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Exploring the Anti-biofilm Metabolites from *Actinomycetes* Through Metabolomics Tool

(Meneroka Metabolit Anti-biofilem daripada *Actinomycetes* Melalui Alat Metabolomik)

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ABSTRACT

Biofilms, which are robust microbial populations enclosed in self-produced extracellular polymeric substance (EPS) matrices, are a major source of be concern in the healthcare, industry, and environmental sectors because they are resistant to conventional therapies. It is essential for individuals to increase their awareness of antibiotic-resistant strains in relation to oral biofilm-associated diseases, such as periodontitis and gingivitis. *Actinomycetes*, particularly *Streptomyces* species, are prolific makers of secondary metabolites which is a natural products and have strong antibiofilm characteristics. This antibiofilm compound causes fewer negative effects than synthetic medications. Advances in metabolomics tools, including as liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy, have significantly expedited the identification and characterisation of novel bioactive compounds. However, limitations such as poor metabolite yields, scalability concerns, and regulatory restrictions prevent their widespread use. Screening *Actinomycetes* from diverse environments is key to identifying antibiofilm metabolites, with co-culture systems and high-throughput screening accelerating the discovery of novel compounds. This scoping review paper focuses on current breakthroughs, applications, and limits in using *Actinomycetes* and metabolomics tools to discover novel antibiofilm agents. Understanding the mechanisms of antibiofilm action is critical for designing treatment methods, and *Actinomycetes*-derived metabolites provide interesting options for combating biofilm-associated infections in medical and industrial settings. Future studies will concentrate on optimising the production of these metabolites, assessing their complete range of biological functions, and increasing their therapeutic effectiveness.

Keywords: Antibiofilm metabolite; extracellular polymeric substances; metabolomics; oral pathogens

ABSTRAK

Biofilem yang merupakan populasi mikrob kukuh yang tertutup dalam matriks bahan polimer ekstrasel (EPS) yang dihasilkan sendiri, merupakan sumber kebimbangan utama dalam sektor penjagaan kesihatan, industri dan alam sekitar kerana ketahanannya terhadap terapi konvensional. Adalah penting bagi individu untuk meningkatkan kesedaran terhadap strain tahan antibiotik yang berkaitan dengan penyakit yang berpunca daripada biofilem oral, seperti periodontitis dan gingivitis. *Actinomycetes*, khususnya spesies *Streptomyces* adalah pengeluar utama metabolit sekunder yang merupakan produk semula jadi dan mempunyai ciri antibiofilem yang kuat. Sebatian antibiofilem ini menghasilkan kesan sampingan yang lebih sedikit berbanding ubat sintetik. Kemajuan dalam alat metabolomik seperti kromatografi cecair-spektrometri jisim (LC-MS) dan spektroskopi resonans magnet nuklear (NMR) telah mempercepatkan dengan ketara proses pengecaman dan pencirian sebatian bioaktif baharu. Namun, terdapat beberapa kekangan seperti hasil metabolit yang rendah, isu skalabiliti dan sekatan peraturan yang menghalang penggunaannya secara meluas. Saringan *Actinomycetes* daripada pelbagai persekitaran adalah kunci dalam mengenal pasti metabolit antibiofilem, dengan sistem kokultur dan teknik saringan berkelajuan tinggi mempercepatkan penemuan sebatian baharu. Makalah ulasan skop ini menumpukan kepada kemajuan semasa, aplikasi dan batasan dalam penggunaan *Actinomycetes* serta alat metabolomik bagi penemuan agen antibiofilem baharu. Memahami mekanisme tindakan antibiofilem adalah kritikal untuk merangka strategi rawatan dan metabolit yang diperoleh daripada *Actinomycetes* menawarkan pilihan yang menarik dalam menangani jangkitan berkaitan

biofilem dalam bidang perubatan dan industri. Kajian masa hadapan akan memberi tumpuan kepada pengoptimuman penghasilan metabolit ini, penilaian terhadap keseluruhan fungsi biologinya serta peningkatan keberkesanan terapeutiknya.
Kata kunci: Bahan polimer ekstrasel; metabolit antibiofilem; metabolomik; patogen oral

INTRODUCTION

Biofilms are structured microbial ecosystems which adhere to surfaces and are surrounded by a self-produced matrix of extracellular polymeric substances (EPS). This matrix, made up of polysaccharides, proteins, and extracellular DNA, offers structural stability and protects the bacteria inside from external threats including drugs and host immunological reactions (Chowdhury et al. 2023). As a result, biofilm-associated bacteria have resistance levels up to 1,000 times greater than their planktonic counterparts (Emwas et al. 2019). Biofilms are dynamic structures that communicate between cells via quorum sensing, allowing bacteria to coordinate their activity, share resources, and collectively resist treatments. Biofilms present a substantial issue in healthcare, industry, and environmental management due to their unique features (Highmore et al. 2022).

Biofilms are responsible for more than 80% of chronic infections in the hospital setting. These include infections of indwelling medical devices like catheters, prostheses, and endotracheal tubes, as well as chronic wounds and respiratory tract infections in disorders such as cystic fibrosis (Rather, Gupta & Mandal 2021). These infections are difficult to identify and cure, frequently necessitating extended antibiotic therapy or surgical intervention. The economic impact is significant, with biofilm-associated infections leading to longer hospital admissions, greater medical expenditures, and higher mortality rates. For example, catheter-associated urinary tract infections (CAUTIs) make up a large portion of global healthcare spending (Highmore et al. 2022).

Industrial biofilms are also challenging. Biofouling in pipes, heat exchangers, and water treatment systems lowers operating efficiency, increases energy consumption, and requires regular maintenance (Penesyan et al. 2021). Biofilms in maritime settings cause biofouling on ship hulls, which increases hydrodynamic drag and fuel consumption, typically by 10-15% and in some cases up to 30% depending on severity (Highmore et al. 2022; Shree et al. 2023). Similarly, biofilms in food processing plants can cause contamination, jeopardising food safety and quality. Addressing these issues necessitates novel approaches that go beyond conventional chemical treatments, which are either ineffectual or ecologically hazardous (Shree et al. 2023).

Environmental biofilms present distinct problems, especially in aquatic habitats. These microbial communities can host infections, damage water quality, and speed up eutrophication by trapping nutrients like nitrogen and phosphorus. The ensuing algal blooms can destabilise aquatic ecosystems, jeopardising biodiversity and access to water (Penesyan et al. 2021).

To address the various issues provided by biofilms, researchers are increasingly turning to natural products for answers. *Actinomycetes*, a kind of Gram-positive filamentous bacteria, have emerged as a potentially valuable resource. These microorganisms, particularly those of the genus *Streptomyces*, are well-known for their capacity to create a diverse range of secondary metabolites. *Actinomycetes* have historically played an important role in the creation of antibiotics, antifungals, and immunosuppressants. Notable examples are streptomycin, the first antibiotic effective against tuberculosis, and erythromycin, which is often used to treat respiratory infections (Selim, Abdelhamid & Mohamed 2021).

A recent study has shown that *Actinomycetes* have potential in antibiofilm applications. These microbes generate compounds with distinct chemical structures and bioactivities that disrupt biofilms by a variety of mechanisms, including quorum sensing (a cell-to-cell communication mechanism that enables bacteria to coordinate gene expression and biofilm formation) suppression, EPS breakdown, and direct bactericidal activity (Mishra et al. 2020; Rinschen et al. 2019). Surfactin-like lipopeptides, for example, have been found to interfere with quorum-sensing pathways in *Pseudomonas aeruginosa*, inhibiting biofilm formation (Rinschen et al. 2019). Other metabolites, such as actinomycin derivatives, attack the structural integrity of the EPS matrix, effectively destroying the biofilm architecture (Mishra et al. 2020).

The development of metabolomics has transformed the discovery and characterisation of these bioactive compounds. Metabolomics is the complete study of tiny molecules in biological systems, which provides information on the chemical variety of *Actinomycetes'* metabolites. Analytical methods such as liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy have enabled the discovery and structural elucidation of novel antibiofilm agents. Recent metabolomics studies have further demonstrated distinct metabolic signatures associated with antibiofilm activity, where multivariate analyses such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) showed clear separation between biofilm-inhibiting and non-inhibiting strains, indicating specific metabolite clusters responsible for biofilm disruption (Boccard et al. 2021; Chowdhury et al. 2023).

Despite these advancements, significant obstacles remain. The scalability of metabolite synthesis is a considerable hurdle, as many actinomycete-derived substances have poor yields (Zhao et al. 2024). Furthermore, regulatory restrictions for novel bioactive compounds might cause delays in their conversion from laboratory

to clinical or industrial uses. Future research should Solve these constraints by combining metabolomics with complementary technologies like genomics and synthetic biology. Researchers can discover innovative solutions to biofilm-related worldwide concerns by utilising *Actinomycetes'* entire biosynthetic capacity (Chowdhury et al. 2023).

BIOFILMS AND THEIR CHALLENGES

Biofilms are complex microbial communities encased in a self-produced extracellular polymeric substance (EPS) matrix that offers structural integrity and resistance to external harm. The EPS matrix, which is made up of polysaccharides, proteins, and extracellular DNA, is essential for biofilm resilience, making it substantially more resistant to antibiotics and environmental stresses than its planktonic counterparts (Shree et al. 2023). These robust forms are entangled in a wide range of challenges in healthcare, industry, and the environment.

Healthcare implications of biofilms

Biofilms contribute to over 80% of chronic and recurrent illnesses (Rather, Gupta & Mandal 2021). These infections become more dangerous when biofilms build on indwelling medical devices such as catheters, prosthetic joints, and endotracheal tubes. For example, catheter-associated urinary tract infections (CAUTIs) are among the most prevalent healthcare-associated infections HAIs globally, with biofilms on catheters serving as reservoirs for pathogenic bacteria that are resistant to antibiotic therapy. Similarly, biofilm growth on endotracheal tubes frequently causes ventilator-associated pneumonia, complicating therapy and increasing patient mortality (Mishra et al. 2020).

Chronic wounds, such as diabetic foot ulcers and venous leg ulcers, contain biofilms that inhibit healing and contribute to long-term inflammation. The EPS matrix protects biofilm-associated bacteria in these wounds from antibiotics, prompting the development of novel therapeutic techniques. *Pseudomonas aeruginosa* biofilms in the respiratory tract aggravate cystic fibrosis development by resisting both drugs and the immune system's efforts to eliminate the infection (Penesyan et al. 2021).

Industrial challenges

Biofilms are similarly harmful in industrial environments, where they cause biofouling in pipelines, cooling towers, and water filtering systems. Biofouling diminishes operating efficiency, increases energy consumption, and requires regular maintenance. For example, biofilm growth in heat exchangers can drastically limit heat transfer efficiency, increasing energy expenses. In the food and beverage business, biofilms on processing equipment represent significant contamination dangers, affecting product safety and quality. Microbial biofilms can remain after cleaning and sanitisation, demonstrating their resilience to conventional chemical treatments (Rather, Gupta & Mandal 2021).

Biofilms also pose substantial issues in marine settings. Biofouling on ship hulls generated by microbial biofilms increases drag, lowering vessel speed and efficiency. Biofouling on ship hulls increases drag, leading to higher fuel consumption typically ranging from 10-15% and potentially reaching up to 30% under severe conditions, resulting in greater greenhouse gas emissions and operational costs (Highmore et al. 2022; Shree et al. 2023). Anti-biofouling coatings and cleaning procedures are used to address these challenges, although their efficacy is frequently restricted.

Environmental implications

In aquatic habitats, biofilms provide two functions. On the one hand, they contribute to natural nutrient cycling and ecosystem function. They can, however, house harmful bacteria and aggravate eutrophication, especially in nutrient-contaminated water bodies. Biofilms trap nitrogen and phosphorus, causing algal blooms that deplete oxygen and disturb aquatic ecosystems. These impacts are far-reaching, resulting in biodiversity loss and water resource degradation (Penesyan et al. 2021).

Biofilms also pose issues in water treatment plants, where their existence might result in microbial contamination of treated water. Biofilms in distribution systems serve as reservoirs for opportunistic pathogens, putting water quality and public health at risk.

Conventional approaches and limitations

Chemical disinfectants, antibiotics, and physical cleaning procedures are the traditional means of combating biofilms. However, these techniques frequently fail to completely eradicate biofilms due to their intrinsic resistance mechanisms. The EPS matrix works as a diffusion barrier, keeping disinfectants and antibiotics out of the biofilm's microbial cells. Furthermore, latent 'persister' cells inside the biofilm can survive treatment and regenerate the biofilm when circumstances improve (Shree et al. 2023).

Biofilm resistance to antibiotics is especially challenging since it requires larger dosages or longer treatment regimens, increasing the likelihood of adverse effects and the formation of antimicrobial resistance (AMR). Overuse of strong chemical cleansers to fight biofilms in industrial settings can have significant environmental repercussions, such as hazardous chemicals being released into ecosystems (Mishra et al. 2020).

Opportunities for novel solutions

Given the limits of conventional techniques, novel biofilm management solutions are becoming increasingly important. Natural products, particularly those produced from *Actinomycetes*, provide interesting alternatives. These bioactive compounds have distinct modes of action that target the structural and functional components of biofilm. For example, some metabolites interfere with quorum sensing, a bacterial communication system required for biofilm development and maintenance. Other

compounds break down the EPS matrix or kill bacteria in biofilms (Rinschen et al. 2019). Metabolomics advances have allowed researchers to discover and describe these compounds, paving the way for targeted antibiofilm therapy.

ACTINOMYCETES: A RICH SOURCE OF BIOACTIVE METABOLITES

Actinomycetes are Gram-positive, filamentous bacteria known for their exceptional capacity for producing a diverse range of secondary metabolites. These microorganisms, particularly those of the *Streptomyces* species, have emerged as one of the most abundant sources of bioactive compounds, such as antibiotics, antifungals, anticancer agents, and immunosuppressants. Their metabolic diversity, along with their distinct biosynthetic routes, has established them as a key player in the quest for novel antibiofilm agents (Selim, Abdelhamid & Mohamed 2021).

Actinomycetes' evolutionary success is intimately related to their capacity to adapt to various settings. These bacteria are typically found in soil, marine ecosystems, and harsh environments including deserts and hydrothermal vents. Their adaptability is seen in their vast genomes, which frequently contain between 20 and 50 biosynthetic gene clusters (BGCs) that produce secondary metabolites (Chowdhury et al. 2023). These clusters feature sophisticated enzymatic pathways that produce structurally varied and biologically active molecules. *Actinomycetes* gain a competitive edge in resource-limited situations by producing such metabolites, which allow them to suppress rival bacteria while also securing necessary nutrients.

A recent study has shown that *Actinomycetes* have potential in antibiofilm applications. Many of these metabolites have novel modes of action that directly affect biofilm formation and function. Surfactin-like lipopeptides, for example, have been shown to inhibit quorum sensing in *Pseudomonas aeruginosa*, a bacterial communication mechanism required for biofilm development and maintenance. These compounds inhibit biofilm development and bacterial pathogenicity by disrupting quorum-sensing pathways (Rinschen et al. 2019). Similarly, polyketides, another kind of secondary metabolite, have demonstrated promising results in targeting the extracellular polymeric substance (EPS) matrix. By eroding the matrix, these compounds expose the embedded bacteria to environmental stressors, increasing the effectiveness of antimicrobial treatments (Penesyan et al. 2021) (Figure 1).

Actinomycins, a class of chromopeptide lactones generated by *Streptomyces* species, have also shown potent antibiofilm activity. These compounds impair the structural components of the biofilm matrix, rendering bacteria more vulnerable to antibiotics and immunological responses. Non-ribosomal peptides (NRPs), like daptomycin, have

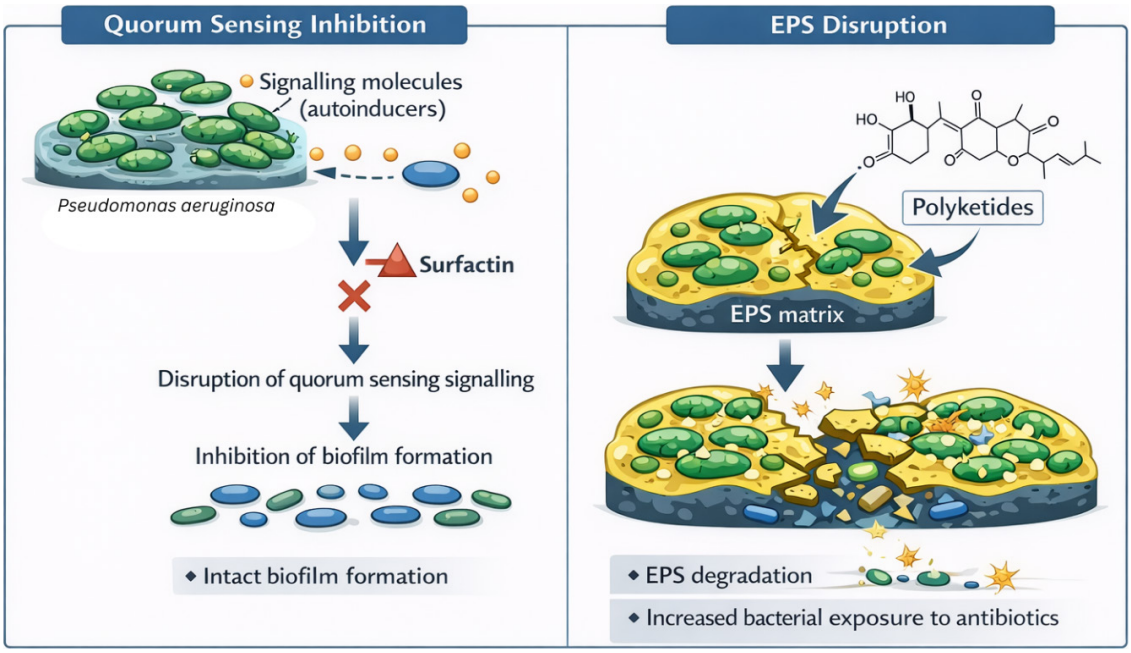

FIGURE 1. Mechanisms of antibiofilm activity of actinomycete-derived metabolites. On the left, surfactin-like lipopeptides disrupt quorum sensing (bacterial communication), thereby preventing biofilm formation in *Pseudomonas aeruginosa*. On the right, polyketides degrade the extracellular polymeric substance (EPS) matrix, leading to structural breakdown of the biofilm and increased exposure of bacteria to antimicrobial agents

been demonstrated to disrupt bacterial membranes and undermine biofilm stability, making them extremely effective against biofilm-associated infections (Jagannathan et al. 2021). Furthermore, some actinomycete-derived metabolites can stimulate the development of biofilm-degrading enzymes, such as DNases and proteases. These enzymes degrade important biofilm structure components, allowing for greater dissemination and improving the efficacy of other treatments (Chowdhury et al. 2023).

Several actinomycete strains have been identified as potential sources of antibiofilm metabolites. Marine-derived *Actinomycetes*, for example, have unique metabolic capabilities as a result of their adaptation to extreme salinity and pressure in marine settings. A strain of *Streptomyces* obtained from maritime sediments was discovered to create polyketides that may suppress *Pseudomonas aeruginosa* biofilms by up to 70% (Tenebro et al. 2021). Soil-derived *Actinomycetes*, such as *Streptomyces coelicolor* have also demonstrated outstanding promise. *S. coelicolor* generates actinorhodin, a polyketide having antimicrobial and antibiofilm properties that impede quorum sensing and bacterial attachment to surfaces (Selim, Abdelhamid & Mohamed 2021). Strains isolated from intense environments, such as deserts and hydrothermal vents, hold further potential due to the distinct chemical structures of their metabolites. For example, a thermophilic *Actinopolyspora* strain generates lipopeptides that are thermostable and active at high temperatures, making them suited for industrial applications (Chowdhury et al. 2023).

Despite these promising discoveries, a number of difficulties prevent *Actinomycetes* from being fully exploited for antibiofilm applications. One significant constraint is the limited production of many bioactive metabolites, which limits their scalability for industrial or clinical applications. To overcome this, researchers are looking into ways to optimise fermentation conditions and use genetic engineering approaches to increase metabolite synthesis (Zhao et al. 2024). Another problem is the intricacy of biosynthetic pathways, which are frequently poorly understood. Advances in genomes and transcriptomics are shedding light on these pathways, allowing for targeted alterations to promote synthesis of desirable compounds (Mishra et al. 2020).

Regulatory constraints also impede the development and promotion of actinomycete-derived antibiofilm agents. These compounds must undergo extensive safety and effectiveness testing before receiving approval for clinical or industrial usage. This procedure is time-consuming and costly, emphasising the need for simplified regulatory frameworks to hasten the conversion of laboratory findings into real-world applications (Penesyan et al. 2021).

Future *Actinomycetes* research will most certainly benefit from the combination of metabolomics with other omics technologies, such as genomics and proteomics. CRISPR-based genome editing advances have enabled researchers to activate previously silent biosynthetic gene clusters, resulting in the development of novel metabolites. Furthermore, co-culture systems in which *Actinomycetes* coexist with other microbes have been found to drive the development of novel secondary metabolites with increased bioactivity (Chowdhury et al. 2023). These techniques show considerable promise for overcoming the present constraints and realising *Actinomycetes'* full potential as a source of novel antibiofilm agents.

METABOLOMICS: DISCOVERY OF NEW DRUGS FROM DIFFERENT TOOLS

Metabolomics is a transformational discipline that studies tiny molecules, or metabolites, in biological systems. This strategy has substantially accelerated natural product development, notably in the context of *Actinomycetes*, by allowing for the identification and characterisation of novel secondary metabolites with antibiofilm activity. The discipline produces specific metabolic profiles using advanced analytical technologies such as liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy. These profiles shed light on the chemical variety of metabolites and their functional roles in biological processes, giving researchers an effective tool for identifying compounds with substantial bioactivities (Emwas et al. 2019).

A key benefit of metabolomics is its capacity to offer a high-resolution picture of an organism's metabolic status under certain environmental or physiological conditions. Traditional metabolite discovery approaches relied heavily on bioassay-guided fractionation, which was time-consuming and inefficient. In contrast, metabolomics enables the simultaneous identification and structural characterisation of hundreds of metabolites in a single analysis, considerably speeding up the discovery process. This advantage is especially important for *Actinomycetes*, which often adjust their metabolite synthesis in response to changes in their growing environment. For example, comparative metabolomics has shown that *Actinomycetes* isolated from marine ecosystems develop unique secondary metabolites from their terrestrial counterparts, several of which have powerful antibiofilm characteristics (Zhao et al. 2024). Polyketides and lipopeptides produced from marine *Streptomyces* strains have been demonstrated to successfully disrupt biofilm development in pathogens such as *Pseudomonas aeruginosa* (Chowdhury et al. 2023).

LC-MS and NMR spectroscopy are two of the most important analytical technologies in metabolomics. LC-MS combines liquid chromatography's ability to separate complex mixtures with mass spectrometry's ability to detect metabolites with high resolution and sensitivity. This makes LC-MS an excellent tool for evaluating the many secondary metabolites generated by *Actinomycetes*. For example, LC-MS was used to detect actinomycin derivatives from *Streptomyces* extracts, which have substantial antibiofilm action by destroying the extracellular polymeric substance (EPS) matrix of biofilms (Rinschen et al. 2019). Similarly, NMR spectroscopy may show comprehensive structural

information on metabolites, such as stereochemistry and functional groups, without the need for substantial sample preparation. This approach has proved beneficial for identifying complex metabolites such as polyketides and non-ribosomal peptides, which are frequently crucial to biofilm disruption processes (Chowdhury et al. 2023).

In addition to LC-MS and NMR, emerging technologies such as matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) and Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry are broadening metabolomics applications. These technologies enable high-throughput analysis and spatial information on metabolite distributions, allowing researchers to trace bioactive chemicals produced inside microbial colonies or biofilm environments. Such developments not only make metabolite identification easier, but also give vital insights into how these compounds work in complex biological systems (Xiao, Zhou & Ressom 2012).

The combination of metabolomics with other omics technologies, including genomics, transcriptomics, and proteomics, has increased its usefulness in natural product discovery. Genomics allows researchers to find biosynthetic gene clusters (BGCs) that contain enzymes for secondary metabolite synthesis, whereas transcriptomics exposes how these genes are expressed under various circumstances. Together, these techniques give a comprehensive understanding of the biosynthetic processes involved in metabolite formation. For example, genome mining of *Streptomyces coelicolor* showed multiple previously silent BGCs that were triggered during co-culture, leading to the identification of novel antibiofilm polyketides (Zhao et al. 2024). Proteomics complements these approaches by identifying enzymes involved in metabolite biosynthesis, providing targets for genetic engineering for enhanced production.

Metabolomics has benefits in antibiofilm research that go beyond the identification of particular bioactive compounds. For example, metabolomic profiling of marine-derived *Streptomyces* strains showed lipopeptides capable of suppressing quorum sensing in *Pseudomonas aeruginosa*. By interrupting bacterial communication mechanisms, these metabolites inhibit biofilm development and pathogenicity. Similarly, comparative metabolomics of soil-derived *Streptomyces* strains showed non-ribosomal peptides that break down the EPS matrix, reducing biofilm stability and increasing conventional antibiotic effectiveness (Mishra et al. 2020; Tenebro et al. 2021). These findings emphasise metabolomics' potential not just for identifying novel antibiofilm agents but also for developing synergistic treatments that combine various metabolites for increased effectiveness.

Despite its transformational potential, metabolomics presents significant hurdles in the study of *Actinomycetes*. The intricacy of metabolite mixtures, which can comprise hundreds of structurally identical compounds, can make analysis and characterisation challenging. To solve this issue, researchers are using machine learning methods and cheminformatics software to examine complex data sets more rapidly. Another issue is the low abundance of many bioactive metabolites, which can make identification challenging and restrict their scalability for industrial purposes. Current research focuses on improving the sensitivity and resolution of analytical equipment, as well as using genetic engineering to boost metabolite yields (Zhao et al. 2024).

Looking ahead, the combination of metabolomics and synthetic biology presents promising potential for furthering antibiofilm research. Researchers may now activate silent biosynthetic pathways using CRISPR-based genome editing, allowing them to produce hitherto inaccessible novel metabolites. Furthermore, co-culture systems in which *Actinomycetes* coexist with other microorganisms have been found to drive the development of novel secondary metabolites with increased bioactivity (Chowdhury et al. 2023). Portable metabolomics technologies might improve on-site analysis in remote or harsh circumstances, allowing for the identification of novel metabolites from hitherto unexplored habitats. These developments have the potential to broaden the area of natural product discovery, opening the door to novel therapeutic and industrial uses for actinomycete-derived antibiofilm agents.

DRAWBACKS AND BENEFITS OF METABOLOMIC TOOLS

Metabolomic methods have revolutionised the study of tiny molecules, showing previously unknown details about the chemical variety of *Actinomycetes'* metabolic products. However, while these approaches have many advantages, their limits must be addressed when evaluating their efficacy in natural product discovery and antibiofilm studies.

The fundamental advantage of metabolomic techniques is their ability to do high-throughput, thorough analyses of complex metabolic mixtures. Technologies such as liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy enable the simultaneous identification and quantification of hundreds of metabolites, even at low concentrations. This feature is especially important for *Actinomycetes*, which develop structurally varied secondary metabolites with antibiofilm potential. For example, LC-MS has been useful in profiling actinomycin derivatives, demonstrating their potent antibiofilm action against pathogens embedded in biofilms (Rinschen et al. 2019). Furthermore, the structural accuracy given by NMR allows for the elucidation of complex chemical structures such as those seen in polyketides and non-ribosomal peptides (Chowdhury et al. 2023). These improved analytical capabilities have sped the discovery process by decreasing the need for traditional, time-consuming bioassay-guided fractionation.

Another benefit of metabolomics is its adaptability. Metabolomic analysis may be performed on a variety of biological systems, including actinomycete cultures, microbial co-cultures, and environmental isolates. This

versatility enables researchers to investigate how growth circumstances and ecological factors affect metabolite production. Comparative metabolomics has been particularly useful for discovering environment-specific metabolites. For example, investigations comparing marine and soil-derived *Streptomyces* strains showed polyketides peculiar to marine isolates that impede quorum sensing and rupture biofilms (Tenebro et al. 2021). These findings are useful in broadening the known repertoire of bioactive compounds.

Notwithstanding their revolutionary promise, metabolomic techniques have several limitations. One notable constraint is the intricacy of the data produced during analysis. *Actinomycetes* develop hundreds of metabolites, many of which have identical structures or are closely related, making it challenging to distinguish and precisely identify all molecules. This intricacy is heightened by isomeric metabolites, which need sophisticated separation and identification methods. While advances in computational tools and machine learning have enhanced data interpretation, these solutions continue to need significant computing resources and expert expertise (Zhao et al. 2024). Furthermore, low-abundance metabolites frequently fall below the detection limits of current analytical systems, thus, masking significant discoveries. Improving the sensitivity and resolution of metabolomic techniques is a significant area for development.

Another disadvantage is the accessibility of metabolomic technology. Many laboratories cannot afford high-resolution devices like LC-MS and NMR spectrometers (Emwas et al. 2019). Their operation and maintenance need specialised training, further restricting access, especially in resource-constrained environments (Zhao et al. 2024). Furthermore, metabolomic investigations create huge datasets that require the use of modern bioinformatics tools for effective analysis. These criteria create practical obstacles, particularly for researchers working at impoverished institutions or in remote areas (Chowdhury et al. 2023).

However, as technology and technique develop, issues connected with metabolomic tools are being addressed more often. Portable metabolomics devices, for example, are gaining recognition as a novel option that allows researchers to do on-site investigations in the field. These tools are especially useful for studying unexplored areas such as deserts, deep-sea vents, and other extreme ecosystems, where *Actinomycetes* with unusual biosynthetic capabilities are frequently encountered. Furthermore, combining metabolomics with genomics and proteomics has shown promise in addressing some of the intrinsic complexity of metabolic data. Genomics generates a map of biosynthetic gene clusters, whereas proteomics reveals the enzymes involved in metabolite production. These alternative techniques, when combined with metabolomics, provide a complete perspective of secondary metabolite synthesis, making it easier to uncover novel bioactive compounds (Emwas et al. 2019).

In addition to technological breakthroughs, methodological improvements are broadening the scope of metabolomics. Co-culture systems, which simulate natural microbial interactions, have been demonstrated to increase the synthesis of metabolites that are quiescent under typical laboratory settings. Metabolomic profiling of co-cultures has shown previously unknown compounds with increased antibiofilm activity (Chowdhury et al. 2023). Similarly, genome-editing technologies such as CRISPR are being utilised to activate dormant biosynthetic pathways, allowing for the development of novel compounds that contribute to biofilm separation.

Despite these optimistic advancements, cost remains a significant barrier. The procurement and operational costs of metabolomic tools, along with the demand for specialised personnel, continue to impede their broad implementation (Emwas et al. 2019; Zhao et al. 2024). However, as technology progresses and prices fall, metabolomic technologies are anticipated to become more accessible to a wider scientific community (Chowdhury et al. 2023).

In conclusion, while metabolomic tools have certain limitations, their advantages greatly exceed their disadvantages, particularly in the context of antibiofilm research. Metabolomics has enabled swift and precise characterisation of secondary metabolites, opening up new pathways for understanding and utilising *Actinomycetes'* chemical diversity. As analytical tools and integrative methodologies progress, metabolomics' potential to generate novel solutions to biofilm-related challenges will only increase.

DISCOVERING ANTIBIOFILM METABOLITES

Screening *Actinomycetes* from various habitats is a critical step in identifying antibiofilm metabolites. *Actinomycetes* survive in a variety of ecological niches, including soil, marine sediments, and harsh environments, each with a distinct set of metabolites. For example, marine-derived *Streptomyces* strains have shown outstanding antibiofilm characteristics, generating compounds that impair quorum sensing and damage biofilm integrity (Tenebro et al. 2021). These distinct environments frequently promote the evolution of specialised biosynthetic pathways, resulting in the development of novel bioactive compounds that cannot be produced under typical laboratory conditions. Researchers are increasingly looking for metabolites with unique chemical profiles and antibiofilm capabilities in underexplored ecosystems such as mangroves, deserts, and deep-sea hydrothermal vents (Chowdhury et al. 2023).

The use of co-culture systems has aided the discovery process by simulating natural microbial interactions that promote the development of distinct secondary metabolites. When *Actinomycetes* are cultivated with other microorganisms, interspecies competition frequently activates silent biosynthetic gene clusters, resulting in the production of novel metabolites. For example, co-culture

of *Streptomyces* with *Bacillus subtilis* led to the discovery of novel lipopeptides with significant antibiofilm action against *Pseudomonas aeruginosa* (Zhao et al. 2024). This method has been particularly useful in identifying molecules that are undetected in monoculture systems, highlighting the relevance of ecological mimicry in metabolite discovery.

High-throughput screening approaches have also transformed the discovery of antibiofilm metabolites. Automation and downsizing advances have made it possible to swiftly evaluate huge libraries of actinomycete extracts for antibiofilm activity. These screens frequently utilise microtiter plates and fluorescent dyes to quantify biofilm biomass, allowing researchers to find extracts that dramatically reduce biofilm development or disrupt pre-existing biofilms (Mishra et al. 2020). When numerous metabolites are examined together, high-throughput approaches not only speed up the discovery process but also make it easier to identify synergistic effects. This is especially critical in biofilm-associated infections, where multidrug methods are frequently required to overcome biofilm resistance (Penesyan et al. 2021).

Metabolomics is critical to the identification of antibiofilm metabolites because it allows for the thorough analysis of metabolic alterations related with biofilm inhibition. Metabolomics is important for finding antibiofilm metabolites because it allows for a full examination of metabolic changes linked with biofilm suppression. Recent LC-MS-based metabolomics studies have discovered significant bioactive compounds such as lipopeptides (e.g., surfactin and fengycin) and polyketides, including actinorhodin, that are highly linked with antibiofilm activity (Chowdhury et al. 2023; Selim, Abdelhamid & Mohamed 2021). Chemometric analyses such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) have shown clear metabolic differences between treated and control samples, indicating significant metabolite shifts caused by biofilm disruption (Boccard et al. 2021). Furthermore, co-culture metabolomics with *Streptomyces* and *Bacillus* species has shown the production of novel or increased metabolites not seen in monoculture systems, implying the activation of silent biosynthetic pathways (Chowdhury et al. 2023; Liang et al. 2023).

Genome mining is another novel strategy to identifying antibiofilm metabolites, which uses bioinformatics tools to uncover biosynthetic gene clusters in *Actinomycetes'* genomes (Chowdhury et al. 2023). This approach has identified multiple previously unknown gene clusters that encode enzymes for the manufacture of structurally novel metabolites. The activation of these gene clusters via genetic engineering or environmental modification has resulted in an abundance of novel antibiofilm compounds. For example, genome mining of *Streptomyces coelicolor* identified an unseen biosynthetic route that, when triggered, provided a suite of lipopeptides with potent antibiofilm activities against *Staphylococcus aureus* biofilms (Zhao et al. 2024). Genome mining not only increases the chemical variety of known metabolites, but it also speeds up the discovery of bioactive molecules with therapeutic applications.

Despite tremendous progress in identifying antibiofilm metabolites, some hurdles remain. One of the major challenges is the limited yield of many bioactive metabolites, which restricts their scalability for industrial or clinical applications (Penesyan et al. 2021). To overcome this issue, researchers are using fermentation optimisation and synthetic biology methods to increase metabolite synthesis (Mishra et al. 2020). Another hurdle is transferring *in vitro* results to real-world settings, where biofilms are frequently more complex and diverse. The development of biofilm models that closely resemble clinical and industrial contexts is essential for assessing the effectiveness of newly identified metabolites under realistic conditions (Rinschen et al. 2019).

In summary, the study of antibiofilm metabolites is a dynamic and multidisciplinary subject that incorporates ecological research, cutting-edge analytical methods, and novel screening methodologies. *Actinomycetes*, with their extraordinary metabolic diversity, continue to be a rich source of bioactive compounds, which show significant potential for addressing biofilm-related global concerns. As approaches advance, the combination of metabolomics, genomics, and high-throughput technologies will speed up the discovery process, opening the door for innovative solutions to biofilm-related issues affecting healthcare and industry.

MECHANISMS OF ACTION

Understanding how these metabolites destroy biofilms is critical for developing them as effective therapeutics. Metabolomics not only assists in the discovery of bioactive substances but also sheds light on their modes of action. For example, certain *Actinomycetes*-derived metabolites were discovered to interfere with quorum sensing - a bacterial communication mechanism required for biofilm formation - while others hindered extracellular matrix creation or directly caused structural damage to the biofilm (Rinschen et al. 2019).

APPLICATIONS AND FUTURE DIRECTIONS

The discovery of antibiofilm metabolites in *Actinomycetes* has important implications for medicinal and industrial uses. In medicine, these chemicals may lead to novel therapies for persistent infections caused by biofilms, such as those found in cystic fibrosis, chronic wounds, and implanted medical devices. Antibiofilm compounds might be employed in industrial applications to avoid biofouling in water systems, pipelines, and other important infrastructure (Jagannathan et al. 2021).

Future study will concentrate on improving the synthesis of these metabolites, understanding their complete spectrum of biological functions, and assessing their

safety and efficacy in clinical settings. The combination of metabolomics and other omics technologies, including genomics and proteomics, can help us better understand the intricate connections between *Actinomycetes* and biofilms, opening the path for the development of novel antibiofilm medicines.

CONCLUSION

The study of *Actinomycetes* using metabolomics methods has shown a wealth of antibiofilm chemicals that have the potential to solve the rising problem of biofilm-related infections. As researchers continue to leverage the potential of metabolomics, the discovery of new bioactive metabolites from *Actinomycetes* is anticipated to play an important role in the development of next-generation antimicrobial treatments. The future of antibiofilm research depends on the integration of sophisticated technology and the continuous discovery of nature's microbial variety.

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