

## SCIENTIFIC ARTICLE

### Anti-biofilm compounds from Actinobacteria

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### Introduction

Biofilm is a major concern in current perspective. They are ubiquitous in nature. It is an aggregation of microorganisms in which cells adhere to each other on a surface area. These adherent cells are frequently fixed within a self-produced matrix of extracellular polymeric substance (EPS). Non-cellular materials have provided better support for the development of biofilm, may also be found in the biofilm matrix. Additionally, biofilm also colonize many household surfaces in bath and kitchen, including toilets, sinks, pipelines and medical devices. Poor disinfection practices and ineffective cleaning processes may increase the prevalence of illnesses associated with pathogenic organisms encountered during normal household activity.

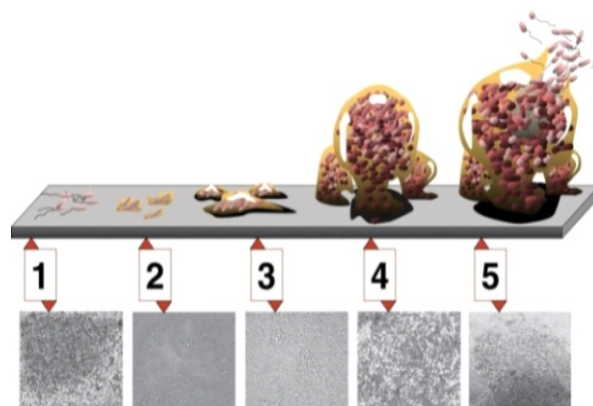
Biofilm can comprise single microbial species or multiple microbial species and can form on a range of biotic and abiotic surfaces. Although mixed-species biofilm predominate in most environments, single-species biofilm exist in a variety of infections and on the surface of medical implants (Adal and Farr, 1996). Hence, single-species biofilm are the focus of most current research. Among the various, single-species biofilm-forming bacterium, *Pseudomonas aeruginosa*, gram-negative bacterium emerge as the most studied. Although, as detailed in this review, the other gram-negative bacteria, *P. fluorescens*, *Escherichia coli*, and *Vibrio cholerae* have also been studied in detail. The gram-positive biofilm-forming bacteria that have been studied include *Staphylococcus epidermidis*, *S.aureus*.

### Biofilm Formation

Formation of a biofilm starts with the attachment of free-floating microorganisms on a surface. The development of a biofilm let for an aggregate cell colonies to become antibiotic resistant. Cell-cell communication or quorum sensing (QS) has been shown to be involved in the formation of biofilm in several bacterial species (Rodney, 2002).

The following are the five stages of biofilm

development. Stage 1 initial attachment; stage 2 irreversible attachment; stage 3 maturation I; stage 4 maturation II and stage 5 dispersion. The photomicrograph show the development of *Pseudomonas aeruginosa* biofilm formation.



(Image Source: Boundless. "Biofilms and Infections." *Boundless Microbiology*.)

All photomicrographs are shown to same scale.

### Impact of Biofilm on Environment

Biofilm are an integral part of the natural environment and can also serve very beneficial purposes, such as in the treatment of drinking water, wastewater, detoxification of hazardous waste and commercial application of microbial leaching (Kokare *et al.*, 2009).

However, biofilms have great negative impacts on the world's economy and pose serious problems to industry, marine transportation, public health and medicine due to increased resistance to antibiotics and chemical biocides, increased rates of genetic exchange, altered biodegradability and increased production of secondary metabolites. In hospitals, biofilms are a particularly important problem. Bacterial biofilms can be colonized in several medical devices, such as catheters, heart valves, prostheses, surgical pins etc., and become the sources of infections. For example, there are more than one million cases of catheter-associated urinary tract infections (CAUTI) reported every year, many of which can be attributed to biofilm-associated bacteria (Davies, 2003). Moreover, many chronic infections, like diabetic, ulcers or lung infections in patients with cystic fibrosis, result from bacteria growing as biofilm. Biofilm have been found to be involved in a wide variety of microbial infections in the body, nearly 80% of all infections. Recently various approaches were proposed and expected to be effective in directly preventing or eliminating bacterial biofilm.

Anti-biofilm activities of some small molecules are tabulated below.

Small molecules – Anti-biofilm activity (Pooi and Yien, 2014)		
Name	Activity against	Properties
Aryl rhodanines	<i>Staphylococcal aureus</i> and other Gram-positive bacteria, but not Gram-negative bacteria	Inhibit the early stages of biofilm development by preventing attachment of the bacteria to the surfaces
Ethylene glycol tetraacetic acid (EGTA)	<i>S. aureus</i> strains	The chelators prevented biofilm formation
Trisodium citrate (TSC)	<i>S. aureus</i> strains	The chelators prevented biofilm formation
Cis-2-Decenoic acid (C2DA)	Methicillin-resistant <i>S. aureus</i> (MRSA), in addition to other Gram-positive and Gram-negative bacteria.	Potentially control initiation of biofilm formation in addition to dispersion of existing biofilm
D-amino acids	<i>S. aureus</i> , <i>Bacillus subtilis</i> and <i>Pseudomonas aeruginosa</i>	A mixture of D-amino acids reportedly triggered biofilm disassembly

### Anti-biofilm agents

2-Aminoimidazoles are an emerging class of small molecules that inhibit biofilm formation and disperse bacterial biofilm (Richards and Melander, 2009). Nitroxide compounds were found to significantly suppress biofilm formation of *P. aeruginosa* and also mixed-culture biofilms (Stefanie-Ann *et al.* 2015). Saima and Rabih (2011) demonstrated that, both *in-vitro* and in a pilot clinical trial, the role of Nacetylcysteine (NAC) in the treatment of bacterial biofilms on the surface of intravascular catheters. Dispersin B (DspB), a Matrix-targeting enzymes, prevented the formation of *S. epidermidis* biofilm. It is being commercially developed as a wound care gel and medical device coating. External and internal catheters that were coated with a combination of Dispersin B and triclosan. These catheters were shown to be effective at preventing bacterial infection (Fazekas *et al.*, 2012). Enzymes, like DNase I,  $\alpha$ -amylase and DspB are biofilm-dispersing agents that degrade the biofilm matrix, permitting increased penetration of antibiotics. DNase I, which is also a Matrix-targeting enzymes, cleaves eDNA in the biofilm matrix and prevents biofilm formation on abiotic surfaces, such as glass, plastic, and titanium surfaces (Mann *et al.*, 2009).

Nanoparticles also have a significant property of anti-biofilm activity. Silver ions and silver nanoparticles have been used as anti-biofilm and antimicrobial agents in the treatment of burns and chronic wounds. The antimicrobial property of silver ion is known as an oligodynamic effect, a process in which metal ions interfere with the growth and prevent the biofilm formation. Several *in vitro* studies have been carried

out to document the effectiveness of silver ion at preventing infection, both in coating form and as nanoparticles dispersed in a polymer matrix (Markowska *et al.*, 2013). Zinc ions and ZnO nanoparticles are also found to remarkably inhibit the biofilm formation of *Pseudomonas aeruginosa* (Jin-Hyung *et al.*, 2014).

### Need for implementation of anti-biofilm

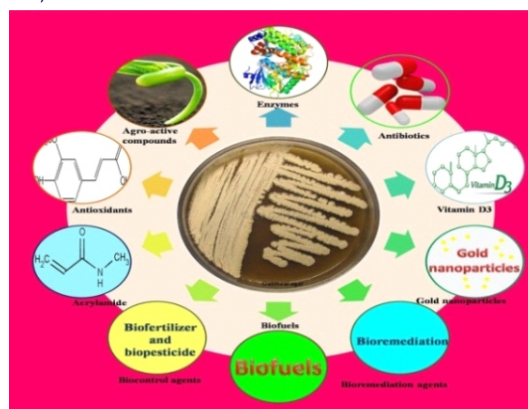
Currently, there is awareness on the use of and protection against biofilms. Due to the lack of effective anti-biofilm, novel alternative compounds or strategies are urgently required for biofilm control.

### Actinobacteria

Actinobacteria are well-known producer of several novel medicinally useful compounds. Actinobacteria are widely distributed in terrestrial and in aquatic ecosystem (Goodfellow, 1988). Actinobacteria are one of the most efficient groups of secondary metabolite producers. Among its various genera, *Streptomyces*, *Saccharopolyspora*, *Amycolatopsis*, *Micromonospora* and *Actinoplanes* are the major producers of commercially important biomolecules. Around 33,500 bioactive secondary metabolites produced by microorganisms, among that 13,700 are known to be produced by actinobacteria, representing 40 % of total bioactive microbial metabolites discovered. The bioactive secondary metabolites produced by actinobacteria include antibiotics, antitumor agents, immunosuppressive agents and enzymes

### Actinobacteria role in anti-biofilm development

Actinobacteria have been looked upon as potential sources of anti-biofilm compounds too, and the work done earlier has shown that actinobacteria play a prominent role in the development of anti-biofilm compounds (Venugopal *et al.*, 2013). You *et al.* (2007) demonstrated that inhibition of *Vibrio*



### Potential applications of actinobacteria

(Image Source : Shivlata and Satyanarayana, 2015)

biofilm formation by a marine actinobacteria A66, *Streptomyces albus*.

Coral-associated actinobacteria (CAA) are seen to be a promising source of anti-biofilm compounds, for developing novel drugs against highly resistant *Staphylococcal* biofilms. Bakkiyaraj and Pandian (2010) evaluated the effect of a coral associated actinomycete (CAA)-3 on *S. aureus* biofilms both *in vitro* and *in vivo*. Methanolic extracts of CAA-3 showed a reduction in *in-vitro* biofilm formation by *S. aureus*, ATCC 11632, methicillin resistant *S. aureus* ATCC 33591 and clinical isolates of *S. aureus*. CAA also represent an interesting source of marine invertebrates-derived antibiofilm agents in the development of new strategies to combat Streptococcal biofilms. Paramasivam *et al.* (2010) proved that, CAA extracts significantly inhibit biofilm formation up to 60–80%. The extract of *Streptomyces akiyoshinensis* displayed efficient antibiofilm activity against all the biofilm forming M serotypes.

Bavya *et al.* (2011) study showed that actinobacteria strain inhibited the biofilm formation. *Streptomyces filamentous* showed good activity against biofilm formation. Marine *Streptomyces* is a potential organism against biofilm produced by *Vibrio spp.* *Streptomyces sp.* inhibited *P. aeruginosa* biofilm formation by 90 % without affecting the growth of planktonic *P. aeruginosa cells*. The experiments on solvent extraction, heat treatment, and proteinase K treatment suggested that hydrophilic compound (s), possibly extracellular peptides or proteins from *Streptomyces sp.* cause the biofilm reduction of *P. aeruginosa* (Kim *et al.*, 2012). Terrestrial actinobacteria also have the ability to inhibit the biofilm formation. Manickam *et al.* (2014) emphasized the role of *Streptomyces spp.* crude fatty acid extract, inhibiting the biofilm formation of *S. pyogenes* ATCC 19615. An attempt was made to study the *in-vitro* inhibition of biofilm forming ESBL (extended spectrum beta lactamase) pathogens such as *Escherichia coli*, *Pseudomonas sp.* and *Klebsiella sp.* by actinobacterial extracts by Hemachandran *et al.* (2011). The extracts isolated produced from the actinobacterial strain MA7 inhibited all the biofilm forming ESBL pathogens. The recent finding of anti-biofilm studies proved that, we anticipate that these new strategies and approaches will be eventually developed from actinobacterial world, for use in the treatment of problematic biofilm-related infections in the clinical settings and industries

## Conclusion

Biofilm is a current challenging problem in environmental and public health concern. Researchers in the fields of clinical, food and water, and environmental microbiology have begun to investigate microbiologic processes with a biofilm perspective. As the pharmaceutical and health-care industries embrace this approach, novel strategies for biofilm prevention and control will undoubtedly emerge. Actinobacteria have been looked upon as potential sources of anti-biofilm compounds. The earlier and current works have shown that actinobacteria are the promising producer for anti-biofilm compounds.

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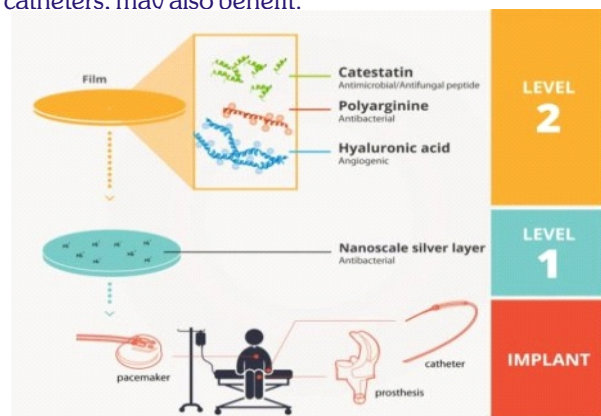
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<http://thescienceofnutrition.me/2015/03/13/natural-anti-biofilm-agents/>

### RESEARCH REPORTS

#### Antimicrobial film for future implants

The implantation of medical devices is not without risks. Bacterial or fungal infections can occur and the body's strong immune response may lead to the rejection of the implant. Researchers at Unit 1121 "Biomaterials and Bio-engineering" (Inserm/Strasbourg university) have succeeded in creating a biofilm with antimicrobial, antifungal and anti-inflammatory properties. It may be used to cover titanium implants (orthopaedic prostheses, pacemakers...) prevent or control post-operative infections. Other frequently used medical devices that cause numerous infectious problems, such as catheters, may also benefit.



#### Levels of implantation.

(Image Credit: Inserm / E.Falett)