A Research Study on ‘‘Testing of Antimicrobial Activity of Microbial Biosurfactants’’

Riyajuddin[1], Ashish Jha[2]
[1,2]*Amity institute of Biotechnology, India*

Abstract- The efficiency of traditional antibiotics has come under scrutiny due to the increasing global rise of multidrug-resistant bacteria. This is seen, for example, in ESKAPE infections, whose many resistance mechanisms have reduced the number of effective treatment choices. The possible application of biosurfactants is one of the novel approaches intended to reduce the prevalence of antibiotic-resistant bacteria. A class of distinct amphiphilic compounds of microbial origin, these surface-active agents can interact with the lipidic components of microorganisms. The way that biosurfactant interact with various surfaces can change their hydrophobic characteristics, which in turn can change how well microorganisms adhere to surfaces and create biofilms. Biosurfactants are potentially appropriate for targeted usage in pharmaceutical and medical applications because, in contrast to synthetic surfactants, they exhibit low toxicity, great biodegradability, and stability under extremes of pH and temperature. This article examines the development of biosurfactants for use as antimicrobial and antibiofilm agents in biomedical and therapeutic settings, as well as the possible synergistic effects of biosurfactants in conjunction with antibiotics.

I. INTRODUCTION

Biosurfactants are surface-active molecules produced by microorganisms, including bacteria, fungi, and yeast. They are composed of both hydrophilic (water-attracting) and hydrophobic (water-repelling) components, which allows them to reduce surface and interfacial tension between different phases, such as between oil and water.[1,2,3]. Biosurfactants can be categorized based on their chemical structure, including glycolipids, lipopeptides, phospholipids, and others. Unlike synthetic surfactants, which are derived from petrochemicals, biosurfactants are biodegradable, less toxic, and environmentally friendly, making them an attractive alternative in various industries.[4,5,6,7]

II. APPLICATIONS OF BIOSURFACTANTS

1. Environmental Remediation: They are used to clean up oil spills, break down pollutants, and treat wastewaters.
2. Agriculture: Used as eco-friendly agrochemicals to enhance soil health and plant growth.
3. Food Industry: Biosurfactants have applications as natural emulsifiers, preservatives, and food additives.
4. Pharmaceuticals and Cosmetics: They are used for their antimicrobial properties and as ingredients in lotions, creams, and other products.

The ability of a material or living thing to suppress or eradicate microorganisms, such as bacteria, fungi, viruses, and parasites, is known as antimicrobial activity.[8] Numerous environments on Earth, including soil, water, and the human body, are home to microorganisms. Numerous microorganisms are benign or even helpful, but some are harmful and can cause a variety of infections, illnesses, and ailments in people, animals, and plants.[9]The development of these dangerous microbes is inhibited by antimicrobial agents, which include antibiotics, antivirals, antifungals, and antiparasitic. Understanding antimicrobial activity is important not only in the healthcare industry but also in other fields where microbial growth control is critical, like agriculture, food safety, and environmental management.[10]

By lowering the death and morbidity rates linked to infectious diseases, the discovery of antimicrobial agents has transformed contemporary medicine. Infections that are now easily treated with medications could frequently result in death prior to the development of antibiotics. The effective treatment of bacterial infections, fungal diseases, and viral infections, among other conditions, has been made possible by the development of antimicrobial agents.

Antibiotics like tetracycline and penicillin, antivirals like acyclovir, and antifungals like fluconazole, for example, have greatly decreased the prevalence of microbial infections. However, the overuse and abuse of these antimicrobial agents has resulted in antimicrobial resistance (AMR), a serious and expanding worldwide problem. AMR happens when microbes develop defences against medications intended to eradicate them or stop their growth. Previously treatable infections may become difficult or impossible to treat as a result of this resistance, increasing the risk of death, prolonging illnesses, and raising healthcare expenses. Finding novel antimicrobial agents and tactics is therefore more important than ever.[11]

III. REVIEW LITERATURE

What is Biosurfactant

Biosurfactants, which are surface active agents derived from microorganisms, can take the place of their synthetic counterparts in industries like oil, pharmaceuticals, cosmetics, and agriculture. They have lower critical micelle concentration values, are biodegradable, and are less toxic. Biosurfactants are classified into two groups according to their mass: low molecular weight compounds, like glycolipids and lipopeptides, are typically used to reduce surface tension; high molecular weight compounds, like lipoproteins and polymeric biosurfactants, are effective emulsifiers; and hydrophilic heads, which typically contain amino acids, mono-, di-, or polysaccharides, and hydrophobic tails, which are composed of chains of 10–18 carbon atoms or fatty acids.[31] use biosurfactants. Biosurfactants are utilized in froth flotation in mineral processing because of their hydrophilic nature, which comes from sugars, phosphates, carboxylic acids, amino acids, and cyclic peptides, and their hydrophobic qualities, which come from long-chain fatty acids. These properties facilitate selective adsorption, typically via physical and/or chemical interactions with mineral surfaces, allowing for good recovery of the target minerals and selectivity. Because of their surface-active qualities, which allow them to reduce surface tension in a manner comparable to that of synthetic surfactants, biosurfactants can be used as alternatives to synthetic surfactants.

Biosurfactants have superior temperature and pH tolerance, emulsifying capabilities, antimicrobial qualities, and the capacity to form micelles, which makes them suitable for use as drug delivery vehicles. Because of their molecular makeup, biosurfactants can be used in emulsification and solubilization procedures, support the adsorption of bioactive molecules through biological membranes, and reduce surface tension—the ability of a liquid to withstand external forces. They come together to form micelles when their concentration rises above a threshold known as the critical micelle concentration (CMC). In polar solvents like water, the hydrophilic heads align, while the hydrophobic tails congregate in the centre of the micelle. This characteristic is employed to lessen impurities, such as when extracting oil from soil or water. Microorganisms like bacteria, filamentous fungi, and yeast ferment materials and undergo enzyme-substrate reactions to produce biosurfactants. Various substrates, such as glucose, fructose, alkenes, citrates, etc., can be used to create biosurfactants with various characteristics.[31]

Classification of Biosurfactant

Surfactants can reduce surface and interfacial tension in water-oil and oil-water systems because they are amphiphilic molecules with a hydrophobic head and a hydrophobic tail. Numerous industrial processes that involve emulsification, foaming, detergency, wetting, dispersing, or solubilization use these compounds. Synthetic surfactants are made from chemically based materials, while biosurfactants are made from biologically based materials.

A variety of physical and chemical characteristics, including law toxicity, biodegradability, foaming ability, stable activity at pH and temperature extremes, and the capacity to accumulate between fluid phases, are characteristics of biosurfactants, which are amphiphilic compounds produced principally on microbial cell surfaces or excreted extracellular hydrophobic and hydrophilic moieties that reduce surface and interfacial tension at the surface and interface, respectively. These biosurfactants have enormous uses in a variety of industries, including medicine, food, agriculture, cosmetics, oil recovery, and pharmacy. Biosurfactants have anticancer and antimicrobial properties.[32] Because they can break down the biofilm, they are also employed as anti-adhesive agents. Additionally, it exhibited antiviral

properties. Additionally, they have demonstrated appropriate use as cleaning products, emulsifiers in food, dispersants in pesticides, anti-fungal agents, environmental bioremediation, and enhanced oil recovery technologies, as well as anti-aging and wound-healing agents. The structural makeup of biosurfactants is extremely varied. Based on the physical effects of surfactants, there are several techniques for a general screening of strains that produce biosurfactants. As an alternative, it may be investigated whether strains can obstruct hydrophobic surfaces. Both qualitative and quantitative results can be obtained from the screening techniques. The interfacial or surface activity is the basis for the screening techniques for microorganisms that produce biosurfactants. The techniques used to screen for microorganisms that produce biosurfactants include Direct Surface/Interfacial Tension Measurements, such as the Axisymmetric Drop Shape Analysis by Profile, Pendant Drop Shape Technique, Stalagmometry Method, and Du-Nosy-Ring Method, along with Measurements based on the emulsification, drop collapse, oil spreading, haemolytic, and bacterial adhesion to hydrocarbons (BATH) assays.[32]

Pseudomonas and Burkholder species are the primary producers of rhamnolipids, which are low-molecular-weight secondary metabolites of glycolipids. However, P. aeruginosa is the most common bacterial species that produces rhamnolipids. These compounds are composed of a hydrophobic tail made up of one or two fatty acid chains and a hydrophilic head made up of one or two rhamnose sugar molecules connected by an o-glycosidic bond [33]. Depending on how many rhamnose residues they contain, Pseudomonas species produce two different kinds of rhamnolipids: mono-rhamnolipids, which have one rhamnose, and di-rhamnolipids, which have two rhamnose. Rhamnolipids that occur naturally typically take the form of mixtures of various congeners, such as mono- and di-rhamnolipids, with fatty acid chain structures that vary from C8 to C16. Chlorolipids, the second most commonly reported glycolipid biosurfactant, are produced by certain yeasts, including Candida capicola and Tremella bombykol. In addition to having different degrees of acetylation on the soporose moiety, they can be laconic or acidic. Chain length, saturation level, and hydroxylation position (terminal or subterminal) can all vary for the hydroxy fatty acid component, which normally contains 16–18 carbons [38]. Chlorolipids, like rhamnolipids, have high surfactant activity, which lowers the surface tension of both acidic and laconic water from 72 MN/m to 40 MN/m.

Application of Biosurfactant

A lack of new drug development and the abuse of antimicrobial agents have sped up the emergence of antibiotic-resistant pathogens. The rise of new resistant pathogens is a significant threat to the healthcare environment, and methicillin-resistant Staphylococcus aureus has grown to be a significant public health concern over the last 20 years. Many resistance mechanisms and the ability to transmit resistance through horizontal gene transfer make infections caused by Enterococcus aecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter Baumann, Pseudomonas aeruginosa, and Enterobacter spp. (ESKAPE pathogens) extremely difficult to control. The CDC has created a comprehensive report that highlights the most common resistant organisms and divides them into three threat categories (urgent, serious, and concerning) according to their level of pathogenicity in an effort to intensify the fight against antimicrobial resistance. Combining antibiotics with other substances, like biosurfactants, is one method suggested to lower the incidence of antibiotic-resistant infections. Biosurfactants are naturally occurring amphiphilic substances with surface activity that are produced by bacteria, yeasts, and fungi as secondary metabolites. As complex substrates like hydrocarbons undergo biodegradation, these organisms can release biosurfactants as a by-product, which aids in the solubilization and utilization of the substrates and improves the capacity of microbes to degrade them. Following their dissolution, biosurfactants can effectively adsorb to surfaces by reducing the solution's surface tension. These substances have several benefits over chemical surfactants, such as low toxicity, high biodegradability, environmental compatibility, and specific activity at high salinity, pH, and temperatures. As a result, they are more stable than synthetic surfactants. Because of these characteristics, biosurfactants have been investigated as possible replacements for certain goods in the detergent, cosmetics, pharmaceutical, and healthcare sectors.

Although the precise antimicrobial mechanism or mechanisms of biosurfactants are still unknown, it is hypothesized that they interact with bacterial cell membranes through multiple mechanisms. The polar groups of the positively charged surfactant and the negative charges of some of the molecules that make up bacterial membranes (such as lipopolysaccharides in Gram-negative bacteria and lipoteichoic acid in Gram-positive bacteria) have been suggested to interact electrostatically to bind rhamnolipids to the bacterial membrane. According to a different theory, the surfactants' alkyl chains engage in hydrophobic interactions with the membrane's lipid bilayer, upsetting the membrane's structure and permitting the passage of intracellular materials across it, which causes cytoplasmic leakage and ultimately dies cells. A class of special amphiphilic molecules with microbial origins, biosurfactants can interact with the lipidic components of microorganisms to change their physicochemical characteristics. Several of these substances have been shown to possess biological characteristics (antimicrobial and antifungal activity), which may make them appropriate for use in pharmaceutical, medical, and agricultural applications. As mentioned earlier, biosurfactants are characterized by their high biodegradability and low toxicity, especially because they are composed of simple sugars, fatty acids, or polypeptides. These properties ensure the safety of drug formulations and reduce the possibility of side effects, while also preserving the effectiveness of bioactive substances. Surfactant, a lipopeptide produced by the microbial species B. subtilis, has been proposed as a potentially useful substitute in detergent and soap formulations. According to, surfactant has low-toxicity and non-irritant properties, making it versatile in many everyday household products.

IV. MATERIAL METHOD

4.1 Composition of BSM:

Basal salt medium BSM [(per L); $NaNO_3$ 1g, KH_2PO_4 1 g, K_2HPO_4 2 g, $MgSO_4$ 1 g, $CaCl_2$ 0.02 g, $FeSO_4$ 0.002 g, KCL 1 g, Trace Elements SL6 1 mL] and minimal salt medium MSM [(per L); Na_2HPO_4, 980 mg; KH_2PO_4, 170 mg; $MgSO_4$, 4.87 mg; H_3BO_3, 0.006 mg; $(NH_4)_2SO_4$, 100 mg; $CaCO_3$, 0.20 mg; $FeSO_4$, 0.05 mg; $CuSO_4.5H_2O$, 0.016 mg; $ZnSO_4$, 0.08 mg; pH 6.8] were used for production of biosurfactant from bacteria.

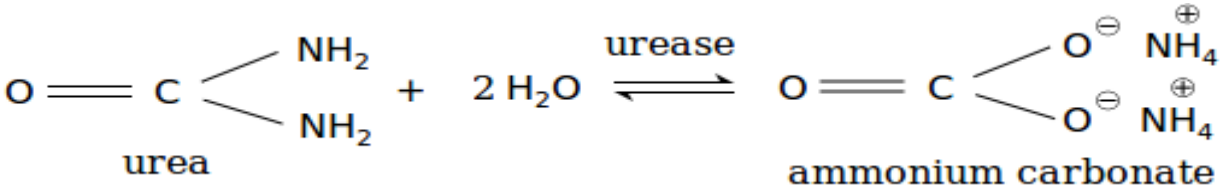
4.2 Isolation of biosurfactant producing bacteria

Soil samples were collected from waste dumping site, Lucknow and transported to the laboratory at ambient temperature. Ten gram of soil samples were serially diluted from 10-3 to 10-5 and spreader onto LB agar plates. The plates were incubated for 48 h at 37 °C. Morphologically different bacterial colonies were selected and inoculated in LB broth for further studies.

4.3 Biochemical Characterization:

Colony morphology and biochemical characterization of biosurfactant producing bacteria was performed as described by Berge's Manual of Determinative Bacteriology. In brief, for pure colony, colour, form, shape, and elevation, the bacterial isolate was streaked on a nutrient agar plate and incubated at 30 °C for 24 h.

4.3.1 Urease : The urease enzyme found in certain bacteria breaks down urea (also known as carbamide) into NH_3 (ammonia) and CO_2 (carbon dioxide), which when combined with water creates ammonium carbonate. View the formula for a chemical reaction.[36]



One way to find out if the bacteria express urease is to grow them in urease medium with a pH indicator. Urea will be changed to ammonium carbonate and the medium will become alkaline if the bacteria have urease. As a result, the colure will turn red (cerise).

4.3.2 Catalase : The catalase enzyme is produced by the majority of facultatively anaerobic bacteria as well as by many aerobic bacteria. Hydrogen peroxide (H_2O_2), which is produced by superoxide dismutase from the superoxide radical, is detoxified by this enzyme.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

After adding one drop of 3% H_2O_2 to the bacteria, watch the suspension. Because H_2O_2 is corrosive, handle it carefully! Positive test result: The presence of catalase in the bacterium is indicated by gas formation (O_2) in the form of bubbles.

No gas formation is the result of a negative test. For example, it can be used to differentiate catalase-positive Staphylococcus and Micrococcus bacteria from catalase-negative Streptococcus and Enterococcus bacteria. The catalase test is mainly used for gram-positive bacteria.[36]

43.3 Citrate: Certain bacteria can use citrate as their sole carbon source.

Add a tiny quantity of bacteria to a tube filled with citrate medium. A deep inoculation or streaking into a "Simmons citrate tube" is another option. Incubate for 24 hours at 30–37 oC. Growth in citrate medium or growth with a blue colure shift in Simmons citrate tube indicate a positive test result. A negative test result means that there is no growth in the citrate medium or growth, but the colure of Simmons citrate tube remains green.

4.3.4 Indole test: Bacteria that produce the enzyme tryptophanase are able to hydrolyse tryptophan into ammonia, pyruvic acid, and indole. Kovac's reagent or the spot indole test can be used to demonstrate the presence of indole. An indole-p-dimethylaminocinnamaldehyde reaction in the spot test results in a blue to blue-green product. The p-dimethylamine benzaldehyde in Kovacs reagent reacts with indole to form a red complex.[36].

4.4 Screening method of Biosurfactant:

4.4.1 Drop collapse:

Without surfactants, a drop of water applied to a hydrophobic surface will form a bead, as seen in. The polar water molecules are repelled from the hydrophobic surface, forming the bead. Surfactant, on the other hand, reduces the force or interfacial tension between the water drop and the hydrophobic surface, causing the water drop to spread over the hydrophobic surfactant of the various [6].

4.4.2 Oil spreading:

Morikawa et al. created the oil spreading assay. This assay involves adding 10µl of crude oil to a petri dish containing 40 ml of distilled water in order to create a thin layer of oil. Following that, 10µl of culture or culture supernatant is carefully applied to the oil layer's centre. The presence of biosurfactant in the supernatant causes the oil to be displaced, creating a clearing zone. On the oil surface, the diameter of this clearing zone is correlated with surfactant activity, also known as oil displacement activity. A linear relationship between surfactant quantity and clearing zone diameter is found for pure biosurfactant.

The oil spreading method only needs a small volume of sample, is quick and simple to use, and doesn't require any special equipment.36 When the biosurfactant's activity and quantity are low, it can be used. Plaza et al.36 and Youssef et al.44 showed that the oil spreading technique is a trustworthy way to find out which microorganisms are producing biosurfactants. Additionally, used the assay for screening.[7]

4.4.3 Emulsification Test

The emulsification ability of biosurfactants is the basis for another widely used assay that Cooper and Goldenberg created. This characteristic is measured by adding kerosene to an aqueous sample. Two minutes of high-speed vertexing are applied to the mixture. Following a 24-hour period, the stable emulsion layer's height is measured.[7] The height of the emulsion layer divided by the total height of the liquid yields the emulsion index E 24.

The emulsification assay of the biosurfactant generated by both strains in a crude oil-containing medium was evaluated using the method described by Patil and Saima in opposition to crude oil. After adding 1 mL of crude oil and 1 mL of cell-free supernatant containing a culture that produces biosurfactants, the mixture was vigorously vortexed for 5 minutes and allowed to stand for 30 minutes at 35 °C. The emulsification activity was then measured.[15]

4.4.4 Surface tension:
When the surface tension of the cell-free culture broth was measured, it decreased. Assays for oil spreading, drop collapse, and surface tension were found to be directly correlated. Just a little strain that was active in one of these methods was active in the other two. Using the drop collapse method and surface tension, and Miller-Maier reported a similar direct correlation. Staphylococcus and Bacillus produced positive results with surface tensions of 42 m N/m and 38 m N/m, respectively, while E. coli produced negative results.

4.5 Production of Biosurfactants by selected isolates:
A loop of each selected isolates was grown in 100 ml of nutrient broth as the inoculum. The culture was incubated 37 C 120rpm for 24 h. Then 2% (v/v) of the inoculum was transferred into 100 ml fermentation medium with the same compositions as used glucose 2% (v/v) was used as the sole carbon source. The PH of the medium was adjusted to 7.0 using 1M NaOH and 1M HCL before autoclave. The medium incubated at same condition for 2-5 day. At the end of the incubation period, the culture were centrifuged at 10,000 rpm at 4℃for 15 min to separate cells from the broth. The cell free supernatant was used in recovery of biosurfactant. The pellet was dried in the drying oven until constant weight to determine the cell dry weight using equation below.

4.6 Extraction Recovery of Biosurfactant:

For extraction of biosurfactant, the extracts were obtained by acid preparation cell- free supernatants were obtained from MSM culture broths (as mentioned above) by centrifuging at 10,000 rpm for 15 min at 4℃. Supernatants were then acidified adjusting the pH 2.0 with concentrated hydrochloric acid (HCl) followed by overnight refrigeration at 4℃. Precipitated cell-free supernatants were again centrifuged at 10,000 rpm for 20 min to collect the pellet precipitates and pH 7.0 was maintained crude biosurfactants were then recovered from the pelleted precipitates through chloroform and methanol (2:1) extraction followed by rotary evaporation at 40℃.

4.7 Antimicrobial Activity of biosurfactant :

The antimicrobial activity of biosurfactant against 4 different pathogenic strain, namely *E.coli, B. subtills, k. pneumonia, and Enterobacter* were determined by using plate assay. The bacterial inoculum was prepared by culturing the strains in nutrient broth and incubated overnight at 37℃ on a rotatory shaker at 150 rpm overnight grown culture were spreader onto LB Agar plates and a drop 5 µl of obtained biosurfactant at different concentration ranging from 40 µg/µL to160 µg/µL were spotted onto the plates. Furthermore, the plates were incubated at 37℃ for 24 h and observed for the formation of bacterial growth inhibition zone.

V. RESULTS

5.1 Bacteria isolation:

Safter enrichment of samples, a Total of 15 bacterial strain were isolated from enrichment of samples using glucose as a sole carbon source.

5.2 Biochemical Characterization:

The selected strain was tested for its biochemical characterization. the selected strain was Gram negative, citrate and catalase positive while urease and indole negative suggesting.

Table 1: Biochemical characterization of isolated strain LN3

Strain	Gram staining	Urease	Indole	Citrate	Catalase
Strain LN3	G-ve	-ve	-ve	+ve	+ve

5.3 Screening of biosurfactant producing strain

The selected strain LN3 showed potential in the screening method opted for testing their potential for biosurfactant production. The strain reduced surface tension from 72N/m to 42N/m with increasing emulsification index of 51%. Furthermore, the strain LN3 collapse drop towards hydrophobic source and displaced the oil up to 55mm.

Table 2: Screening of Strain LN3

Strain	Drop collapse	Oil spreading (mm)	Emulsification index (%)	Surface tension (N/m)
Strain LN3	++	55	51	42

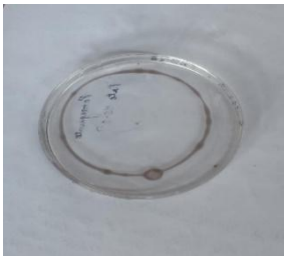
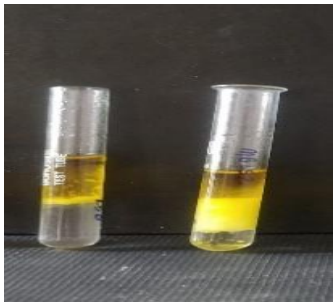
a.  b.  c. 

Fig1. Results of screening: a. drop collapse (left: control, right: strain LN3), b. oil spreading, c. emulsification index (left: control, right: strain LN3).

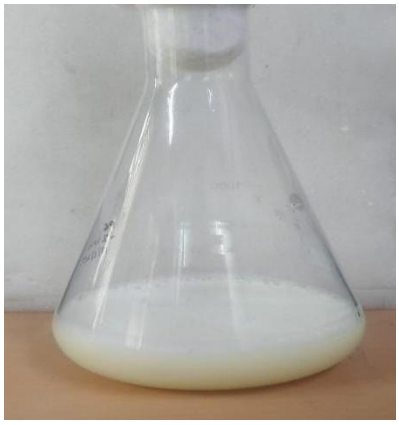
a.  b. 

Fig2. Production of biosurfactant; a. Production flask, b. extraction of biosurfactant

3.2 Production of Biosurfactants:

The isolated strain LN3 produced 1g/L of biosurfactant using 1.5% glucose as a sole carbon source.

3.3 Antimicrobial Activity:

Plate assay for antibacterial potential of strain LN3 was tested against different pathogenic strains namely 40µg/µl 60µg/µL the results showed the formation of inhibition zone suggested the bactericidal potential against the tested strains of pathogenic bacteria. For E.coli

and subtilise the inhibition zone was observed at each selected concentration however, K. Pneumonia showed inhibition zone at 100 and 150 rpm.

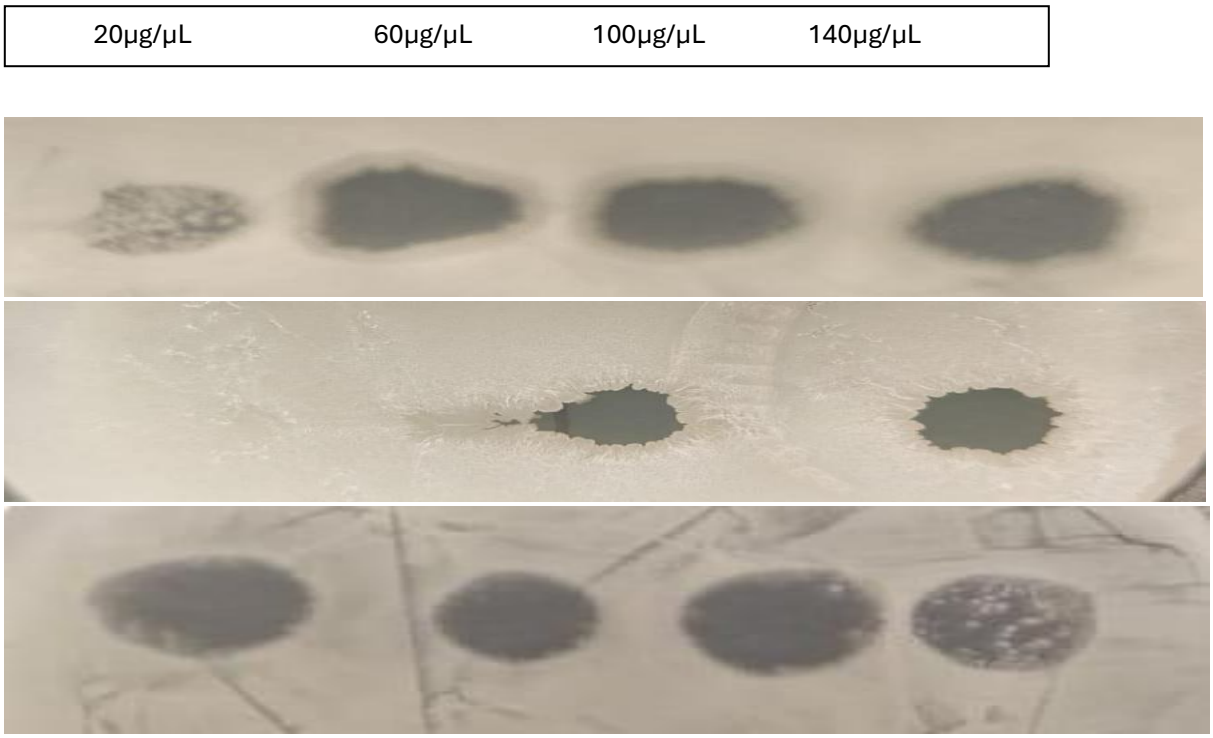


Fig. 3 Plate assay for antibacterial potential of strain LN3 [E.coli, *B.subtilis* , K*Pneumonia]*

VI. CONCLUSION

The tested substance or substances showed varied levels of effectiveness against the chosen microorganisms, according to the antimicrobial activity testing. The clear zones of inhibition in the agar plates demonstrated that biosurfactant successfully inhibited the growth of pathogenic microorganisms. The greatest potency against microorganism was demonstrated by microbial biosurfactant suggesting that it has the potential to be a successful antimicrobial agent.

Some microbes, however, showed resistance, indicating that more research is required to examine the underlying mechanisms of resistance and the potential for improving the antimicrobial activity. The findings offer important new information about how biosurfactant might be used to treat or prevent infections brought on by pathogenic strains.

The Strain LN3 was has potential in utilizing glucose as a sole carbon source for yr. production of biosurfactant. It reduced the surfactants up to oil spreading 55, emulsification 51, surface tension 42 with an emulsification index moreover, it also formed inhibition zone against different pathogenic strains suggesting its application in pharmaceuticals.

REFERENCE

[1] Zafar, S., Bilal, M., Khan, I.M., Ijaz, A., Aslam, A., Shah, A.A., Ashraf, M.A. and Rasheed, R., Use of Biosurfactants in Nanoparticle Preparation and their Role in Drug Delivery. In *Microbial Surfactants in Pharmaceuticals and Cosmetics* (pp. 189-205). CRC Press.

[2] Makkar, R. and Cameotra, S., 2002. An update on the use of unconventional substrates for biosurfactant production and their new applications. *Applied microbiology and biotechnology*, *58*, pp.428-434.

[3] Desai, J.D. and Banat, I.M., 1997. Microbial production of surfactants and their commercial

potential. *Microbiology and Molecular biology reviews*, *61*(1), pp.47-64.

[4] Singh, P., & Cameotra, S. S. (2004). "Synthesis of Biosurfactants from Environmental Pollutants." *Current Science*, 87(1), 38–44.

[5] Pereira, D. A., & Santos, V. M. (2009). "Biosurfactants: Properties, Applications, and Environmental Benefits." *Environmental Biotechnology*, 5(3), 129–140.

[6] Sarubbo, L.A., Maria da Gloria, C.S., Durval, I.J.B., Bezerra, K.G.O., Ribeiro, B.G., Silva, I.A., Twigg, M.S. and Banat, I.M., 2022. Biosurfactants: Production, properties, applications, trends, and general perspectives. *Biochemical Engineering Journal*, *181*, p.108377.

[7] Ron, E.Z. and Rosenberg, E., 2002. Biosurfactants and oil bioremediation. *Current opinion in biotechnology*, *13*(3), pp.249-252.

[8] Davies, J. and Davies, D., 2010. Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews*, *74*(3), pp.417-433.

[9] Berdy, J., 2005. Bioactive microbial metabolites. *The Journal of antibiotics*, *58*(1), pp.1-26.

[10] Pereira, D. A., & Santos, V. M. (2009). "Biosurfactants: Properties, Applications, and Environmental Benefits." *Environmental Biotechnology*, 5(3), 129–140.

[11] Lourenço, M., Duarte, N. and Ribeiro, I.A., 2024. Exploring Biosurfactants as Antimicrobial Approaches. *Pharmaceuticals*, *17*(9), p.1239.

[12] Puyol McKenna, P., Naughton, P.J., Dooley, J.S., Ternan, N.G., Lemoine, P. and Banat, I.M., 2024. Microbial biosurfactants: antimicrobial activity and potential biomedical and therapeutic exploits. *Pharmaceuticals*, *17*(1), p.138.

[13] De Giani, A., Zampolli, J. and Di Gennaro, P., 2021. Recent trends on biosurfactants with antimicrobial activity produced by bacteria associated with human health: different perspectives on their properties, challenges, and potential applications. *Frontiers in microbiology*, *12*, p.655150.

[14] EZ Gomaa · 2013 · Cited by 119 — The aim of the present *study* was to investigate the *antimicrobial effect* of the *biosurfactant* produced by Bacillus licheniformis strain M104 grown on.

[15] AA Bodour · 1998 · Cited by 648 — A *drop-collapse method* has been refined for use as both a qualitative assay to screen for *surfactant*-producing microbes, and as a quantitative assay to ...

[16] Q Shu · 2021 · Cited by 96 — *Glycolipid biosurfactants are natural amphiphiles* and have gained particular interest recently in their biodegradability, diversity, .

[17] Lee, K., Silva, E.A. and Mooney, D.J., 2011. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *Journal of the Royal Society Interface*, *8*(55), pp.153-170.

[18] Silva, M.D.G.C., Medeiros, A.O., Converti, A., Almeida, F.C.G. and Sarubbo, L.A., 2024. Biosurfactants: promising biomolecules for agricultural applications. *Sustainability*, *16*(1), p.449.

[19] Mulligan, C.N., 2005. Environmental applications for biosurfactants. *Environmental pollution*, *133*(2), pp.183-198.

[20] Thakur, A. and Koul, B., 2022. Impact of oil exploration and spillage on marine environments. In *Advances in Oil-Water Separation* (pp. 115-135). Elsevier.

[21] Reiling, H.E., Thanei-Wyss, U., Guerra-Santos, L.H., Hirt, R., Käppeli, O. and Fiechter, A., 1986. Pilot plant production of rhamnolipid biosurfactant by Pseudomonas aeruginosa. *Applied and Environmental Microbiology*, *51*(5), pp.985-989.

[22] Microbial biosurfactants: Novel applications in medicine and food." *Environmental Science and Pollution Research, 21*(18), 10244-10263.

[23] Desai, J.D. and Banat, I.M., 1997. Microbial production of surfactants and their commercial potential. *Microbiology and Molecular biology reviews*, *61*(1), pp.47-64.

[24] Desai, J.D. and Banat, I.M., 1997. Microbial production of surfactants and their commercial potential. *Microbiology and Molecular biology reviews*, *61*(1), pp.47-64.

[25] Antimicrobial activity of biosurfactants produced by bacteria isolated from oil-contaminated environments." *International Journal of*

Environmental Research and Public Health, 15(7), 1465.

[26] "Biosurfactants: Properties, applications, and future potential." *Springer Nature, 2019.*

[27] Varela, M.F., Stephen, J., Lekshmi, M., Ojha, M., Wenzel, N., Sanford, L.M., Hernandez, A.J., Parvathi, A. and Kumar, S.H., 2021. Bacterial resistance to antimicrobial agents. *Antibiotics*, *10*(5), p.593.

[28] Khodaie, L., Patel, P., Deore, S., Surana, V. and Byahatti, V., 2024. Synergistic effects of plant extracts for antimicrobial therapy. In *Herbal Formulations, Phytochemistry and Pharmacognosy* (pp. 55-76). Elsevier.

[29] Nabadda, S., Kakooza, F., Kiggundu, R., Walwema, R., Bazira, J., Mayito, J., Mugerwa, I., Sekamatte, M., Kambugu, A., Lamorde, M. and Kajumbula, H., 2021. Implementation of the World Health Organization global antimicrobial resistance surveillance system in Uganda, 2015-2020: mixed-methods study using national surveillance data. *JMIR Public Health and Surveillance*, *7*(10), p.e29954.

[30] Akhtar, K., Farhana, N., Hossain, A. and Khanam, F., 2024. Molecular characterization of antimicrobial resistance organisms from drinking water and wastewater in a metropolitan city. *medRxiv*, pp.2024-04.

[31] Shoeb, E., Akhlaq, F., Badar, U., Akhter, J. and Imtiaz, S., 2013. Classification and industrial applications of biosurfactants. *Academic Research International*, *4*(3), p.243.

[32] Dini, S., Bekhit, A.E.D.A., Roohinejad, S., Vale, J.M. and Agyei, D., 2024. The physicochemical and functional properties of biosurfactants: a review. *Molecules*, *29*(11), p.2544.