Molecular Characteristics, Antibiotic Sensitivity, and Substrate Specificity of Biosurfactant-Producing Bacterial Isolates from Crude Oil Spill Sites in Niger Delta: A Comprehensive Review

Emeanuru P. C.* Ogbulie J. N. Adieze I. E. Nwachukwu I. N. Braide W. and Nwosu C. J.
Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria
* Corresponding author: peacesylversonco@gmail.com

Abstract: The Niger Delta area, renowned for its biodiversity, has suffered severe environmental degradation due to crude oil spills resulting from oil exploration and exploitation activities. To address this, researchers have explored biosurfactant-producing bacteria as eco-friendly solutions for remediating polluted sites. Indigenous strains, such as *Pseudomonas aeruginosa* and *Bacillus subtilis*, have been identified for their hydrocarbon-degrading and biosurfactant-producing capabilities. Molecular techniques, including 16S rRNA gene sequencing, aided in their identification. Biosurfactants, surface-active compounds produced by microorganisms, enhance hydrophobic pollutant biodegradation by reducing interfacial tension. These biosurfactants also exhibit diverse substrate specificities, enabling them to degrade various hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs). Despite their environmental benefits, concerns have expressed regarding antibiotic sensitivity due to rising global antibiotic resistance. Studies in the Niger Delta have revealed varying antibiotic sensitivity profiles among these bacteria, including resistance to common antibiotics like ampicillin and ciprofloxacin. Mechanisms contributing to this resistance include efflux pumps, enzymatic degradation, genetic mutations, and mobile genetic elements such as plasmids. This resistance raises environmental concerns, as these strains, if released during bioremediation, could transfer resistance genes to other bacteria, including pathogens. To mitigate this, integrated approaches combining genetic studies, metagenomics, and environmental modeling are crucial. Responsible antibiotic use in clinical and agricultural practices is pivotal. This review critically examines the molecular characteristics, antibiotic sensitivity patterns, and substrate specificity of biosurfactant-producing bacterial isolates from crude oil spill sites in the Niger Delta, Nigeria, emphasizing the need for sustainable environmental management strategies.
Key word: Characteristics, Antibiotic Sensitivity, and Substrate Specificity of Biosurfactant-Producing, Bacteria

INTRODUCTION

The Niger Delta, a region known for its rich biodiversity among the rainforest zones of Nigeria, has unfortunately faced severe environmental degradation due to crude oil spills arising from oil activities. In recent years, the search for eco-friendly solutions to remediate these polluted sites has led scientists to explore the potential of biosurfactant-producing bacteria. These studies aimed to identify indigenous microbial strains that can be used for the remediation of chronically polluted soils in the region (Okafor *et al.*, 2021). As a matter of fact various bacterial species, known for their hydrocarbon-degrading capabilities, including *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus* species have been demonstrated to produce biosurfactants that can be used for the remediation of petroleum contaminated soils (Adebusoye *et al*., 2008; Okafor *et al*., 2021; Bharali *et al.*, 2022; Goveas, *et al*., 2022). Molecular techniques such as 16S rRNA gene sequencing have been employed to identify these isolates.

Biosurfactants are surface-active compounds produced by microorganisms that are capable of enhancing the availability and biodegradation of hydrophobic pollutants like crude oil by reducing interfacial tension which in turn increases the surface area of the immiscible phases (Fakruddin, 2012; Aulwar and Awasthi, 2016). This action makes the oil less viscous.

The biosurfactant-producing bacterial isolates identified exhibited diverse substrate specificities for different hydrocarbons, enabling them to interact with a wide range of hydrophobic compounds; with some being able to degrade polycyclic aromatic hydrocarbons (PAHs) (Okafor *et al*., 2021; Futughe *et al*., 2023). Eras-Muñoz *et al.*, (2022) reported that biosurfactants-

producing bacterial isolates in the Niger Delta, Nigeria, effectively emulsified crude oil components, enhancing their bioavailability to indigenous microorganisms which then improved pollutant biodegradation. The findings of these studies highlight the potential of indigenous microbial strains as a bioresource for the remediation of chronically polluted soils in the Niger Delta area. The use of biosurfactant-producing bacteria for the remediation of petroleum-contaminated soil is an eco-sustainable approach that can help manage the environmental problems caused by oil spills in the region (Adebusoye *et al*., 2008; Bharali *et al*., 2022; Aruotu *et al*., 2023).

However, the use of these bacterial-produced surface active compounds, have raised concerns regarding antibiotic sensitivity, particularly in the context of rising global antibiotic resistance. Studies in the Niger Delta region, Nigeria have revealed diverse antibiotic sensitivity profiles among biosurfactant-producing bacterial isolates. Common antibiotics including ampicillin, tetracycline, and ciprofloxacin have been tested against the bacterial isolates to gauge the susceptibility of these isolates. The bacterial isolates showed varying degrees of antibiotic sensitivity, with some being resistant to multiple antibiotics (Okafor *et al*., 2021). The findings indicate a worrying trend of resistance, possibly attributable to the selective pressure exerted by the extensive use of antibiotics in both clinical and agricultural settings.

The mechanisms underlying antibiotic resistance in biosurfactant-producing bacteria are multifaceted. Studies (Ezeonuegbu *et al*., 2018) have highlighted the role of efflux pumps, enzymatic degradation, and genetic mutations in conferring resistance. Mobile genetic elements such as plasmids facilitate the transfer of resistance genes, further aggravating the issue. Understanding these mechanisms is crucial in devising strategies to mitigate the spread of antibiotic resistance in public health and environment.

The antibiotic resistance observed in biosurfactant-producing bacterial isolates poses significant environmental implications. These resistant strains, if released into the environment during bioremediation efforts, could potentially transfer resistance genes to other bacteria, including pathogens. Therefore, stringent monitoring and regulation of biosurfactant applications is crucial to prevent unintended consequences on public health.

To address this challenge, integrated approaches combining genetic studies, metagenomics, and environmental modeling are imperative (Ogukwe *et al*., 2019). These approaches aid in cracking the genetic basis of antibiotic resistance and predicting its potential spread in the environment. Furthermore, promoting responsible antibiotic use in both clinical and agricultural practices is pivotal in curbing the emergence and dissemination of antibiotic-resistant biosurfactant-producing bacteria. This review critically examines the molecular characteristics, antibiotic sensitivity patterns, and substrate specificity of biosurfactant-producing bacterial isolates from crude oil spill sites in the Niger Delta region.

Biosurfactants: Structures and functions in environmental remediation

Biosurfactants are amphipathic molecules produced by microorganisms that have gained considerable attention in recent years due to their diverse structures and remarkable functions (Fakruddin, 2012) in allendevours. Nwaguma *et al*. (2016) investigated the production of biosurfactant from bacteria isolated from hydrocarbon-polluted and pristine soils within Ogoniland in the Niger Delta region of Nigeria and identified based on the 16S rRNA genes pylogenetic analysis, *Pseudomonas* sp. IVN02, *Alcaligenes faecalis* IVN45, *Klebsiella pneumoniae* IVN51, *A. faecalis* IVN61, *Enterobacter sacchari* IVN67 and *P. aeruginosa* IVN74 respectively. These

surface-active compounds have demonstrated significant potential in various applications, particularly in environmental remediation processes in critically polluted areas like the Niger Delta region (Figure 1). Biosurfactants exhibit a wide range of chemical structures, including glycolipids, lipopeptides, phospholipids, and polymeric surfactants. Glycolipids, such as rhamnolipids and sophorolipids, are among the most studied biosurfactants (Makkar and Cameotra, 2016). The structures consist of a hydrophilic sugar moiety linked to one or two hydrophobic fatty acid chains. Lipopeptides (surfactin and iturin are examples) feature cyclic peptides linked to a fatty acid tail. These diverse structures contribute to the surface-active properties of biosurfactants. Rhamnolipids, a class of glycolipid biosurfactants, are composed of one or two rhamnose sugar moieties linked to β-hydroxy fatty acids. These group of biosurfactants exhibit excellent emulsification and foaming properties, making them valuable in environmental applications (Makkar and Cameotra, 2016). Infrared spectroscopy and thin layer chromatography of the biosurfactant produced by a *Bacillus* strain from the study of Al-Rowaihi (2015) suggested a lipopeptide structure for the crude biosurfactant. Therefore, b~~B~~ecause of their amphipathic nature, biosurfactants play pivotal roles in environmental remediation, primarily in the solubilization and degradation of hydrophobic compounds, including petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) (Adebusoye *et al*., 2008; Goveas *et al*., 2022). The study of Bharali *et al*. (2022) revealed that polyaromatic hydrocarbons (PAHs) are more soluble in the presence of biosurfactants produced by *P. aeruginosa*. Phenanthrene had the highest solubility among the tested PAHs, which further increased as biosurfactant doses raised above their respective critical micelle concentrations (CMC). This enhanced solubility promotes the bioavailability of these pollutants to microbial degradation, contributing to the overall bioremediation process (Banat *et al*., 2010). Furthermore, biosurfactants aid in metal remediation by forming complexes with metal ions, making them more accessible for removal processes.

Different strains of the same biosurfactant – producing organism could show different degradation functions. For example, Xiangsheng *et al*. (2012) reported that three strains of *Pseudomonas aeruginosa* producing rhamnolipids showed differing degradation characteristics when grown on a hydrophobic phase.

Apart from environmental applications, biosurfactants find use in various biotechnological fields, including pharmaceuticals, food, and cosmetics. Their natural origin and low toxicity make them attractive alternatives to synthetic surfactants. Moreover, biosurfactants exhibit antimicrobial properties, making them potential candidates for novel antimicrobial agents. Surfactin, a lipopeptide biosurfactant produced by *Bacillus subtilis*, not only possesses surface-active properties, but also demonstrate antimicrobial activities against a wide range of pathogens. This dual functionality makes surfactin a promising candidate for pharmaceutical applications, particularly in antimicrobial drug development (Raaijmakers *et al*., 2010). Biosurfactants, with their diverse structures and multifaceted functions, stand as nature's versatile tools in environmental remediation and biotechnological advancements.

Genetic and Molecular Aspects of Biosurfactant Production in Bacteria

Biosurfactant production in bacteria is a complex process governed by specific genes and pathways. The genes responsible for biosurfactant production vary among different bacterial species. One well-studied biosurfactant is rhamnolipid, produced by *Pseudomonas aeruginosa*. The biosynthesis of rhamnolipids involves several key genes, including rhlA, rhlB, and rhlC. RhlA gene encodes a β-ketosynthase enzyme that catalyzes the synthesis of β-hydroxyalkanoyl-acyl carrier protein (ACP)

from fatty acyl-ACP. The RhlB is a rhamnosyltransferase responsible for the addition of rhamnose, and RhlC is a rhamnosyltransferase responsible for the addition of the second rhamnose molecule, forming the final rhamnolipid structure (Beal *et al*., 2018). Mutations in these genes have been shown to impact the production, quantity, and properties of rhamnolipids produced (Beal *et al*., 2018).

Al-Rowaihi (2015) identified a biosurfactant-producing motile *Bacillus* from a petroleum-polluted environmental samples. Partial sequences of 10 16S rDNA gene clones from the identified strain was highly similar to those of various members of the family Bacillaceae. This strain designated I-15 strain possesses intragenomic heterogeneity in the rrn (RNA) operons. Biosurfactant production is accompanied by morphological and physiological alterations. Chikere and Ekwuabu (2014); Chikere *et al*. (2015) identified bacterial species from crude oil spill sites (*Pseudomonas* spp., *Bacillus* spp., *Rhodococcus* sp., *Achromobacter* sp., *Serratia* sp., *Aeromonas* sp., *Micrococcus* sp., *Acinetobacter* sp., and *Gordonia* sp.) which were found to possess genes involved in hydrocarbon ring cleavage, such as catechol 2,3-dioxygenase. Further, Chikeren *et al*. (2016) detected the aromatic hydrocarbon ring cleavage functional gene (catechol 2,3-dioxygenase) (C23D0) gene in the following organisms: *Brevundimonas naejangsanensis, Pseudomonas pseudoalcaligenes, Pseudomonas* spp., *Aquitalea magnusonii, Achromobacter* sp., *Halomonas lutea, Pseudomonas aeruginosa, Shewanella* sp, *Achromobacter* sp., *Gordonia* sp., *Sphingobacterium* sp. and *Bacillus* sp

Regulation of biosurfactant genes

Biosurfactant production is tightly regulated to ensure energy efficiency and resource allocation. Regulatory proteins and environmental cues influence the expression of biosurfactant genes. For example, in *Bacillus subtilis*, the *ComA* protein in *Bacillus subtilis* acts as a transcriptional regulator for surfactin biosynthesis genes, responding to quorum sensing and environmental signals and modulating the expression of surfactin biosynthesis genes in a manner conducive to the bacterial cell and its surroundings (Nakano *et al*., 2016).

Many bacteria regulate biosurfactant production through quorum sensing, a cell-to-cell communication mechanism that allows bacteria to coordinate their behaviour based on population density. Quorum sensing involves the production and detection of signaling molecules called autoinducers. When the concentration of these molecules reaches a threshold, specific genes, including those involved in biosurfactant production, are activated. In *Pseudomonas aeruginosa*, for example, the Las and Rhl quorum-sensing systems control the expression of genes involved in rhamnolipid biosynthesis, ensuring a coordinated and efficient production of biosurfactants (Davey *et al*., 2003). Therefore, understanding the genetic and molecular aspects of biosurfactant synthesis is crucial for enhancing their production and optimizing their applications in various fields, including environmental remediation and biotechnology.

Antibiotic sensitivity studies in biosurfactant-producing bacterial isolates from crude oil spill sites in the Niger Delta Area: implications of antibiotic resistance patterns

Biosurfactants, crucial in enhancing biodegradation, have raised concerns regarding antibiotic sensitivity, particularly in the context of global rising antibiotic resistance. Common antibiotics including ampicillin, tetracycline, and ciprofloxacin have been tested to gauge the susceptibility of these isolates. Several studies in the Niger Delta region have investigated the antibiotic sensitivity of biosurfactant-producing bacterial isolates and have revealed diverse antibiotic sensitivity profiles. For example, Chikere and Ekwuabu (2014); Al-Rowaihi (2015); Chikere *et al.* (2015); Chikere *et al.*

(2016); Okolo *et al*. (2017); studied bacterial isolates from crude oil spill sites in the Niger Delta for their molecular characteristics, antibiotic sensitivity, and substrate specificity. Using both culture-dependent and molecular techniques, they identified *Pseudomonas* spp., *Bacillus* spp., *Rhodococcus* sp., *Achromobacter* sp., *Serratia* sp., *Aeromonas* sp., *Micrococcus* sp., *Acinetobacter* sp., and *Gordonia* sp. These isolates showed potential for hydrocarbon degradation and were found to possess genes involved in hydrocarbon ring cleavage, such as catechol 2,3-dioxygenase. The isolates also exhibited varying levels of antibiotic sensitivity and substrate specificity, indicating their ability to adapt to different environmental conditions. These studies employed common antibiotics such as ampicillin, tetracycline, and ciprofloxacin to assess the susceptibility of these isolates. The findings revealed diverse antibiotic sensitivity profiles, with some isolates exhibiting resistance to multiple antibiotics. Mechanisms underlying this resistance include efflux pumps, enzymatic degradation, genetic mutations, and mobile genetic elements, such as plasmids.

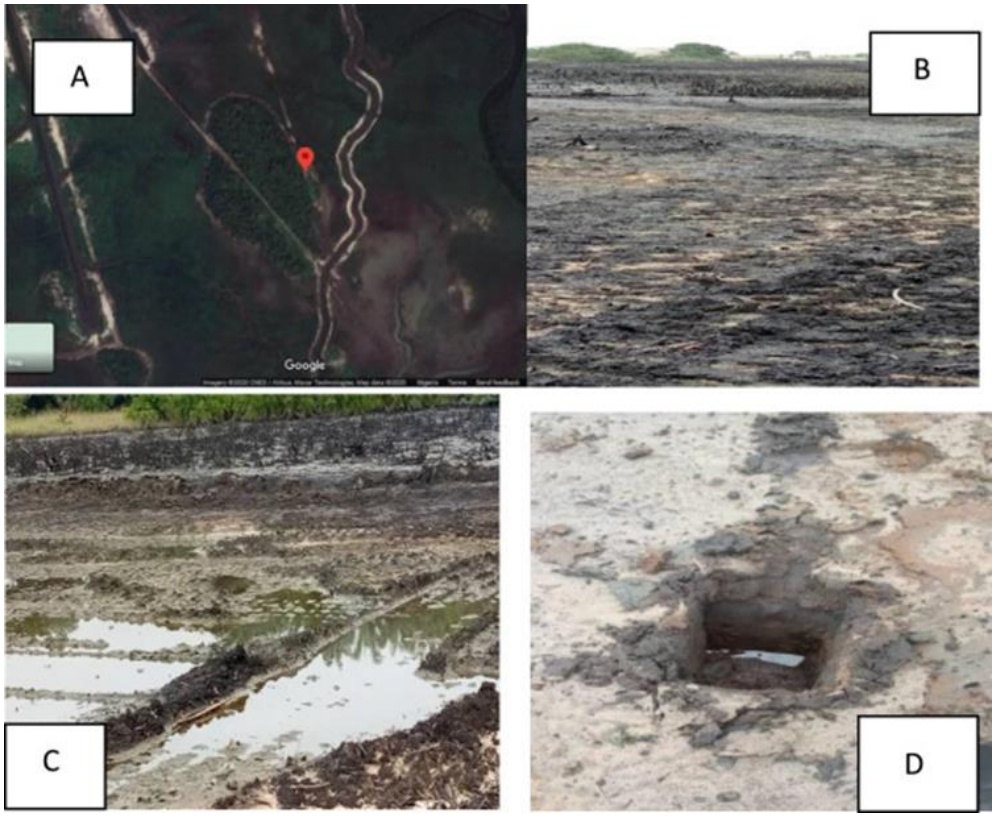

Figure 1: (Okafor *et al*., 2021) Site conditions of abandoned under-commissioned artisanal refining structure in A is the satellite map of the impacted site, B shows the entire field is covered in crude oil tars following government demolition of the site. C is a closer shot showing floating oil sheens and D shows an excavated depth of about 30 cm with crude oil the level of degradation of the environment.

Dick *et al*. (2015) investigated the antibiotic sensitivity pattern and plasmid profile of *Escherichia coli*, *Vibrio* and *Salmonella* species isolated from well and river water sources in Oproama Community in Niger Delta, Nigeria. Antibiotic sensitivity profiles of the bacteria (*Escherichia coli*, *Vibrio* sp. and *Salmonella* sp.) isolated from the water showed high sensitivity to oflaxicin, nalidixic acid and nitrofurantoin and high resistance to amoxicillin, augumentin, cotrimazole and tetracycline. Multi antibiotic resistant index (MARI) as high as 0.375 (*Escherichia coli*): 0.5 (*Vibrio* spp) and 0.75 (*Salmonella* spp.) were recorded after curing the plasmids with sodium

deodecyl sulphate (SDS). The plasmid profiles revealed that 60% of the isolates harboured detectable plasmids with sizes up to 23.130 kb

The study of Asionye *et al*. (2023) on the antibiotic resistance profile of a potable water source in Okerenkoko community, Delta State, Ngeria, a region with reported crude oil spill, revealed that of the culturally identified bacteria, *Proteus* sp. (1) had an MDR of 1.0, *Klebsiella* sp. (3) had 0.67, while *Salmonella* sp. and *Escherichia coli* had a MDR of 0.22 and 0.11. Other identified bacteria: *Bacillus* sp, *Escherichia* sp., *Staphylococcus* sp., *Streptococcus* sp., *Shigella* sp., *Proteus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Vibrio* sp. *Micrococcus* sp. and *Escherichia coli* showed 64% resistance against cefuroxime and ceftazidime, 71% against nitrofurantoin and 36% against ofloxacin.

The antibiotic sensitivity patterns observed in biosurfactant-producing bacterial isolates from crude oil spill sites in the Niger Delta region signify a pressing environmental issue. The genetic basis of this resistance, often mediated by plasmids and integrons, highlights the need for cautious consideration during bioremediation efforts.

Elsewhere, similar resistance genes amongst biosurfactant-producing bacteria isolated from environmental samples have been documented. Sambanthamoorthy *et al.* (2014) assessed the *in vitro* antimicrobial, anti-adhesive and anti-biofilm abilities of biosurfactants produced by *Lactobacillus jensenii* and *Lactobacillus rhamnosus* against clinical multidrug resistant (MDR) strains of *Acinetobacter baumannii*, *Escherichia coli*, and *Staphylococcus aureus* (MRSA). Surface activities for both biosurfactants ranged from 6.25 to 25 mg/ml with clear zones observed between 7 and 11 cm. These compounds from the two tested strains (*L. jensenii* and *L. rhamnosus*) showed antimicrobial activities against *A. baumannii, E. coli* and *S. aureus* at 25-50 mg/ml. Electron microscope studies indicated that the biosurfactant caused membrane damage for *A. baumannii* and pronounced cell wall damage in *S. aureus.*

Uwem *et al.* (2023) reported a heavy metal co-resistance with antibiotics amongst biosurfactant-producing bacteria isolated from petroleum dump sites via similar mechanisms. This synergy has the potential to amplify antibiotics resistance genes in the environment which can be transferred into clinical settings. Identified bacteria isolates were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion technique and the resulting multidrug resistant (MDR) isolates were subjected to heavy metal tolerance test using agar dilution technique with increasing concentrations (50, 100, 150, 200 and to 250 µg/ml). Out of the 20 isolates subjected to antibiotics sensitivity, 50% (n = 10) showed multiple drug resistance and these were *B. subtilis, B. cereus, C. freundii, P. aeruginosa, Enterobacter* sp, and *E. coli* (n = 5). At the lowest concentration (50 µg/ml), all the MDR isolates tolerated all the heavy metals, but at 250 µg/ml, apart from cadmium and lead, all test isolates were 100% sensitive to chromium, vanadium and cobalt.

Chen *et al*. (2020) investigated the distribution of antibiotic resistant genes (ARGs) at different positions in a water-flooding oilfield in China, and found that ARGs were observed in all parts of the investigated system. The surface regions of the water re-injection system were more vulnerable to ARG pollution, and the final ARG concentration was up to 2.2×10^8 gene copies/L, and sulfonamide were the most abundant. However, ARG concentration decreased sharply in the samples from underground part of the re-injection system. The study also indicated that wastewater-recycling process above ground, which proposed to reduce the discharge into environment directly, may pose a risk for ARGs spread.Thus, the potential spread of antibiotic resistance genes among environmental bacteria and their subsequent impact on human health and bioremediation efficacy underscore the need for stringent

monitoring and regulation. Integrating genetic studies, metagenomics, and environmental modeling is crucial for understanding and mitigating the spread of antibiotic resistance in biosurfactant-producing bacteria. Responsible antibiotic use, both in clinical settings and agricultural practices, is imperative to curb the emergence and dissemination of antibiotic-resistant strains, ensuring the sustainable use of biosurfactants for environmental remediation. The findings indicate a worrying trend of resistance, possibly attributable to the selective pressure exerted by the extensive use of antibiotics in both clinical and agricultural settings as well as have serious implications.

Antibiotic-resistant biosurfactant-producing bacteria, if introduced into the environment during bioremediation efforts, pose a risk of transferring resistance genes to other bacteria. This horizontal gene transfer may lead to the emergence of antibiotic-resistant environmental strains, further exacerbating the challenge of antibiotic pollution. The transfer of antibiotic resistance from environmental bacteria to human pathogens raises concerns about public health. If these resistant genes enter the human microbiome, they can compromise the effectiveness of antibiotics in clinical settings, leading to challenges in treating infections. Antibiotic-resistant biosurfactant-producing bacteria may have altered metabolic priorities due to the expression of resistance mechanisms. This alteration could potentially impact their efficiency in hydrocarbon degradation, undermining the effectiveness of bioremediation efforts.

To address this challenge, integrated approaches combining genetic studies, metagenomics, cum environmental model ing cannot be underscored (Das *et al*., 2020). These approaches aid in deciphering the genetic basis of antibiotic resistance and predicting its potential spread in the environment. Additionally, promoting responsible antibiotic use in both clinical and agricultural practices is pivotal in curbing the emergence and dissemination of antibiotic-resistant biosurfactant-producing bacteria.

Antibiotic sensitivity and its significance

The antibiotic sensitivity profile of biosurfactant-producing bacterial isolates is essential for several reasons. Firstly, it helps in assessing the vulnerability of these bacteria to antibiotics, which is crucial for understanding their survival and persistence in contaminated environments. Secondly, knowledge of their antibiotic sensitivity aids in the selection of appropriate antibiotics for experimental purposes and in controlling the growth of these bacteria during bioremediation processes. Additionally, understanding antibiotic resistance patterns is imperative to address concerns related to the potential transfer of antibiotic resistance genes between environmental bacteria and human pathogens.

Substrate specificity of biosurfactant-producing bacteria and their applications in bioremediation strategies

Biosurfactant-producing bacteria possess remarkable substrate specificity, enabling them to target a wide range of hydrophobic compounds, especially hydrocarbons (Aruotu *et al.*, 2023). This specificity makes them valuable assets in bioremediation efforts aimed at cleaning up environments contaminated with petroleum-based pollutants. These diverse hydrocarbons include alkanes, aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and crude oil constituents. Their specificity stems from the enzymes and metabolic pathways involved in hydrocarbon degradation and biosurfactant production. For example, biosurfactant-producing bacteria often possess alkane hydroxylase enzymes, enabling them to break down alkanes present in crude oil. *Pseudomonas aeruginosa* and *Alcanivorax borkumensis* are notable examples with the ability to degrade alkanes efficiently. Bacteria like *Burkholderia cepacia* and *Rhodococcus* spp. are proficient in degrading aromatic hydrocarbons, including benzene, toluene,

ethylbenzene, and xylene (BTEX compounds). Other bacteria, such as *Mycobacterium* and *Sphingomonas* spp., are equipped with enzymes like dioxygenases that facilitate the breakdown of complex PAHs, making them suitable for environments contaminated with these compounds.

Aruotu *et al.* (2023) investigated the distribution of polycyclic aromatic hydrocarbon (PAH) degraders across two different petroleum hydrocarbon-polluted sites - Tombia and Bodo- in the Niger Delta, Nigeria, and the ability of the reconstituted indigenous consortium to utilize these PAHs. Biosurfactant production was measured using the emulsification index technique. Poly aromatic hydrocarbon concentrations of approximately 6000 mg/kg and 9000 mg/kg in Tombia and Bodo were higher than the Department of Petroleum Resources (DPR) intervention limit of 40 mg/kg. A total of 12 bacteria from the genera *Bacillus, Pseudomonas, Micrococcus* and 3 fungal isolates (*Fusarium, Aspergillus* and *Penicillium*) from the 2 sites were able to utilize naphthalene and/or anthracene as sole carbon source. The Tombia site had more microorganisms capable of PAH degradation with the redox indicator 2, 6-dichlorophenol indophenol (DCPIP) (10 bacterial and 3 fungal species), and two bacterial species from Bodo were able to produce biosurfactant.

Bharali *et al*. (2022) studied the efficiency of four different strains of *Pseudomonas aeruginosa* and their biosurfactants in the bioremediation process. The strains were found to be capable of metabolizing a wide range of hydrocarbons (HCs) with preference for high molecular weight aliphatic (ALP) over aromatic (ARO) compounds. The bacterial strains degraded ALP, ARO, and nitrogen, sulphur, oxygen (NSO) containing fractions of the crude oil by 73–67.5, 31.8–12.3 and 14.7–7.3%, respectively. Additionally, the viscosity of the residual crude oil reduced from 48.7 to 34.6–39 mPas. Gas chromatographic analysis confirmed the ability of the individual strains and their consortium to degrade various fractions of crude oil. The consortium designated as 7 and 11 improved the degradation of ALP, ARO, and NSO HCs portions by 80.4–78.6, 42.7–42.4 and 21.6–19.2%, respectively. Further, the addition of biosurfactant increased the degradation performance of consortia by 81.6–80.7, 43.8–42.6 and 22.5–20.7%, respectively. Okafor, *et al.* (2021) reported that biosurfactants- producing bacterial strains from the genera *Pseudomonas, Bacillus, Klebsiella,* and *Enterobacter* utilized aromatics (benzene and naphthalene).

The use of biosurfactant-producing bacteria for the remediation of petroleum-contaminated soil is an eco-sustainable approach that can help manage the environmental problems caused by oil spills in the region (Adebusoye *et al*., 2008; Bharali *et al*., 2022; Aruotu *et al*., 2023). These studies also emphasizes the importance of understanding the molecular characteristics, antibiotic sensitivity, and substrate specificity of bacterial isolates to optimize their use for bioremediation purposes (Okafor *et al*, 2021; Futughe *et al*., 2023). Thus, utilizing these bacteria in bioaugmentation and biostimulation approaches enhances their biodegradation potential, offering sustainable solutions for mitigating the environmental impact of hydrocarbon pollution.

Overview of methodologies in studying biosurfactant-production, molecular characteristics, antibiotic sensitivity, and substrate specificity in bacterial isolates

The understanding of biosurfactant-producing bacteria at a molecular level is essential for harnessing their potential in various applications, from environmental remediation to biotechnology. Studying their biosurfactant production, molecular characteristics, antibiotic sensitivity, and substrate specificity requires a diverse set of methodologies.

Biosurfactant production methods

Biosurfactant production methods include Shake Flask Cultivation, Surface Tension Measurement, Emulsification Index (E24), and Thin Layer Chromatography (TLC) Shake Flask Cultivation: Biosurfactant-producing strains are cultured in shake flasks containing growth media supplemented with hydrocarbons. The isolated bacteria are then selected by using different methods as drop collapse test, oil displacement test, blue agar test, blood hemolysis test, emulsification activity and surface tension (Elazzazy *et al*., 2015). The biosurfactant production is assessed by various methods after cultivation.

Surface Tension Measurement: Surface tension of culture supernatants is measured using a tensiometer, indicating the surfactant activity of the produced compounds. This has been used to demonstrate the ability of *Virgibacillus salarius* to grow and reduce surface tension under a wide range of pH, salinities and temperatures (Elazzazy *et al*., 2015).

Emulsification Index (E24): The ability of biosurfactants to emulsify hydrocarbons is quantified using the E24 index, representing the percentage of emulsified layer height to the total liquid height in a test tube using TLC, FTIR, and GC-MS analyses.

Thin Layer Chromatography (TLC): The TLC is used to separate and visualize biosurfactants based on their polarity, aiding in identifying different biosurfactant types. Other sophisticated and highly accurate characterization techniques such as high performance-liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and gas chromatography–mass spectrometry (GC–MS) have also been employed in its identification (Sambanthamoorthy *et al*., 2014).

Molecular characteristics methods

Polymerase Chain Reaction (PCR) amplification of the 16S rRNA gene is followed by sequencing, allowing for bacterial species identification and phylogenetic analysis. High-quality genomic DNA is extracted using commercial kits or phenol-chloroform extraction methods, providing genetic material for further molecular studies. Specific genes related to biosurfactant production, such as rhlA, rhlB, and rhlC, are amplified using PCR, enabling the detection of biosurfactant-associated genetic markers (Das *et al*, 2020).

Antibiotic sensitivity methods

Antibiotic susceptibility of bacterial isolates is determined using disk diffusion assays, where antibiotic-soaked disks are placed on agar plates inoculated with the isolates. Zones of inhibition indicate sensitivity. The minimum inhibition concentration values are determined using microbroth dilution methods, providing quantitative data on the lowest antibiotic concentration inhibiting bacterial growth.

Substrate specificity analytical methods

Gas Chromatography-Mass Spectrometry (GC-MS) is utilized to analyze the degradation products of hydrocarbons, confirming the ability of biosurfactant-producing bacteria to break down specific hydrocarbon compounds. High-Performance Liquid Chromatography (HPLC) separates and quantifies hydrocarbon degradation intermediates, providing detailed information on substrate specificity. The combination of these methodologies offers a comprehensive approach to studying biosurfactant-producing bacterial isolates. From understanding their genetic makeup and biosurfactant production abilities to assessing antibiotic sensitivity and substrate specificity, these techniques provide essential insights for both fundamental research and practical applications.

Challenges and future directions

Biosurfactant-producing bacteria exhibit immense diversity in terms of species, biosurfactant types, and applications. Understanding this complexity poses a challenge, requiring researchers to study a wide range of microorganisms and their intricate biochemical pathways. In addition,

genetic variations within biosurfactant-producing bacterial populations complicate studies. Identifying key genes and regulatory elements responsible for biosurfactant synthesis requires advanced genomic techniques and in-depth comparative genomics.

Many biosurfactant-producing bacteria are fastidious and challenging to cultivate in laboratory settings. The development of specialized culture media and cultivation techniques is crucial to isolate and study these microorganisms effectively. Accurate quantification of biosurfactant production remains a challenge. Traditional methods often lack precision and sensitivity, necessitating the development of novel analytical techniques for reliable quantification. Furthermore biosurfactant production is influenced by environmental parameters such as pH, temperature, and nutrient availability. Understanding these factors' impact on biosurfactant synthesis is essential for optimizing production processes.

Investigating the role of biosurfactant-producing bacteria within natural microbial communities is another area that can be explored. Studying their interactions with other microorganisms in complex environments can uncover novel biosurfactant-mediated ecological processes and bioremediation strategies. Also, comprehensive studies to assess the environmental impact of biosurfactant applications can be conducted. Factors such as persistence, biodegradability, and ecotoxicity to ensure the safe and sustainable use of biosurfactants in real-world scenarios can be investigated. Utilizing genomics, transcriptomics, proteomics, and metabolomics to comprehensively study biosurfactant-producing bacteria provide holistic insights into genetic mechanisms, gene expression patterns, and metabolic pathways, enhancing our understanding of biosurfactant synthesis. Synthetic biology techniques enable the design and manipulation of microbial genomes, allowing the development of customized biosurfactants for specific applications. Integrating expertise from diverse fields can facilitate innovative approaches and accelerate progress in biosurfactant research.

CONCLUSION

Biosurfactant-producing bacteria in the Niger Delta region of Nigeria hold immense potential for sustainable solutions in bioremediation, agriculture, and industrial applications. Addressing the challenges through innovative research methodologies and interdisciplinary collaborations is essential. Comprehensive studies bridging knowledge gaps will not only deepen our understanding of biosurfactant biology, but also pave the way for novel applications, driving the field toward a more environmentally friendly and economically viable future. A holistic approach, integrating molecular studies, environmental monitoring, and responsible antibiotic usage, is essential to mitigate antibiotic resistance in biosurfactant-producing bacteria. Strict regulations and continuous research efforts are vital to harness the benefits of biosurfactants while minimizing the risks associated with antibiotic resistance in environmental applications.

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