovery of these biosurfactants is both difficult and expensive[13]. Often, the available amount and type of raw material can contribute considerably to the production cost implying thereby that raw materials account for 10–30 % of the total production cost in most biotechnological processes. Thus, to reduce this cost, it is desirable to use low-cost raw materials for the production of biosurfactants[14]. A variety of cheap raw materials, including oils obtained from plants, whey, molasses and oil waste products seemed to influence the increase in the production of biosurfactants[15]. The present study intends to focus on the screening, characterization and production of biosurfactants from the *S. castaneoglobisporus* AJ9 isolated from the Kovalam solar salt works of southern peninsular India.

Material and Methods

The strain *S. castaneoglobisporus* AJ9 (GenBank: KC603900.1) used in this study was already isolated from the sediment sample of the condenser pond in Kovalam (8°05'04.35"N; 77°31'17.07"E) solar salt work, Kanyakumari district, Tamil Nadu, India[16]. The reference strains, *Streptomyces* sp. RJI, RJ2, RJ8 and RJ11 were also used for comparison of *S. castaneoglobisporus* AJ9 which were already isolated from Thamaraikulam (8°07'06.64"N; 77°28'59.26"E) solar salt works and deposited in the laboratory[17].

The biosurfactant detection protocols were performed from the possible haloalkaliphilic *Streptomyces* sp. which was isolated earlier from solar salt works. The protocols followed were: (i) drop collapse assay with mineral oil[18]; (ii) Oil spreading technique by adding weathered crude oil[19]; (iii) Parafilm-M test by adding bacterial extracts to 1 % xylenecyanol in a hydrophobic surface of parafilm M[20]; (iv) Adding kerosene to cell-free extract in equal volume for emulsification activity[21]; and (v) Adding the cell-free extracts in 5 % blood agar plate for hemolytic activity.

For dye degradation potential, a loop full of pure cultures was seeded on the sterile actinomycetes broth and incubated for seven days at 28 °C. After adequate growth of the organism, filtered sterile orange MR, direct violet, cotton red, reactive yellow and nitro green azo dyes (50 mg/l) were added and incubated for 24 h in static conditions. The culture supernatant was obtained by spinning down the culture at 10,000 rpm for 20 min. The degradation activity of the organism was determined by measuring the absorbance of the culture supernatant (2 ml) at OD 542 nm using a UV spectrophotometer[22]. The percentage of decolourization by the organism was calculated using the formula:

$$\text{Decolourization (\%)} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

The emulsification activity index was calculated by following the protocol of Cooper and Goldenberg[21] adding 2 mm of different oils such as coconut oil, gingelly oil, olive oil, crude oil, kerosene, petrol and diesel. The cell-free extract was added in equal amounts of oil and blended in a vortex mixture for two minutes. After 24 h, emulsification stability and emulsification index were measured by calculating the height from the total height of the emulsion layer and then multiplying by 100. The activity was compared with the control by using SDS as a standard.

$$E_{24}\ (\%) = \frac{\text{Total height of the emulsified layer}}{\text{Total height of the liquid layer}} \times 100$$

Based on the potential biosurfactant production, biodegradation and emulsification activities, the biosurfactant was extracted and purified from *S. castaneoglobisporus* AJ9. The biosurfactant was extracted from the cell-free broth by step-by-step purification of acid precipitates using adsorption chromatography from the culture broth after 72 h. After that, 6N HCl was added to the supernatant for acid precipitation to get a pH of 2.0 at 4 °C. The mixture was pelleted by centrifugation at 10,000 rpm for 20 min and the pellet was dissolved in the distilled water. Finally, the biosurfactant was freeze-dried, weighed and sorted. The biosurfactant from *Streptomyces* sp. was again extracted with acetone and dried with the acid in a rotary evaporator under vacuum[23].

In-vitro antibacterial activity was performed by the *Streptomyces* sp AJ9 which yielded biosurfactants against a few human important pathogenic bacteria using the agar diffusion method. Antifungal activity was determined by inoculating the fungal spores into the Potato Dextrose Agar (PDA), incubated for 48 h at 35 °C and measuring the zone of inhibition[24]. To study antiviral activity, biosurfactant was incubated with White Spot Syndrome Virus (WSSV) for 3 h and injected into shrimps. Shrimp mortality wasinvestigated with double-step PCR detection[25].

The Indian white shrimp, *Fenneropenaeus indicus* was injected with a biosurfactant incubated WSSV suspensions (29 °C for 3 h) intramuscularly. Hemolymph was bled from the shrimp after 3rd day post-injection and genomic DNA was extracted[26]. Double-step diagnostic PCR was performed from the genomic DNA template using the WSSV VP28 primer designed by Namita *et al*.[27] and standard PCR protocols were followed. The anticancer activity was performed in HCT-118 colon cancer cell lines with the purified biosurfactants[28].

In order to optimize the production of biosurfactants, Solid State Fermentation (SSF) culture was adopted by supplementing with 1 to 5 % agro-industrial waste including rice bran, wheat bran, ground nut oil cake and oil seed cake. The sterilized solid substrate was inoculated with 2.5 ml of the *S. castaneoglobisporus* AJ9 spore inoculums containing 1.7×10^5 spores. The contents were mixed properly and incubated at 30 °C for 7 days. The culture was harvested from the SSF, biosurfactants were extracted and antibacterial and antifungal activities were performed following the same protocol mentioned in the earlier section. Response Surface Methodology (RSM) and residual plot analysis (Minitab 17 software package) were used to analyze the critical control factors influencing the biosurfactant production of *S. castaneoglobisporus* AJ9 using 1 to 5 % agro-industrial wastes[29,30]. Each independent variable was investigated at a -2 very lower level, -1 lower, 0 medium, +1 higher and +2 very higher levels. Control center points run in the matrix were also included.

Results

Among the different *Streptomyces* sp. screened for various biosurfactant production assays, the *S. castaneoglobisporus* AJ9 was highly positive for drop collapse, oil displacement, parafilm-M test, emulsification and hemolytic assays (Table 1). Next to *S. castaneoglobisporus* AJ9, *Streptomyces* sp. RJ11 had moderate biosurfactant production and the other strains had very little or nil activity.

The dye degradation potential result revealed that *S. castaneoglobisporus* AJ9 had effectively degraded the dye including orange MR, direct violet, cotton red, reactive yellow and nitro green compared to the other *Streptomyces* strains in 48 h. The higher bacterial growth observed in the dyes indicated that degradation was efficient and two ANOVA revealed that degradation potential significantly differed (F = 27.90 (column) and 105.43 (row); P <= 0.001) among the different strains (Fig. 1a).

Better emulsification activities (E_{24}) were observed in *S. castaneoglobisporus* AJ9 against various oils due to higher biosurfactant production compared to other strains including *Streptomyces* sp. RJ2 and RJ11. The E_{24} observed 61.2, 67.2, 64.3, 68.3, 41.11, 28.42 and 31.08 against coconut, gingili, olive, crude, kerosene, petrol and diesel oils, respectively by *S. castaneoglobisporus* AJ9 and the values significantly differed (F = 20.45 (column) and 15.51 (row); P <= 0.001) among all the tested groups (Fig. 1b).

The structural characterization, the peak 6.41 with quality of 96.32 % concluded that the compound was confirmed to be tetradecamethylcycloheptasiloxane with the molecular formula, $C_{14}H_{42}O_7Si_7$ with a molecular weight of 518 (Fig. 2).

Table 1 — Screening of biosurfactants from haloalkaliphilic *Streptomyces* strains isolated from solar salt works

Streptomyces Strains	Biosurfactant screening methods				
	Drop Collapse	Oil Displacement	Parafilm-M test	Emulsification Activity	Hemolytic Activity
AJ9*	+++	+++	+++	+++	++
RJ1**	+	+	-	-	++
RJ2**	++	+	+	-	-
RJ8**	+	-	-	-	+
RJ11**	++	++	+	++	++

-: No activity; +: Low activity; ++: Medium activity and +++: Higher activity; *: *S. castaneoglobisporus* AJ9 isolated from Kovalam solar salt works; **RJ1, RJ2, RJ8 and RJ11: Reference Streptomyces strains isolated from Thamaraikulam solar salt works

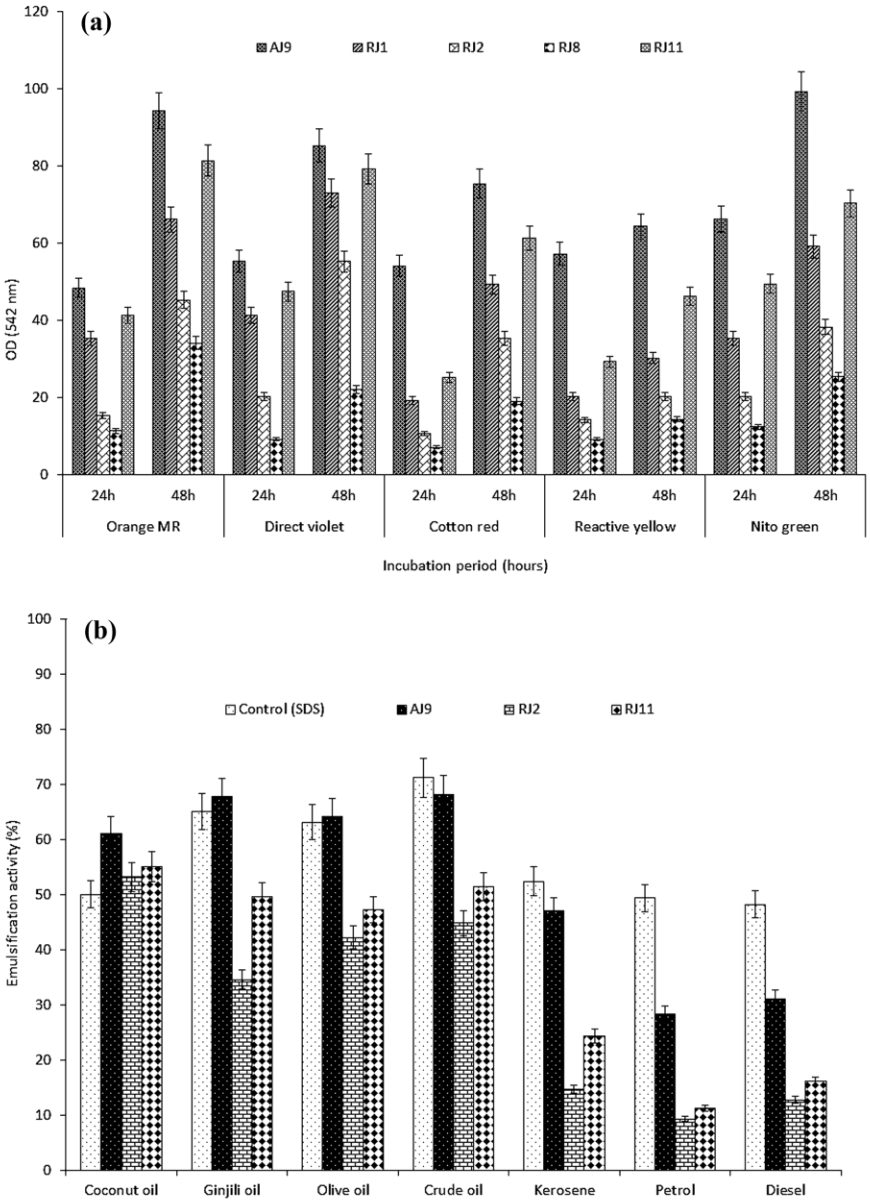

Fig. 1 — a) Dye degradation potential of the cell-free extracts of *Streptomyces* strains. The values differ significantly (F = 27.90 (column) and 105.43 (row); P <= 0.001) – Two-Way ANOVA;and b) Emulsification activity of biosurfactants extracted from *Streptomyces* strains (AJ9, RJ2 and RJ11). The values differ significantly (F = 20.45 (column) and 15.51 (row); P <= 0.001) – Two-Way ANOVA.

The biosurfactant tetradecamethylcycloheptasiloxane effectively suppressed the bacterial pathogens *in vitro*. The antibacterial activity displayed 9.40, 8.73, 11.13, 10.03, 12.90, 15.50, 13.33 and 17.15 mm of the zone of inhibition against *A. hydrophila*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus*, *V. harveyi* and *V. parahaemolyticus*, respectively and differed significantly (P < 0.001). These had antifungal activity against *A. niger*, *Fusarium* sp. and *Penicillium* sp. (Table 2). The double-step PCR detection of WSSV VP28 gene amplification result revealed that there were 100 % PCR signals observed in the control shrimps, whereas the PCR signals significantly (P < 0.0001) reduced to higher contractions of biosurfactant-treated shrimps. The 100 µg biosurfactants treatment helped 100% reduction of PCR positive signals which confirmed that the biosurfactant had a potential antiviral effect against WSSV (Fig. 3a). The anticancer effect is shown in the Figure 3(b) which indicates 100 % cell viability in the control cancer cells. The cell viability had significantly (P < = 0.001) decreased to 93.02, 81.89,

Table 2 — *In vitro* antibacterial and antifungal activity of biosurfactants extracted from *S. castaneoglobisporus* AJ9

In vitro antimicrobial activity (mm of the zone of inhibition)			
Bacterial pathogens	Activity	Fungal pathogens	Activity
Aeromonas hydrophila	9.40 ± 0.70 [a]	*Aspergillus niger*	9.15 ± 0.42 [a]
Bacillus subtilis	8.73 ± 0.65 [b]	*A. flavus*	7.25 ± 0.67 [b]
Escherichia coli	11.13 ± 0.76 [c]	*Candida albicans*	8.64 ± 0.25 [c]
Pseudomonas aeruginosa	10.03 ± 0.45 [d]	*Fusarium* sp	11.98 ± 0.56 [d]
Salmonella typhi	12. 90 ± 1.07 [e]	*Penicillium* sp	14.33 ± 0.95 [e]
Staphylococcus aureus	15.50± 0.85 [f]	*Pythium* sp	6. 52 ± 0.11 [f]
Vibrio harveyi	13. 33± 1.04 [g]		
V. parahaemolyticus	17. 15 ± 1.15 [h]		

The same superscript means (a-h) not differed among the treatment groups ($P < 0.001$) - One way ANOVA

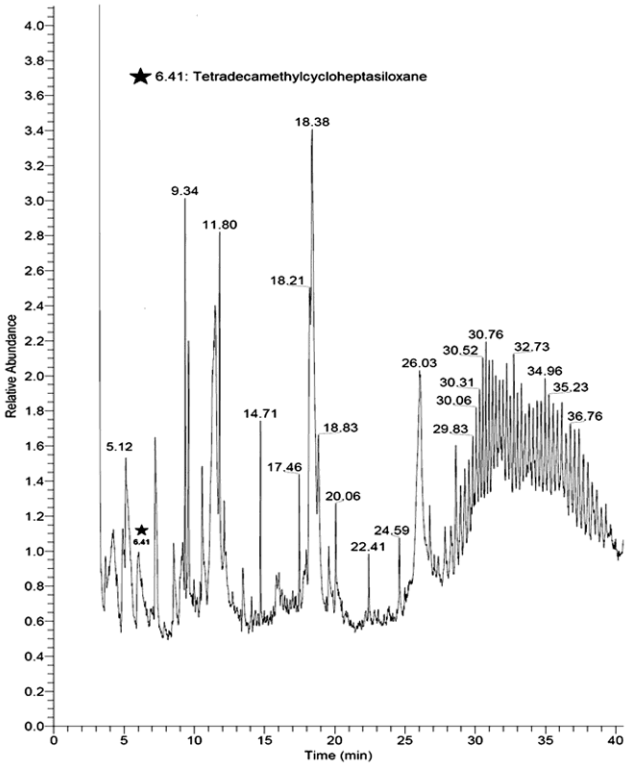

Fig. 2 — Tetradecamethylcycloheptasiloxane, a silicone type of biosurfactant identified from *S. castaneoglobisporus* AJ9 by GC analysis.

75.23, 53.16, 39.78 and 26.11 % in the 10, 20, 40, 60, 80 and 100 µg/ml in biosurfactant treated cancer cells.

The pathogenic bacteria *V. parahaemolyticus* and the fungi *Penicillium* sp. were selected for optimization studies using SSF based on higher antimicrobial activity. Analysis of Variance (ANOVA) revealed that SSF helped to enhance the biosurfactant production and higher antibacterial activity against *V. parahaemolyticus* (F = 2.55; $P \leq 0.080$) and higher antifungal activity against *Penicillium* sp. (F = 3.68; $P \leq 0.28$). The R^2 value observed against *V. parahaemolyticus* was 67.1 % and it was concluded that the model was stronger and predicted a better response (Table 3). The coefficient of regression equation was calculated as:

$$Y = 10.8871 - 0.099\ A + 0.0225\ B - 0.2425\ C + 0.9967\ D + 0.3901\ A^2 + 0.3288\ B^2 - 0.6687\ C^2 + 0.8913\ D^2 - 1.2350\ AXB - 0.2\ AXC - 0.1637\ AXD + 1.4675\ BXC - 0.3087\ BXD + 0.1187\ CXD$$

The R^2 value observed against *Penicillium* sp. was 74.1 % and showed the model to be stronger inferring that it could better predict the response (Table 3). The coefficient of regression equation calculated thereof is given below:

$$Y = 9.30714 - 0.055\ A + 0.43582\ B + 1.065\ C - 0.50167\ D + 0.98530\ A^2 - 0.41595\ B^2 - 0.01595\ C^2 + 0.82155\ D^2 + 0.42375\ AXB - 0.625\ AXC - 0.0725\ AXD - 0.19250\ BXC + 1.0\ BXD - 0.41875$$

The residual plot analysis also clearly indicated that agricultural waste was highly influential in increasing the production of biosurfactants which reflected higher antibacterial and antifungal activities (Fig. 4a, b).

Discussion

Marine Streptomyces have been known to produce protein and polysaccharide-type bioemulsifiers[31]. A few actinomycetes strains had produced biosurfactants on oils and hydrocarbons as substrates which were isolated from the coast of Alibag, Janjira and Goa coastal regions of India[32]. Marine endosymbiotic fungi *Aspergillus ustus* (MSF3) which produced a very high yield of biosurfactant which was isolated from the marine sponge *Fasciospongia cavernosa* collected from the peninsular coast of India[33]. Amongst screening exercises from different isolates, some of the *Streptomyces* strains such as AJ9, RJ2 and RJ11 isolated from the solar salt works had the ability for biosurfactant production. A potential biosurfactant producer *Nocardiopsis lucentensis*

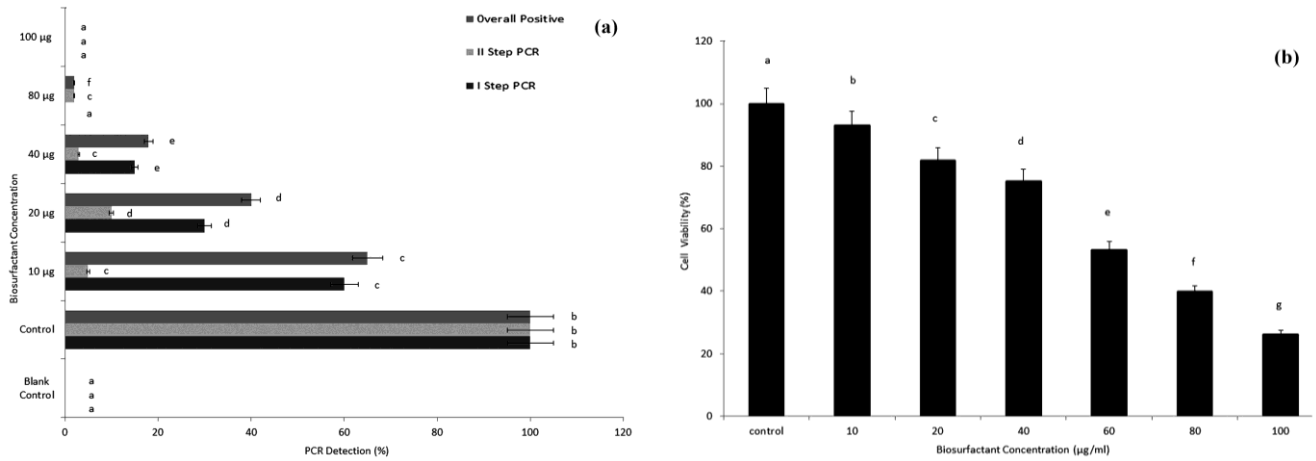

Fig. 3 — a) PCR detection of haemolymph samples of *Fenneropenaeus indicus* after injection with biosurfactants (10 – 100 µg) incubated with WSSV. Statistical differences ($P < 0.0001$) between treated and control groups are indicated by a-f superscripts; error bars are standard errors- One-way ANOVA; and b) Anticancer activity performed in HCT-118 colon cancer cell lines by treating biosurfactants. Means with the same superscripts (a – g) do not differ from each other ($P <= 0.001$).

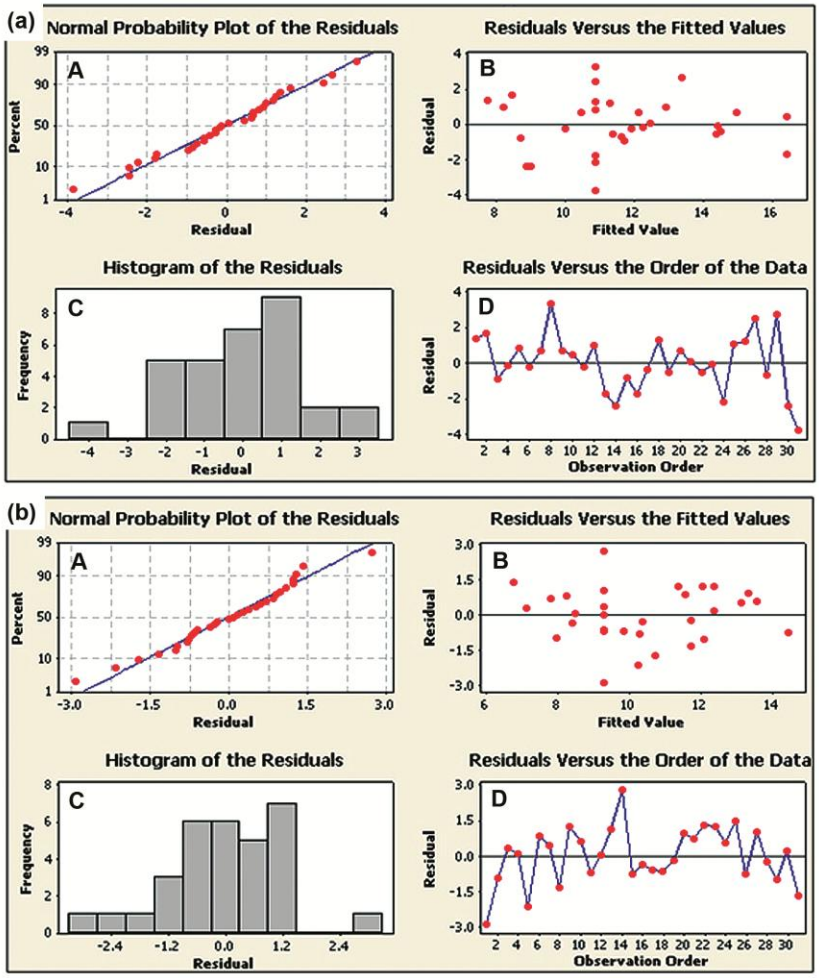

Fig. 4 — Residual plot analysis for a) *V. parahaemolyticus*, and b) *Penicillium* sp.versus rice bran (A), wheat bran (B), ground nut oil cake (C), and oil seed cake (D).

Table 3 — Response Surface Regression Coefficients estimated for *Vibrio parahaemolyticus* and *Penicillium* sp versus rice bran (A), wheat bran (B), ground nut oil cake (C) and oil seed cake (D)

Term	Coefficient	SE coefficient	*T* value	*P* value
		Vibrio parahaemolyticus		
Constant	10.8871	0.8273	13.160	0.000
A	-0.9900	0.4468	-2.216	0.042
B	0.0225	0.050	0.4468	0.960
C	-0.2425	0.4468	-0.543	0.595
D	0.9967	0.4468	2.231	0.040
A*A	0.3901	0.4093	0.953	0.355
B*B	0.3288	0.4093	0.803	0.434
C*C	-0.6687	0.4093	-1.634	0.122
D*D	0.8913	0.4093	2.178	0.045
A*B	-1.2350	0.5472	-2.257	0.038
A*C	-0.2000	0.5472	-0.366	0.720
A*D	-0.1637	0.5472	-0.299	0.769
B*C	1.4675	0.5472	2.682	0.016
B*D	-0.3087	0.5472	-0.564	0.580
C*D	0.1187	0.5472	0.217	0.831
S = 2.189		R^2 = 67.1%		R^2(adj) = 38.3%
		Penicillium sp.		
Constant	9.30714	0.6117	15.216	0.000
A	-0.05500	0.3303	-0.166	0.870
B	0.43583	0.3303	1.319	0.206
C	1.06500	0.3303	3.224	0.005
D	-0.50167	0.3303	-1.519	0.148
A*A	0.98530	0.3026	3.256	0.005
B*B	-0.41595	0.3026	-1.374	0.188
C*C	-0.01595	0.3026	-0.053	0.959
D*D	0.82155	0.3026	2.715	0.015
A*B	0.42375	0.4046	1.047	0.310
A*C	-0.62500	0.4046	-1.545	0.142
A*D	0.07250	-0.4046	-0.179	0.860
B*C	-0.19250	0.4046	-0.476	0.641
B*D	1.00000	0.4046	2.472	0.025
C*D	-0.41875	0.4046	-1.035	0.316
S = 1.618		R^2 = 74.1%		R^2(adj) = 51.4%

MSA04 was isolated from the marine sponge *Dendrilla nigra*[34]. Satpute *et al*.[35] suggested that more screening methods were essential to identify different types of biosurfactants from potential biosurfactant producers as biosurfactants are heterogeneous in nature. In our study, drop collapse, oil displacement, parafilm-M and emulsification activity methods were employed for biosurfactant screening. The drop collapse test and oil displace test was indicative of surface wetting activity[36,37]. Haemolytic assay, emulsifying activity and surface tension measurement proved that biosurfactant production was possible in the alkaliphilic bacterium, *Cronobacter sakazakii*[38].

Many of the halobacterial species isolated from contaminated soils and oil fields were able to degrade chemicals including aromatic compounds. Halophilic archaeon (*Haloferax volcanii* D1227) had the ability to degrade benzoate, cinnamate and 3-phenylpropionate which were isolated from contaminated brine oil. Li *et al*.[39] had isolated a moderate haloalkaliphilic *Planococcus* sp. ZD22 from contaminated oil field soil which effectively degraded the chlorobenzene, bromobenzene, iodobenzene from benzene and BTEX (xylene) in the presence of 0.5 to 25 % NaCl. *Halomonas* sp isolated from solar salt works had effectively degraded the aromatic compounds of cinnamic acid, benzoic acid, ferulic acid, salicylic acid, phenol, *p*-coumaric acid and *p*-hydroxybenzoic acid[40]. The biosurfactant-producing nature of *S. castaneoglobisporus* AJ9 made it capable of degrading the dyes very fast compared to other tested bacterial strains. In general, microbial biosurfactants/ bioemulsifiers and extracellular enzymes could be involved in the degradation of chemical compounds. *Halomonas organivorans* effectively degraded benzoate and phenol because of the cluster of genes[41].

The *S. castaneoglobisporus* AJ9 effectively degraded a host of dyes including orange MR, direct violet, cotton red, reactive yellow and nitro green comparing other sister strains. Mostly, the hydrocarbon metabolism is well developed in halophilic microbes, citing *S. castaneoglobisporus* AJ9 for high biodegradation efficiency. Bertrand *et al*.[42] reported the isolation of halophilic hydrocarbonoclastic bacteria, demonstrating that hydrocarbon metabolism could occur in hypersaline conditions too. *Haloarcula* sp. D1 was shown to metabolize p-hydroxybenzoic acid[43].

Normally, proteins or other related molecules present in biosurfactants play a vital role in emulsification with hydrocarbons and oils[44,45]. These chemical properties are useful for emulsification with respect to oils, toxic chemicals and hydrocarbons from contaminated soil and wastewater. The present study inferred that biosurfactants obtained from *S. castaneoglobisporus* AJ9 are highly efficient to degrade gingelly oil, crude oil, coconut oil and olive oil. Moreover, these would act as carbon sources to induce bacterial cell growth and the production of biosurfactants. A lipopeptide biosurfactant producing *Azotobacter chroococcum* isolated from marine environment had grown well on crude oil, waste motor lubricant oil and peanut oil cake. The biosurfactant also had the ability to emulsify crude oil, waste motor lubricant oil, kerosene, diesel, xylene, anthracene and naphthalene[46,47]. The hydrophobicity of the cells grown in hydrocarbon-containing media was higher than cells grown in water-soluble substrates. This indicated that biosurfactants not only aid in emulsification but also have a role in the change of cell surface hydrophobicity to improve the affinity of microbial cells for the substrate to facilitate their bioavailabilities[48]. Pure paraffin oil and cottonseed oil were used as substrates for the production of surfactin using *Bacillus subtilis*[49]. The polymeric nature of the biosurfactant-producing microbes was generally highly stable with high emulsifying activities. In the present study, *S. castaneoglobisporus* AJ9 yielding the polymeric nature of the biosurfactant tetradecamethylcycloheptasiloxane had high emulsification activity against the different waste oils. Biopolymers obtained from halophilic microbes had higher stability under extreme temperatures and pH conditions and this would be significantly useful in the oil industry for better emulsification[50,51].

The identified biosurfactant molecule tetradecamethylcycloheptasiloxane is usually used in the cosmetic industry including antiperspirants, deodorants, skin creams, and shampoos[52] and pharmacological properties thereof. This biosurfactant characterized from the leaf extract of *Mimusops elengi* is reported to possess antioxidant activity[53]. Also, the siloxanes and silane derivatives are reported to have anticancer[54], antifungal [55] and oil degradation activities[56].

Inrecent years, biosurfactants are divulged to possess several interesting properties of therapeutic and biomedical significance[57]. In the field of pharmacology, biosurfactants act as have antibacterial, antifungal, antiviral, anticancer, immunomodulator and anti-adhesive properties[37]. Due to the antimicrobial potential of the tetradecamethylcycloheptasiloxane in the biosurfactant obtained from *S. castaneoglobisporus* AJ9, it effectively inhibited bacterial pathogens including *E. coli*, *P. aeruginosa*, *S. typhi*, *V. harveyi*, *V. parahaemolyticus*, and the fungal pathogens, *Fusarium* sp. and *Penicillium* sp. Glycolipid biosurfactant derivatives obtained from *Nocardiopsis* MSA13A inhibited the formation of biofilms in the pathogenic Vibrios[58]. Cycloheptasiloxane, tetradecamethyl- and 4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one identified from the latex of *Argemone ochroleuca* had potent antifungal activity against *Candida albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *Drechslera halodes*[55].

The biosurfactants octadecenamide derivatives characterized from the halophilic *Bacillus* sp. BS3 and *Halomonas* sp. BS4 had effectively suppressed the WSSV *in vivo* levels[37,59]. Surfactin was an effective antiviral characteristic biosurfactant which had controlled several viruses including Herpes Simplex Virus (HSV), simian immunodeficiency virus, feline calicivirus, encephalomyocarditis virus, vesicular stomatitis virus, Semliki Forest virus and Suid herpes virus[60]. *S. castaneoglobisporus* yielded biosurfactants that might inhibit the transcription and translation of the WSSV leading to further multiplication arrest.

A few microbial surfactants, such as lipopeptides and glycolipids, selectively inhibit the proliferation of cancer/ tumour cells and damage the cell membranes leading to the lysis of apoptosis pathways. Surfactin molecules were able to inhibit the proliferation of cancer cells and arrested the cell cycle by inducing pro-apoptotic activity[61]. Biosurfactant molecules of

(Z)-9-octadecenamide, 8-Methyl-6-nonenamide and 1, 2-Ethanediamine N, N,N',N'-tetra characterized from *Halomonas* sp. BS3 was able to inhibit the growth of mammary epithelial carcinoma cells at 50%[59].

SSF, which involves the growth of microorganisms on moist solid substrates in the absence of free-flowing water, recently received considerable attention due to several advantages over submerged fermentation[62]. SSF helps to maximize biosurfactant production and develop efficient bioprocesses using cheaper and wasted under-utilized substrates. In our study, cheaper agricultural wastes including wheat bran and oil cakes were used. Based on the results, the combinations of wheat bran, ground nut oil cake and oil seed cake were highly responsible for improved production of biosurfactants and the coefficient observed of more than 1.00000 compared to other combinations. In general, the production economy is governed by factors such as raw material costs, ready availability and copious yield. Patel and Desai[63] used molasses and corn steep liquor as the primary carbon and nitrogen source to produce rhamnolipid biosurfactants from *P. aeruginosa* GS3. Oils obtained from plant origin could be better low-cost alternatives for carbon sources for the production of biosurfactants[64]. There was an improved biosurfactant production by using plant-derived oils such as jatropha oil, castor oil, jojoba oil, canola oil and cottonseed oil on *Virgibacillus salexigens* halophilic bacteria isolated from marine waters[65]. Among the different agricultural wastes, combinations of wheat bran as the cheapest carbon source and groundnut oil cake as the nitrogen source highly induced the production of biosurfactants which reflected higher antimicrobial activity. Carbon–nitrogen combination is one of the crucial factors that highly influenced to induce the production of secondary metabolites in microbes. Kiran *et al*.[34] reported that biosurfactant production was influenced by different substrates including oil seed cake, tannery-treated sludge, pretreated molasses, treated molasses, wheat bran and tannery pre-treated sludge with kerosene. It is suggested that haloalkaliphilic *S. castaneoglobisporus* AJ9 which produce stetrad ecamethylcycl-oheptasiloxane would act as the best antibacterial, antifungal, antiviral and anticancer effectagent. The baseline study could help to the furtherance of producing these particular biosurfactants at a larger scale for commercial supplies in future.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

MBSD & RR played a major role for sample collection and overall experimental procedures of this study. MMB carried out the statistical analysis. GM involved optimization and production work with Response surface methodology (RSM) and residual plot analysis. TC involved experimental design and overall responsibility.

Ethical Statement

The experimental works does not involve live subjects including human and animal models.

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