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Structural Characterization and Stability of Biosurfactant Produced by *Achromobacter* sp. Isolated from Tannery Effluent

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Abstract

This study investigates the structural characteristics and stability of biosurfactants produced by *Achromobacter* specie. FT-IR spectra revealed key functional groups indicative of an amphiphilic biosurfactant, including hydroxyl groups (3279 cm^{-1}), aliphatic C-H stretches (2923 cm^{-1}), carbonyl groups (1640 cm^{-1}), and glycosidic linkages (1220-1019 cm^{-1}). These features suggest a lipopeptide molecular structure. GC-MS profiling further suggests the presence of fatty acid derivatives and high-molecular-mass fragments associated with lipopeptide biosurfactants, identifying compounds such as hydroxydecanoic acid, trans-2-dodecenoic acid, and 3-hydroxydecanoic acid. The chromatographic patterns corresponded with reference spectra of lipopeptide standards. Stability assessment showed that the biosurfactants maintained activity across varying temperatures, pH levels, and NaCl concentrations, demonstrating high thermal, pH, and salt tolerance. These findings highlight *Achromobacter* sp. as a promising producer of structurally diverse and highly stable biosurfactants suitable for industrial and environmental applications.

Keywords: Tannery effluent**,** *Achromobacter* sp.; Biosurfactant, FT-IR; GC-MS, and lipopeptide

Introduction

The global leather industry, while economically significant, is a major contributor to environmental pollution, primarily through the discharge of highly contaminated wastewater known as tannery effluent [1]. The tanning process involves extensive use of water and a complex array of chemicals, resulting in effluent characterized by high levels of organic matter, elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD), total suspended solids (TSS), and total dissolved solids (TDS) [2]. Critically, tannery effluent is also laden with toxic substances, including chlorides, sulfates, sulfides, and heavy metals, most notably chromium in its trivalent (Cr (III)) and highly toxic hexavalent (Cr (VI)) forms [2], [3]. The uncontrolled release of this wastewater poses a severe threat to aquatic ecosystems and human health, necessitating the development of robust and sustainable remediation technologies [1].

Traditional physicochemical methods for treating such complex industrial wastewater are often energy-intensive, costly, and can generate secondary pollutants [4]. Consequently, there is a growing interest in bioremediation strategies, which leverage the metabolic capabilities of microorganisms to detoxify and clean up contaminated sites [5]. Within this context, biosurfactants surface-active compounds produced by microorganisms have emerged as promising alternatives to synthetic chemical surfactants [6]. Biosurfactants are amphiphilic molecules that reduce surface and interfacial tension, facilitating the emulsification of hydrophobic compounds. Their key advantages include high biodegradability, low toxicity, and the ability to function effectively under extreme environmental conditions, such as high salinity, temperature, and pH [7].

The isolation of biosurfactant-producing microorganisms from contaminated environments, such as tannery effluent, is a strategic approach, as these organisms are naturally adapted to the harsh conditions present in their native habitat [8]. The genus *Achromobacter*, a group of Gramnegative bacteria, has been identified as a potent producer of biosurfactants, often exhibiting a remarkable tolerance to environmental stressors [9]. For instance, various *Achromobacter* strains have been shown to produce glycolipid biosurfactants, such as rhamnolipids, which are known for their exceptional stability across a broad range of temperatures (up to 121 °C), pH (6-12), and salt concentrations (up to 5% NaCl) [9], [10]. This inherent robustness makes the biosurfactant produced by *Achromobacter* sp. an ideal candidate for application in the challenging matrix of tannery effluent, which is characterized by high pH and salinity [11]. This study was aimed to characterize and determine stability of biosurfactants produced by *Achromobacter* sp. isolated from tannery effluent.

Materials and methods

Fourier Transform Infra-Red Spectroscopy (FT-IR) analysis

FT-IR analysis FTIR (Fourier Transform Infra-Red Spectroscopy) is a sensitive and primarily technique for identifying the functional groups present in the sample. FTIR spectra of the extracts were measured using carry Agilent technology 360 FTIR machine. KBr pellet of crude extracts were made and the frequency range were measured at wave numbers (4000 – 500 cm^{-1}) with 32 scans and a resolution of 4 cm^{-1} [12].

Gas Chromatography Mass Spectrometry (GC-MS) analysis

Gas Chromatography Mass Spectrometry analysis was performed on GC-(Agilent technologies 7890B model) and MS- (Agilent technologies 5977Å MSD model) to determine the active compounds that are present in the biosurfactants. The apparatus consists of a mass spectrometer interfaced with a gas chromatograph, The following conditions were used with the instruments:elite-1 fused silica capillary column infusion (30x0.25 mm IDx1 EM df, composed of 100% dimethyl polysiloxane), operating in electron impact mode at 70ev; helium (99-999%) were used as carrier gas at a constant flow of 1 ml/min an injection volume of 0.5 El were employed (split ratio of 10:1 injector temperature 250 ^{0}C .The oven temperature was programmed from 110^0 C (isothermal for 2 min) with an increase of 10^{0}C / min , to 200 ^{0}C then 5 ^{0}C / min 280 ^{0} C , ending with a 9 min isothermal at 280 ^{0} C Mass Spectra were taken at 70 Ev; a scan interval of 0.5s and fragments from 40 to 550 Da. Analysis of the mass spectrum The National Institute of Standards and Technology (NIST) database, which contains more than 62,000 patterns, were used for GC-MS analysis. The known components' spectra that were kept in the NIST library were compared to the unknown component's spectrum. The components of the test materials' names, molecular weights, and structures were determined [12].

Biosurfactant Stability Test

Stability studies were carried out as described by [13]. Cell free broth would be obtained by centrifuging the cultures at 5000rpm for 20 minutes. The stability of the biosurfactants against pH, temperature and salt (NaCl) would be determined. (i) The pH of the biosurfactant were adjusted to acidic (2 using HCl) and alkaline (7, 9 and 11 using NaOH). Emulsification index (E_{24}) was later determined after 24 hours [13].

Result and Discussion

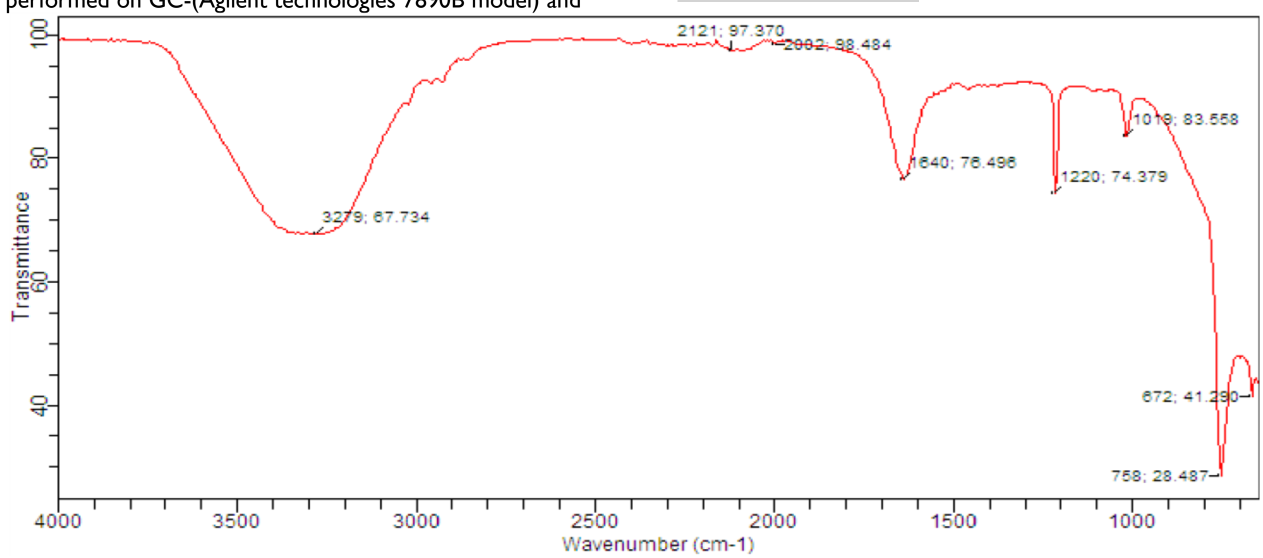

Figure 1: FT-IR Result of Biosurfactants Produced by *Achromobacter* sp.

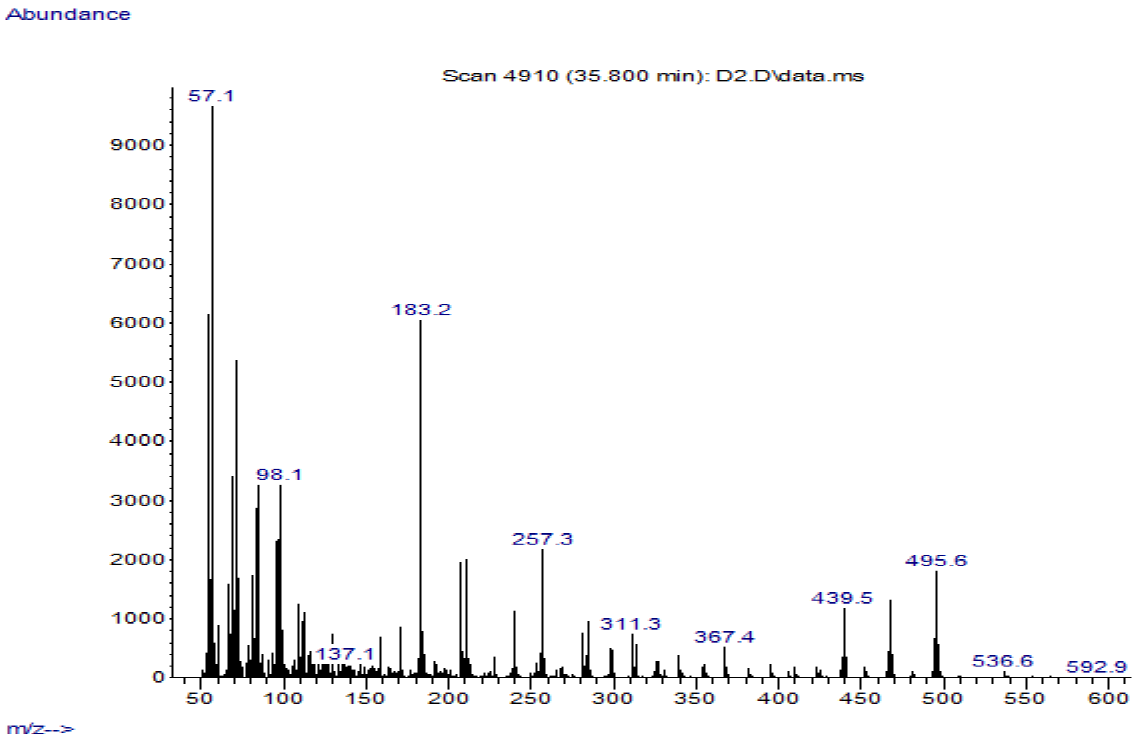

Figure 2: GC-MS Result of Biosurfactants Produced by *Achromobacter* sp.

Table 1: Stability Test of the Biosurfactant Produced by *Achromobacter* sp. (D2) at Different Temperature (˚C), Salt Concentration (%) and pH.

Temp. (° C)	Average E24 (%)	NaCl%	Average E24 (%)	pH	Average E24 (%)
45	71	2	57	2	59
55	65	4	54	6	65
65	62	6	65	8	65
75	60	8	49	10	72
85	75	10	51	12	69

Key: E24 (%) = Emulsification index in percentage

Figure 1 shows the result of FT-IR analysis of Biosurfactants produced by *Achromobacter* sp. revealed that, The FT-IR spectrum of the biosurfactant produced by *Achromobacter* sp. demonstrates the characteristic amphiphilic nature of microbial surfactants. The broad absorption at 3279 cm^{-1} corresponds to O-H stretching vibrations, confirming the presence of hydroxyl groups often associated with sugar residues or peptide bonds. Similar broad bands have been reported for lipopeptide biosurfactants, where hydroxyl groups contribute to hydrophilicity and emulsification capacity [14], [15]. The distinct band at 2923 cm^{-1} indicates C–H stretching of aliphatic chains, representing the hydrophobic lipid tail. This finding aligns with reports of glycolipids such as rhamnolipids and sophoro lipids, where long fatty acid chains are responsible for reducing surface tension and stabilizing emulsions [16],[17]. According to [18]. The C=O stretching at 1640 cm^{-1} is particularly important, indicating amide or ester carbonyl groups typically found in lipopeptides or glycolipids. Comparable peaks have been reported for surfactants-like lipopeptides and for glycolipids with ester linkages between sugar and lipid moieties [19]. The peaks at 1220 cm^{-1} and 1019 cm^{-1} correspond to C–O or C–N stretching and glycosidic bonds, respectively, confirming the presence of sugar residues in the biosurfactant structure. This strongly suggests a glycolipid-type component, consistent with earlier reports of *Achromobacter* species producing glycolipid biosurfactants with emulsifying potential [11], [20]. The low-intensity bands at 758 cm^{-1} and 672 cm^{-1} reflect C–H bending vibrations, indicative of aromatic or alkene groups associated with fatty acid tails or peptide moieties. These peaks reinforce the amphiphilic structure, comprising hydrophilic groups (–OH, C=O) and hydrophobic hydrocarbon chains, which enable the molecule's surface-active behavior. The FT-IR results suggest that the *Achromobacter* biosurfactants may belong to either glycolipid or lipopeptide classes, in agreement with reports highlighting the genus as a versatile producer of structurally diverse biosurfactants with strong emulsification and bioremediation potential [11].

Figure 2 shows the Gas Chromatography Mass Spectroscopy (GC-MS) result of Biosurfactants produced by *Achromobacter* sp., which revealed characteristic ion fragments consistent with lipopeptide-type biosurfactants. The low-mass fragment at 57 (m/z) corresponds to alkyl fragments ($C_4H_9^+$), which are diagnostic of cleavage along long fatty acyl chains. Such fragments are widely reported in biosurfactants with hydrocarbon tails, where they represent the hydrophobic moiety responsible for surface activity [15]. The fragments at 183 and 257 (m/z) indicate fatty acid chain derivatives, confirming the presence of lipid components. Similar observations have been described in the GC–MS profiles of surfactin families, where fatty acid residues are crucial for membrane interaction and emulsification potential [15], [21]. The Intermediate-to-large fragments at 311 and 367 (m/z) suggest partial structures containing both fatty acyl chains and portions of the polar head group, such as peptide residues. This is a hallmark of lipopeptides, where peptide linkages form the hydrophilic region of the molecule [22]. The detection of high molecular ion peaks at 439–593 (m/z) corresponds to lipopeptide molecules. Such high-mass fragments have been previously reported for biosurfactants like surfactin, fengycin, and other peptide-linked surfactants, confirming the amphiphilic structure [23].

The result of gas chromatography mass spectroscopy (GC-MS) biosurfactant samples revealed the presence of different fatty acids at different peaks including hydroxydecanoic acids, decanoic acid, trans- 2-dodecenoic acid, n-pentadecanol, 3-decanoic, acetic acid dichloro, Butyl fluoroformate 2-butanoic, 2- methoxy methyl ester, methyl chloride acid and 3-hydroxydecanoic acid (Figures 2). The chromatograms were also in conformity with reference library standard of lipopeptide biosurfactants. This had agreed with the finding of [24] who worked screening and characterization of biosurfactants producing bacteria isolated from hydrocarbon-contaminated soil. The result of this present study also agreed with findings of [25], who conducted the research on Characterization of a New Glycolipopeptide Biosurfactant Produced by a Chrysene-Degrading Strain *Achromobacter aegrifaciens*.

The biosurfactants produced by *Achromobacter* sp. in this study were stable at various temperatures (˚C), pH levels, and NaCl salt concentrations (%), according to stability studies (Table 1). The stability study findings for temperature in this study are aligned with those for pH and NaCl (%) from [26]. There are several reports on the stability of biosurfactants at extreme conditions of pH, temperature and NaCl that make biosurfactant stable and ideal for industrial application [27], who worked on simultaneous production of lipases and biosurfactants by submerged and solid-state bioprocesses. Biosurfactants and their surface action are safe towards natural factors such as, temperature and pH. [28] reported that biosurfactant Produced by *Bacillus licheniformis* was stable to temperature up to 50°C, pH in the vicinity of 4.5 and 9.0 and NaCl and Ca concentration up to 50 and 25mg/ml. Another biosurfactant produced by *Arthrobacter protophormiae* was observed to be both thermostable (30-100°C) and pH (2 to 12) stable. Since, industrial procedures include extremes of temperature, pH and weight [29].

Conclusion

The structural analyses conducted using FT-IR and GC–MS suggest that *Achromobacter* sp. produces a lipopeptide Biosurfactant. FT-IR spectra revealed key functional groups including hydroxyl, carbonyl, glycosidic, and aliphatic chains indicating a strongly amphiphilic molecule typical of microbial surfactants. GC–MS profiling further detected diverse fatty acid derivatives and high-molecular-mass peptide-linked fragments consistent with established lipopeptide biosurfactants. These findings demonstrate that *Achromobacter* sp. synthesizes structurally complex biosurfactants comparable to known lipopeptides. Additionally, the biosurfactant exhibited high stability across wide ranges of temperature, pH, and salinity, underscoring its suitability for applications in bioremediation, wastewater treatment, petroleum processing, and other industries that require chemically and thermally robust surface-active agents. *Achromobacter* sp. represents a promising microbial candidate for sustainable biosurfactant production and future biotechnological applications.

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