A REVIEW ON BIOFILM METHODS AND EMERGENT CONTROL OF BIOFILM PRODUCING BACTERIA FROM WATER PLUMBING SYSTEM

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INTRODUCTION

Sri Lanka has a well-established drinking water distribution and wastewater drainage system in several parts of the country when compared with other developing countries. Microbiological contamination of drinking water in the plumbing system occurs either due to planktonic (free-floating) bacteria in flowing water or bacterial biofilms grown on the inner surfaces of water pipes (Ullah *et al*., 2021). A water plumbing system consists of a complex network of water supply pipes, drain pipes, vent pipes, and more. Pipelines in the plumbing system are more prone to bacterial biofilm colonization. A biofilm is an accumulation of microbial organisms that are irreversibly attached to a surface and generally enclosed in a polysaccharide matrix. When the microbial organisms are attached to surfaces, they produce a thick and sticky mucus-like substance for anchorage. These slimy secretions help microorganisms to permanently attach to the surface. The drinking water gets contaminated with biofilm bacteria when it detaches from the pipelines with the flow of water. Drain lines in the plumbing systems are the perfect environment for biofilm to grow. They provide constant nutritional sources and a suitable wet environment for the bacteria (Papciak *et al*., 2019). *Acinetobacter* spp, *Pseudomonas* spp, *Klebsiella* spp, *Escherichia coli*, *Staphylococcus aureus* are the common biofilm producers in water plumbing systems (Mahapatra *et al*., 2015). Moreover, the damaging of old wastewater pipelines and unsafe drainage systems is one of the main reasons for biofilm bacteria entering the environment. Consequently, since biofilm harbors antibiotic-resistant bacteria, people will be exposed to them if they get into contact with such environments as soil, floods, wells, and lakes (Blaustein *et al*., 2016).

If a person is infected with biofilm-producing bacteria, the secreted thick mucus acts as a protective layer on the outside of the bacterial cells and protects them from host immune responses and antibiotics. The microbial biofilms constitute a public health problem as the microorganisms in the biofilms are difficult to treat with antimicrobial agents and this causes infections to become more severe and persistent. If the infection is not treated, it may eventually lead to severe disease. Therefore, biofilms have major medical significance as they decrease the susceptibility to antimicrobial agents. The colonization of biofilm bacteria in water plumbing systems has a major impact on causing waterborne diseases in humans such as diarrhoea, cholera, dysentery, typhoid, and polio (Ulla *et al*., 2021). The knowledge on biofilm bacteria in water plumbing systems will lead to identifying many potential platforms and strategies to prevent or reduce getting biofilm infections through the water plumbing system causing waterborne diseases.

METHODOLOGY FOR THE DETECTION OF BIOFILM

Detection of Biofilm Production and Formation

Conventional methods are economical and reliable for routine screening and diagnosis. Among those, the Tissue Culture Plate (TCP), Tube (TM), and Congo Red Agar (CRA) methods are the most extensively utilized biofilm detection methods in research (Hassan *et al*., 2017). Previously, biofilm was detected by visual observation only. Recently qualitative and quantitative methods have been introduced. TCP technique is considered as the most reliable method for the detection of biofilm as it is a quantitative method. Since CRA and TM are qualitative methods, both can be used as general screening methods for the detection of biofilm-producing bacteria (Kırmusaoğlu., 2019). However, advances in microscopic imaging techniques have led to the acquisition of much knowledge about biofilm formation. In particular, scanning electron microscopy (SEM) is uniquely designed to capture compelling, high-resolution images of a sample's surfaces. Therefore, SEM is capable of providing a detailed image of bacterial morphology, bacterial adhesion, biofilm formation, the presence of inorganic products within the biofilm, and the overall shape of microorganisms composing the biofilm. Hence, SEM is considered the gold standard method for the detection of biofilm (Gomes and Mergulhao, 2017; El Abed *et al*., 2012). Besides that, molecular methods considerably broadened the spectrum of microbes detected in biofilms. The new test methods that are currently used for biofilm detection are DNA-based methods, including quantitative real-time PCR, multiplex PCR, Sanger sequencing, Next Generation Sequencing (NGS), and microarray analysis. Further, confocal scanning laser microscopy with molecular probes, Fluorescence *In Situ* Hybridization (FISH) or labelled antibodies-electron microscopy could also be used to detect biofilms (Magana *et al*., 2018; Petrachi *et al*., 2017).

Molecular Identification of Biofilm Bacteria and Its Genes

Modern NGS techniques are beneficial in studying highly diverse bacterial communities (Kotaskova *et al*., 2019). NGS targeting 16S rDNA is able to analyse the bacterial composition in biofilm-related infections and therefore suggested as a promising diagnostic tool (Okuda *et al*., 2018). 16S rDNA sequencing is the most common housekeeping genetic marker to identify bacterial species. The 16S rDNA nucleotide sequence is highly conserved; however, it is interspersed with variable regions which are specific to genus or species (Jenkins *et al*., 2012). There are specific key genes responsible for biofilm formation, such as the *cup A* gene in *Pseudomonas aeruginosa*, *ica* gene in *Staphylococcus aureus*, and the *esp* gene in *Enterococcus faecalis*. The *ica A* and *ica D* genes in *S. aureus* are thought to be essential for intercellular adhesion and the formation of a bacterial multilayer. These genes are also associated with slim production in biofilm formation (Murugan *et al*., 2015). Therefore, these genes could be employed as essential markers for biofilm detection in future applications.

STRATEGIES FOR PREVENTING BIOFILM BACTERIA

According to the prevailing literature, many antibiotics and disinfectants have been widely studied to eliminate biofilm. UV and ozone are used for the removal and prevention of biofilm from water systems. Hypochlorite has also been proven to have little effect on biofilms in addition to dosing of chlorine dioxide at a constant low level (Mahapatra *et al*., 2015). Furthermore, biofilms could be prevented by introducing quorum quenching or anti-quorum sensing molecules into water supply systems, such as the UW85 strain of *Bacillus cereus* (Mahapatra *et al*., 2015). In addition, it has been proven that silver nanoparticles control the formation of biofilms and kill the biofilm bacteria (Martinez-Gutierrez *et al*., 2013). Karunanayake *et al* 2019 reported that silicon nitrate coating reduced the bioburden on both low and high contamination surfaces. Hence, silver and silicon nanoparticles could possibly be used for the prevention of biofilm formation in the future. Moreover, there is ongoing research on making pipeline materials with anti-adhesive properties to inhibit accumulation of biofilm bacteria on the surface of the pipeline. On the other hand, it is suggested to introduce probiotics into the water plumbing system to ensure a good, stable microbial community to compete with biofilm producing bacteria. The good bacteria as probiotics would compete with the biofilm bacteria for nutrition and survival, and consequently become dominant against them (Neu *et al*., 2020). The studies showed that, *P. aeruginosa* has the ability to inhibit growth and biofilm production in *Legionella*. Few other studies reported that, the aquatic bacteria such as *Pseudomonas* spp., *Acinetobacter* spp., *Flavobacterium* spp., have the capability of producing bacteriocins or bacteriocin-like substances (BLSs) against *L. pneumophila* growth (Wang *et al*., 2013). However, there are no detailed reports available on probiotic biofilm related studies.

CONCLUSIONS

Biofilm formation in water plumbing systems is a major threat to public health as it causes waterborne illness. The identification of biofilm-associated bacterial species would aid in the development of treatment techniques to reduce biofilm formation in the water plumbing systems. If these bacterial strains are the causative organisms for different infections, this knowledge may be useful to manage patients affected by related organisms, and it may lead to improving patients' health and economic benefits through the reduction of infections. According to the literature published, it is scientifically proven that nanoparticles have the capability to control biofilm production. Therefore, designing nanocoated biofilm-free pipelines using silver and silicon nanoparticles for controlling biofilm in the plumbing system would reduce biofilm infection and consequently improve public health.

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