REVIEW ARTICLE

Natural Products Could be a Promising Remedy against Biofilm-Forming Bacteria

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ABSTRACT

A biofilm is a typical form of naturally occurring bacterial development. Biofilm development could be responsible for developing resistance to harmful environmental conditions, such as developing immunity to antibiotics, medicine and other antibacterial agents (e.g., methicillin, vancomycin, etc). The quorum sensing (QS) process is essential for biofilm development and for preserving environmental equilibrium when bacterial numbers increase. Finding novel antibacterial medications that can regulate the development and expansion of biofilms is imperative because these structures are closely related to the appearance of multidrug and extended drug (e.g., tuberculosis) resistance and infectious illnesses. Natural plant-based chemicals have been found in an increasing amount of studies over the past 20 years to have antibacterial and chemo-preventive properties in the parameter of biofilm growth. The present article will include some herbs with unknown bioactive ingredients or unclear processes, as well as current findings on the identification of natural anti-biofilm chemicals from plants with known molecular addresses or mechanisms. The authors are also focused on developing techniques for locating and isolating naturally occurring anti-biofilm agents. Anti-biofilm treatments awaiting clinical trials are also reviewed. The currently identified natural anti-biofilm compounds are auspicious and may present novel therapeutic strategies for biofilm-associated illnesses.

Keywords: Antibiotics, Resistance, Microorganism.

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INTRODUCTION

Numerous techniques for bacterial survival have developed over billions of years due to selective forces, enabling this organism to adapt to nearly any environmental niche. Over 90% of bacteria are found in a form known as biofilm, which is one of their favored growth phases. Biofilms are multicellular bacterial colonies that have attached themselves to surfaces and become embedded in the extracellular matrix or ECM. It has been demonstrated that quorum sensing (QS), which is the way of cell communication, is necessary for the formation of biofilms with the ECM around them. Biofilm-dwelling bacteria display a significantly stronger outline of adaptive resistance to various antibiotics and disinfectants than their planktonic counterparts. The global increase in adaptive antibiotic resistance is a challenge to the treatment of both acute and chronic illnesses associated with biofilms. These included cases of nosocomial pneumonia, ventilator-associated pneumonia, surgical and burn wound infections, catheter-related infections, etc. As a result, biofilm has instigated a lot of difficulties in the food industry and the medical field. However, the misuse and overuse of antibiotics have also resulted in the development of drug resistance, which may exacerbate bacterial illness. Therefore, new strategies to combat the growth of bacteria and biofilms should be developed without resorting to the usage of antibiotics. Many new strategies, including natural plant-based remedies, have been developed and published in the last 20 years to prevent QS and the formation of biofilm. Research has demonstrated that specific organic plant components possess antimicrobial and chemo-preventive properties.[5] Literature reveals that plant-based medicines have been utilized for thousands of years by various civilizations and that certain of these plant-based products are vital for both treating and preventing infectious diseases. For instance, bacterial infections were regularly treated and prevented with traditional Chinese medicinal herbs. Certain plants, such as tussilago, taraxacum, or scutellara, have antibacterial qualities. Recent research has also demonstrated that plant extracts can inhibit QS and regulate the formation of biofilms. Medicinal herbs may be advantageous and more promising for the extraction of innovative products for fighting against biofilm since there are hundreds of herbs in the world and traditional Chinese medicine has long utilized them to heal

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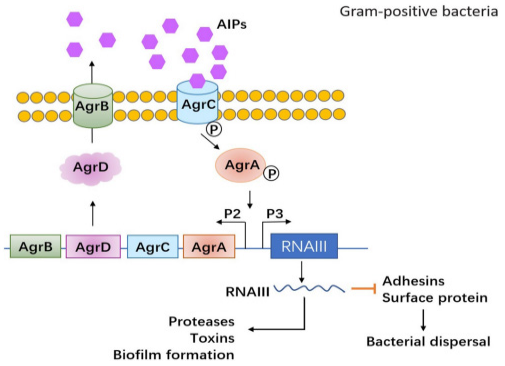

Figure 1: The formation of biofilm and QS

infectious disorders.[1] This review provides brief ideas of the mechanisms underlying the formation of biofilm and QS, as well as recent advancements in the identification and discovery of naturally occurring compounds derived from plants that function as anti-biofilm medications and extraction techniques for the identification of potential constituents.[2]

Formation of Biofilm and its Relation to QS

One of the main factors contributing to bacteria acquiring multidrug resistance is biofilm development. The life cycle of a biofilm comprises four steps: The initial adherence of bacteria, the development of microbial colonies, the growth of microorganisms and the generation of extracellular matrix (ECM), and the mature stage of biofilm, which is followed by the bacteria's dispersal to find new habitats (Figure 1). The host polymerization matrix, which is mostly made up of nucleic acids, protein, and exopolysaccharides, is presented on the outermost layer of the substratum and aids in the bacteria's irreversible attachment. According to a report, the G5 domain of the Aap protein, which has accountability for microbial inter-cell adhesion, was involved in *Staphylococcus epidermidis* starting attachment. In this context, other cell surface-associated proteins, such as SasG, were also mentioned. External factors include the glycosyltransferases (GtfE, GtfG, and GtfH), the extracellular glucan-binding protein, and the surface-exposed protein also has a major impact on the characteristics of cell adhesives. Sortase A (SrtA), a transpeptidase that may bind cell surface proteins, is also responsible for extracellular localization and the formation of biofilms in gram-positive bacteria, including *S. aureus*. The biofilm reaches maturity; the bacteria can break free and form new attachments, so initiating another stage in the biofilm life cycle.[3]

This image shows the four stages of a film life cycle as well as the factors that contribute to biofilm formation and QS. In order to generate biofilm, bacteria first adhere to surfaces, whereupon this attachment turns into an irreversible one. Proteases, cell surface proteins, or proteins related to biofilms are implicated in the beginning of biofilms at these two times, in addition to the cell DNA. The following phases are responsible for the extracellular matrix and the development of biofilm (Figure 2). QS process is involved in the last two phases of biofilm growth. The synthesis of various virulence factors is regulated by a number of autoinducers and the transcriptional receptors that bind to them, helping to preserve environmental equilibrium and prevent the formation of biofilms. If the bacteria spread out across the biofilm to occupy new niches and initiate fresh biofilm growth, the biofilm life cycle might finally be finished. Targeting every stage of the biofilm development process—such as preventing microbial attachment to the adhesive matrix, interfering with extracellular matrix creation, and halting QS signaling—is the main goal of anti-biofilm formation approaches.[4]

It has been demonstrated that the Agr system is the most conventional QS system in gram-positive bacteria. An *S. aureus* has been a well-explored arg system. This system is important because it generates two virulence agents: Proteases and toxins. The Agr system is managed by the Agr operon, which is made up of AgrA, AgrB, AgrC, and AgrD. AgrD is the source of autoinducer peptides (AIPs), a special type of autoinducer found only in gram-positive bacteria. AgrD is released into the extracellular matrix after being modified by AgrB. When the density of bacteria rises, AIPs will trigger the activation of the transmembrane protein known as AgrC. AgrA is further activated by the phosphorylated AgrC, ultimately enhancing the expression of the target gene. AgrA has the ability to control two promoters. P2 is responsible for controlling the Agr proteins, while P3 has the ability to trigger the expression of RNAIII. RNAIII is the main regulator of QS-related genes and proteins which responsible for the formation of biofilms. RNAIII can upregulate the expression of virulence agents such as proteases, toxins, and degradative enzymes. RNAIII, however, can also stop the production of cell- and surface-sticky proteins, which could promote the growth of germs. The Agr system's multiple roles could counteract bacterial infection and swarming. Additionally, this will offer therapeutic targets for the development of antibiofilm drugs, such as RNAIII, Agrs, or AIPs.[5]

Autoinducer acylhomoserine lactones (AHLs) are produced by gram-negative bacteria and are responsible for controlling the expression of particular genes by activating corresponding cytoplasmic receptors. The traditional primary regulators of

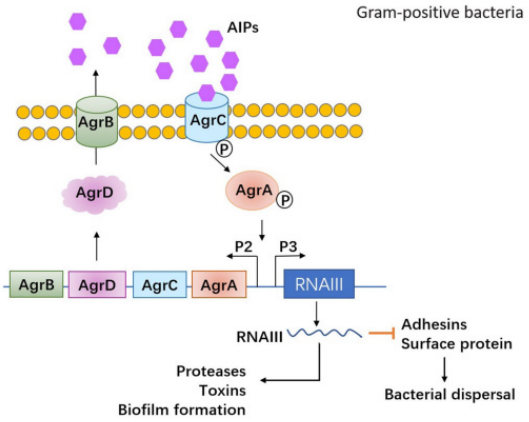

Figure 2: The role of classical QS signaling in gram-positive bacteria's biofilm formation

the QS machinery in gram-negative bacteria are the Luxl/luxR transcriptional factors. AHLs have the ability to activate these factors, which in turn stimulate the expression of target genes, including virulence factors such as toxin, lectin, pyocyanin, elastase, and proteases. Different types of gram-negative bacteria have different QS receptors linked with them (e.g., LasI/LasR, RhlI/RhlR, CqsS and LuxPQ, etc.) and different autoinducers (e.g., pseudo the quinolone signal, CAI-1, AI-2, etc,). Finally, the activation of receptors by particular autoinducers promotes the production of genes like adhesives and virulence variables, which are further implicated in the formation of biofilms.[6]

Natural Anti-biofilm Materials with Distinct Molecular Addresses or Well-defined Processes

In-vitro, some naturally occurring substances sourced from plants showed antibacterial and anti-biofilm characteristics. Many substances derived from organic plant or herbal product extracts were found. The main ways that natural products prevent the formation of QS networks and biofilms are by inhibiting the formation of the polymer matrix, reducing cell binding and attachment, disrupting the generation of extracellular matrix, and dropping the formation of virulence factors. The anti-biofilm substances that were isolated from medicinal plants, including garlic, *Coptis chinensis*, and *Cocculus trilobus,* among others.[7]

Cocculus trilobus

Kim SW and coworkers discovered that the herbal extracts from *C. chinensis* and *C. trilobus* may stop germs from sticking to surfaces coated with fibronectin. They demonstrated anti-adhesin activities by blocking sortase, a membrane enzyme that helped chemically bind surface proteins to amino acids in gram-positive bacteria during the adhesion phase of biofilm development. It was discovered that the ethyl acetate component of *C. trilobus* had the highest capacity to block bacterial adhesin by particularly targeting sortase after screening the ethyl acetate and water components of these two plants.[8]

Cranberry polyphenols

Cranberries in the fruit are a fantastic source of polyphenols. A non-dialysable cranberry fraction rich in high molecular weight polyphenols has been shown to prevent human pathogens—especially cariogenic and lute onto pathogenic bacteria—from adhering to and colonizing in host tissues. It also inhibits the formation of biofilms. Cranberry components also affect the function of enzymes that break down the extracellular matrix, bacterial hydrophobicity, coaggregation, and glucose-binding proteins, all of which are implicated in the creation of biofilms. The cranberry components with large molecules of polyphenols, in particular, may be helpful as bioactive compounds with encouraging qualities for the treatment and/or prevention of oral disorders, such as periodontitis and dental caries, according to the previously mentioned potential advantages of cranberries.[9]

Herba patriniae extract

Fu *et al*. created a luxCDABE-based reporter system to measure the expression of six significant biofilm-associated genes in *P. aeruginosa*. The capacity of 36 herb extracts to inhibit those genes was then examined using this technique. The findings demonstrated that the *H. patriniae* extract effectively suppressed most of these biofilm-related genes and that this decrease in biofilm growth and interference with the mature biofilms' morphology happened at the same time. Furthermore, *H. patriniae* extract decreased *P. aeruginosa's* synthesis of exopolysaccharides. These findings suggested a possible target for the investigation of novel medications to treat illnesses linked to *P. aeruginosa* biofilms.[10]

Ginkgo biloba extract

It has been noted that *G. biloba* extract efficiently prevents *E. coli* biofilms from growing on the surfaces of nylon, polystyrene, and glass membranes at concentrations of 100 µg/mL. According to the methods of inhibitory activities, ginkgolic acid repressed curli and prophage genes in *E. coli*, which was commensurate with decreased fimbriae manufacturing and decreased biofilms. In a different investigation, it was demonstrated that cinnamaldehyde affected the formation of biofilm and structure and decreased the swimming motility of *E. coli*. Cinnamaldehyde and its derivatives influence the pathogenicity, stress response, and biofilm development of *Vibrio* spp., according to Brackman *et al*. By reducing LuxR's ability to bind DNA, the method of QS suppression demonstrated disruption with AI-2 based QS in different *Vibrio* spp.[11]

Phloretin

Apples are rich in phenoletin, an antioxidant. According to Lee *et al*. it had little effect on planktonic cell multiplication but dramatically reduced the *E. coli* strain's ability to generate fimbria and biofilms. Phloretin also prevented *E. coli* O157:H7 from attaching to normal colon epithelial cells and curbed the inflammatory response that was set off by tumor necrosis factor-alpha. The mechanism of phenolretin's inhibitory effects in *E. coli* biofilm cells involved the inhibition of prophage genes, autoinducer-2 importer genetics (lsrACDBF), curli proteins (csgA and csgB), toxin genes (hlyE and stx(2)), and curli genes. This study indicates that phloretin inhibits the formation of biofilms and also acts as an anti-inflammatory agent. Additionally, phenoletin demonstrated 70% inhibitory efficacy against the production of biofilms by *S. aureus* strains, possibly as a result of its ability to target efflux proteins.[12]

Limonoids

Citrus limonoids are one particular kind of secondary metabolite for a triterpenoid. Pure limonoids' capability to affect *Vibrio harveyi* biofilm formation and cell-cell signaling seems to be based more on controlling luxO expression than luxR promoter activity. Isolemonic acid and ichangin are two potent modulators of bacterial cell-cell signaling. Ichangin and isolimonic acid are potent inhibitors of the type III secretion system and biofilm, as demonstrated by the mechanism of

isolimonic acid's inhibitory impact. Furthermore, it seems that isolimonic acid tampers with the AI-3/epinephrine driven cell-cell signaling pathway that is dependent on QseBC and QseA. Zhou *et al*. first demonstrated that hormones inhibited QS-controlled activities, including the generation of signal molecules, and that there was a concentration-dependent decrease in the production of signal molecules.[13]

Hordenine

Hordenine also effectively reduced the expression of genes linked to QS and virulence in *P. aeruginosa* PAO1. According to research, hordenine's anti-QS property works as a novel QS-based medication to guard against viruses found in food and as a rival to signaling molecules. In order to conjugate with hordenine, nanoparticles (NPs) such as AuNPs were also produced. Hordenine-AuNPs shown increased anti-biofilm capabilities on *P. aeruginosa* PAO1, indicating that natural compounds supplied by nanoparticles might be used effectively in biofilm-based infection by microbial species.[14]

Quercetin

Plant polyphenols such as quercetin can be found in a wide variety of fruits, vegetables, and grains. At a lower dosage than that of the majority of previously identified plant extracts and compounds, it was found to significantly prevent the development of biofilms and the generation of virulence factors such as pyocyanin, protease, and elastase. Subsequent analysis of the QS-related transcriptional alterations revealed a marked decrease in the expression levels of RhlI, RhlR, LasI, and LasR, which are implicated in QS signaling. In *P. aeruginosa*, quercetin seemed to be a potent inhibitor of virulence factors and biofilm development. It was also found to be an effective SrtA inhibitor, which by inhibiting sialic acid expression, may severely reduce biofilm creation of *Streptococcus pneumoniae*. The anti-biofilm characteristics of quercetin were also investigated in respect to bacteria that form biofilms, including *S. mutans* and *Enterococcus faecalis*. According to the results, quercetin may find application in human health treatments for antimicrobial infections and anti-carcinogenic effects. Furthermore, it was shown that quercetin-decorated and quercetin-conjugated nanoparticles had stronger anti-biofilm activities, opening up new possibilities for the creation of therapeutic medicines that prevent microbial infections.[15,16]

Plant-based Separation and Extraction of Bioactive Anti-biofilm Constituents

Even though a number of herbal extracts have already demonstrated their capability to hinder biofilm development, more investigation is still required to pinpoint the specific bioactive ingredient or components. As such, disassembling and discarding the constituents of a potent anti-biofilm is essential. To date, it is well observed to identify bioactive components that function as agents to prevent the formation of plant biofilms. These methods include separation by chromatography and structure-based online screening (SB-VS). This has created a solid foundation for the identification of novel substances that deal with bacterial illness and biofilm management. Furthermore, we furnished a synopsis of the techniques employed to differentiate and recognize the bioactive constituents in plant extract. Here, few of the most preferred techniques are mentioned.[5]

Chromatographic separation

According to Kawarai coworkers, tea may prevent *Streptococcus mutans* from adhering to surfaces and forming biofilms. Compared to green tea, Assam tea exhibits stronger anti-biofilm activity toward *S. mutans*. QSI in Assam tea was identified and purified using HPLC and ultrafiltration with centrifugal filter devices. The anti-biofilm impact of Assam tea was higher than that of green tea due to a substance with a molecular weight of less than 10 kDa that was significantly concentrated in galloylated catechins. However, it was found that the pectin found in green tea, which has a mass greater than 10 kDa, encourages the production of biofilms.[17]

Structure-based virtual screening (SB-VS)

Numerous findings have shown that the QS system is a unique and auspicious object for antibacterials. *In-silico* docking analysis and SB-VS were employed to search for potential *P. aeruginosa* QSI against receptors in QS signaling pathways. Five of the best-ranking natural compounds were tested against *P. aeruginosa's* LasR and RhlR receptors out of about 2000 total. Five chemicals that scored highly on the pharmacological effects scale were tested using *in-vitro* bioassays: mangiferin, rosmarinic acid, the substance, and chlorogenic acid, also known as morin. The compounds were evaluated against strain PAO1 and two clinical isolates that were resistant to antibiotics. The majority of these substances considerably reduce the virulence factor production and may also reduce behaviours connected to biofilms. In a different investigation, 3040 natural chemicals and their derivatives were screened for novel QSI candidates using the SB-VS technique. Docking scores and molecular masses were used to identify 22 compounds, with the QS receptor LasR acting as the target. To determine the QSI efficacies of these substances, more research was done. Using a live reporter assay for QS, it was demonstrated that five drugs could decrease QS-regulated gene expression in the bacterium *P. aeruginosa* in a dose-dependent manner. About 46 amino acids, comprising several QS-controlled virulence components in *P. aeruginosa* PAO1, including enzyme IV, chitinase, and pyoverdine synthetases, were also demonstrated to be regulated by these chemicals (19 were upregulated and 27 were downregulated).[18]

Others

In addition to the two approaches mentioned above, other screening methods were also used to find anti-biofilm compounds. After building a group of screening systems known as QSI selectors, Rasmussen *et al*. chose unique QSIs from libraries of synthetic and natural compounds. As a result, the QSI selectors found that the garlic and 4-nitro-pyridine-N-oxide (4-NPO) extracts had the most bioactive ingredients. Additionally, selectivity for QS-controlled genes linked to virulence and pharmacological effects was

demonstrated utilizing a *Caenorhabditis elegans* illness model and GeneChip-based transcriptome analysis.[19]

CONCLUSION AND FUTURE PERSPECTIVES

Rising tolerance to antibiotics and antimicrobial agents has been found in biofilms, which is problematic for human health care. Currently, treating illnesses linked to biofilms is a challenging task for microbiologists and doctors. To address the problem of antibiotic resistance in infectious microbiological diseases, new antimicrobial techniques must be developed.

This evaluation has demonstrated that it offered a comprehensive library for the examination of anti-biofilm chemicals. An increasing amount of research on the advantages of plant-based diets and health benefits has been conducted in recent years. By analyzing their inhibitory effects on the production and growth of bacterial biofilms, a number of researchers have looked at the potential of natural products as alternative treatments for bacterial illnesses. The most recent study found that most natural anti-biofilm medications showed promising preclinical outcomes for anti-biofilm efficacy in a variety of bacterial species. Their most likely regulatory tool was the blockage of QS network or the suppression of every step of biofilm growth. In the past 20 years, methods such as chromatographic separation have been used to screen anti-biofilm compounds, which have made it possible to identify the components that work. Many investigations on plant-based extracts with anti-biofilm effects have not been able to identify the molecular structures of the bioactive chemicals, necessitating further research. Furthermore, the fact that the stages I–IV clinical trials are being conducted on natural anti-biofilm medicines as a means of addressing infectious therapy is hopeful. The primary focus of ongoing clinical research is the external application of oral biofilm resulting from periodontitis, gingivitis, and tooth plaque. In clinical settings, where biofilm infections are firmly ingrained in the tissues, viscera, or other internal organs, it is complicated to assess the safety and effectiveness of plant-based products. However, enhanced specificity, safety, and efficacy are also necessary for the expansion and assessment of organic anti-biofilm medicines in clinical settings, either on their own or in combination with existing antibiotics. This would enhance global health care and have a major positive impact on the management of bacterial illnesses.[20]

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