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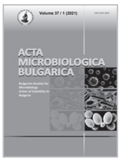

Review

Occurrence and Role of Bacterial Biofilms in Different Systems

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Abstract

Bacterial biofilms are complex communities of bacteria that adhere to surfaces, including living tissues, and form a protective matrix of extracellular polymeric substances (EPS). Biofilms are widespread and play crucial roles in various processes, such as nutrient cycling, bioremediation, and biofouling. They have significant implications for public health. Biofilms provide an ideal environment for bacteria to exchange genetic material, including resistance genes, via horizontal gene transfer mechanisms such as conjugation, transformation, and transduction. Moreover, biofilms can protect bacteria from antibiotics and host immune responses, enabling them to persist and cause chronic infections. The EPS matrix, which can act as a physical barrier, limits the penetration of antibiotics into the biofilm, and the slow-growing or dormant cells within the biofilm are less susceptible to antibiotics than their planktonic counterparts. The significance of bacterial biofilms in the development of antibiotic resistance has prompted research efforts to understand their formation and mechanisms of resistance. Novel strategies to prevent or disrupt biofilm formation are also being explored, including the development of antibiofilm agents and biofilm-disrupting enzymes. Understanding the role of biofilms in the spread of antibiotic resistance is crucial for the development of effective treatment and prevention strategies to combat the chronic infections associated with biofilm-producing bacteria.

Keywords: Bacteria, biofilms, drinking water, distribution system, healthcare facilities, medical devices

Резюме

Бактериалните биофилми са сложни общности от бактерии, които прилепват към повърхности, включително живи тъкани, и образуват защитна матрица от извънклетъчни полимерни вещества (EPS). Биофилмите са широко разпространени и играят ключова роля в различни процеси, като например кръговрата на хранителните вещества, биоремедиацията и биозамърсяването. Те са много важни и за общественото здраве. Биофилмите осигуряват идеална среда за обмен на генетичен материал между бактериите, включително гени за резистентност, чрез механизми за хоризонтален трансфер на гени, като конюгация, трансформация и трансдукция. Освен това, биофилмите могат да предпазват бактериите от антибиотици и имунни реакции на гостоприемника, което им позволява да се задържат и да причиняват хронични инфекции. Матрицата EPS, която може да действа като физическа бариера, ограничава проникването на антибиотици в биофилма, а бавно растящите или спящите клетки в биофилма са по-малко чувствителни към антибиотици, отколкото клетките в планктона. Значението на бактериалните биофилми за развитието на антибиотична резистентност предизвика изследователски усилия за изясняване на тяхното образуване и механизмите на резистентност. Проучват се и нови стратегии за предотвратяване или прекъсване образуването на биофилми, включително разработване на лекарствени формули и ензими, разрушаващи биофилмите. Изясняване ролята на биофилмите в разпространението на антибиотичната резистентност е от решаващо значение за разработването на ефективни стратегии за лечение и превенция на хроничните инфекции, свързани с бактериите, образуващи биофилми.

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Introduction

Bacterial biofilms are complex communities of bacteria that can adhere to surfaces, including living tissues, and form a protective matrix of extracellular polymeric substances. Biofilms play a critical role in various processes, including nutrient cycling, bioremediation, and biofouling. However, biofilms also have significant implications for public health, particularly in the development of antibiotic resistance. In recent years, there has been growing interest in understanding the mechanisms by which bacterial biofilms contribute to the spread of antibiotic resistance. Bacterial biofilms are communities of microorganisms that adhere to surfaces and form a protective matrix composed of extracellular polymeric substances. Biofilms are ubiquitous and are commonly found in medical, industrial, and natural environments. Biofilms play a critical role in the development of antibiotic resistance, and their significance in this process has been extensively studied. Biofilms provide a protective environment for bacteria, shielding them from external stressors, including antibiotics. The extracellular polymeric substances in the biofilm matrix act as a barrier to the penetration of antimicrobial agents, preventing them from reaching the bacteria. This barrier effect is further enhanced by the presence of persister cells, a subpopulation of bacteria that exhibit antibiotic tolerance and can survive in the presence of high concentrations of antibiotics (Goel *et al*., 2021).

Biofilms also promote horizontal gene transfer, a process by which bacteria can exchange genetic material, including antibiotic resistance genes (Reygaert, 2018; Sharma *et al*., 2019). The proximity of bacteria in biofilms facilitates the transfer of plasmids containing antibiotic-resistance genes between cells. Moreover, biofilms provide a selective advantage for the growth of antibiotic-resistant bacteria, allowing them to outcompete susceptible bacteria. Several studies have demonstrated the role of biofilms in the development of antibiotic resistance (El-Halfawy and Valvano, 2015; Andersson *et al*., 2019; Uruén *et al*., 2020; Bello *et al*., 2023). For example, studies have shown that biofilm-forming bacteria exhibit higher levels of antibiotic resistance than their planktonic counterparts (Mah, 2012; Hall and Mah, 2017). Additionally, biofilm formation has been shown to promote the acquisition and spread of antibiotic-resistant genes in clinical settings (Abebe, 2020).

Bacterial biofilms play a critical role in the development of antibiotic resistance. Biofilms provide a protective environment for bacteria, promote horizontal gene transfer, and provide a selective advantage for the growth of antibiotic-resistant bacteria. Understanding the significance of biofilms in the development of antibiotic resistance is crucial for the development of effective strategies to combat antibiotic resistance.

Detrimental effect of bacterial biofilms on different components

Biofilms in drinking water distribution system (DWDS)

Water is an essential part of existence. Only about 2.6 percent of the world's water (1.4×10^9 km^3) is freshwater, and hence available as potential drinking water (DW). Consumption of contaminated DW can result in a variety of diseases and health problems in all persons, but especially in those who are more vulnerable, such as newborns, young children, the elderly, the sick, and those who are immunocompromised. Biofilms are well-known to be one of the most serious microbiological issues in the drinking water distribution system (DWDS) which contributes to the degradation of water quality (Simoes and Simoes, 2013). Microbial contamination of drinking water causes a variety of health issues, including cholera, diarrhea, and death, particularly in children, especially in underdeveloped nations such as Asia and Africa.

Bio-fouling is the unwanted collection of biotic materials on a surface. Bio-fouling in industrial and drinking water has negative consequences such as degrading water quality chemically and microbiologically, causing yield loss, and reducing the effectiveness of heat transmission and exchange, as well as membrane methods. Bacteria associated with forming biofilms in drinking water include *E. coli, Klebsiella pneumoniae, K. oxytoca, Enterobacter cloacae, Helicobacter pylori, Shigella* spp., *Campylobacter* spp., *Salmonella* spp., *Clostridium perfringens, Enterococcus faecium, E. faecalis, Legionella pneumophila, Pseudomonas aeruginosa, P. fluorescens, Aeromonas hydrophila, A. caviae, Mycobacterium avium, M. xenopi,* and so on (Tasneem *et al.,* 2018).

Bio-corrosion of pipes, undesirable water quality changes affecting color, taste, turbidity, and odors, and a reduction in heat exchange efficiency may all result from bacterial cells attaching and developing biofilms on the inner surfaces of piping systems from which cells could be detached into the bulk water (Prest *et al.,* 2016). Sulfate-reducing bacteria, sulfur-oxidizing bacteria, iron-oxidizers, iron-reducers, and manganese-oxidizers are the principal biofilm-producing bacteria known to promote metal corrosion (Kip and van Veen, 2015). Overall, biofilms can compromise the safety of drinking water and cause damage to water infrastructure.

Biofilms in the food industry

Biofilms can form on surfaces that come in contact with or don't come in contact with foods in the food industry. Biofilms are to blame for over 60% of foodborne outbreaks. As a result, biofilms present in food processing environments constitute a serious threat to food safety and the food industry. Different environmental conditions enhance the creation of biofilms during food processing, such as moisture, nutrients, contaminated raw materials, and so on; this reduces shelf life and promotes foodborne diseases (Galie *et al.,* 2018). Intoxications or infections can result from food-borne diseases caused by bacterial biofilms on food matrixes or manufacturing equipment.

The following are the top five foodborne pathogens: *Listeria monocytogens* which cause listeriosis responsible for spontaneous abortion or fetal harm in pregnant women. *Salmonella enterica* is responsible for Gastroenteritis, septicemia, Reiter's syndrome, and even mortality. *E. coli* causes hemorrhagic colitis. *Bacillus cereus* in which toxins cause diarrhea and emetic sickness and *S. aureus, which* causes food poisoning (Galie *et al.,* 2018). Other pathogens that can be found in food include *Pseudomonas* spp., a common spoilage bacterium that produces proteases that are harmful to food. Infection with *Vibrio parahaemolyticus* is most commonly linked to undercooked seafood consumption. *C. perfringens* is a bacterium that produces a variety of toxins. *Campylobacter jejuni* is a common cause of bacterial gastroenteritis in humans.

Shewanella putrefaciens is a bacterium that produces volatile sulfur compounds, amines, and trimethylamine. *Cronobacter* spp. is a species of bacteria that commonly causes illnesses in newborns and immuno-compromised people. *Geobacillus stearothermophilus*, is a common dairy product contamination. Multi-species biofilms, which are more permanent and difficult to regulate, can be formed by these organisms (Galie *et al.,* 2018). Biofilms are also responsible for major technical issues in the food sector, as they can obstruct heat passage across equipment surfaces, raise fluid frictional resistance at the surfaces, and accelerate surface corrosion, resulting in a loss of production efficiency (Meesilp and Mesil, 2019). In a nutshell, biofilms pose a direct risk of pathogenic bacteria contamination in the food industry, as well as the possibility of contamination of instruments and equipment.

Biofilms on textiles

The colonization and proliferation of axillary skin bacteria introduced during sweating, which create biofilms that are difficult to remove by standard washing, causes the typical sweat malodor and discoloration of sweaty and used garments. The bacteria, as well as the perspiration odor and color, persist because cleaning is ineffective. Biofilms build up over time, causing textile quality to deteriorate (Mollebjerg *et al.,* 2021). As a result, pathogenic and non-pathogenic microbes come into contact with fabrics. *Staphylococcus* spp., *Escherichia* spp., *Pseudomonas* spp., *Micrococcus* spp., *Bacillus* spp., Enterobacteriaceae, *Acinetobacter* spp., and *Corynebacteria* spp. are some of the bacteria that influence the adherence of microorganisms on fabrics (Van Herreweghen *et al.,* 2020; Vishwakarma, 2020). *Staphylococcus* spp. was found to be enriched on almost every type of fiber, with *Staphylococcus hominis* having a strong preference for cotton. On polyester, polyester blends, and wool, *Micrococcus luteus* can be found. Many bacteria, including *Enhydrobacter* spp., *Cutibacterium* spp., *Staphylococcus epidermidi*s, and *Micrococcus* spp., grow well in wool (Van Herreweghen *et al.,* 2020).

Bacterial colonization and growth in textiles are dynamic processes that evolve when the fabric is used, dried, cleaned, and worn again. Bacteria attach to textiles more after drying than when wet because the decreasing water content in the textile encourages bacteria adhesion and bacterial development is influenced by nutrition availability and water content (Mollebjerg *et al.,* 2021). Distinct fiber types have different surface characteristics and functional groups, which affect bacterial adhesion and growth as well as the adsorption and retention of volatile substances. Polyester clothing is more susceptible to microbial development than cotton since it is a petroleum-based synthetic material with no natural characteristics and a low adsorption capacity based on the molecular structure. Cotton absorbs more water, whereas polyester absorbs much more sebum and sweat solutes. Polyester fibers absorb bacteria more effectively than cotton fibers.

Malodor is caused by bacteria degrading eccrine sweat and sebum components in the textile. Short-chain volatile fatty acids are produced by the degradation of amino acids and lactic acid in sweat, while short-medium-chain volatile fatty acids are produced by the degradation of fatty acids and triglycerides in sebum. Between eccrine sweat and sebum, sebum is the main source of the odor. Eccrine sweat is 99 percent water, with the remaining 1% made up of electrolytes, amino acids, carbohydrates, and vitamins. Sebum is composed of triglycerides, fatty acids, squalene, cholesterol, wax esters, and cholesterol esters (Mollebjerg *et al.,* 2021).

Biofilms in healthcare facilities

Biofilms have been found on medical device surfaces, dead tissues (e.g., sequestra of bones), and inside living tissues (e.g., lung tissue, tooth surfaces) in healthcare settings. They can form on the surfaces of catheters, artificial heart valves, pacemakers, breast implants, contact lenses, and cerebrospinal fluid shunts, among other biomedical equipment. Gram-positive and Gram-negative bacteria can attach to and create biofilms on the surfaces of these devices, as indicated in Table 1.

Biofilms have a significant impact on human health care. Gram-positive bacteria, such as *S. aureus, S. epidermidis,* and *E. faecalis*, as well as Gram-negative bacteria, such as *Escherichia coli, K. pneumoniae, Proteus mirabilis,* and *P. aeruginosa,* are important microorganisms involved in health-care-associated infections. *S. epidermidis* and *S. aureus* account for over 80% of bacteria involved in material-related contaminations, with the latter causing chronic wounds and other difficulties in particular in connection with surgical site infections (Schule *et al.,* 2021). Bacterial biofilms can also form in water distribution systems in hospitals. In the hospital water system, *P. aeruginosa* can build biofilms on the inner surfaces of metal pipes. Human infections and disorders caused by biofilm-forming bacteria include cystic fibrosis (CF), otitis media, periodontitis, infective endocarditis (IE), chronic wounds, osteomyelitis, and so on (Masters *et al.,* 2019) as shown in Table 2.

Bacterial biofilms have also enhanced antimicrobial resistance and are linked to a variety of chronic illnesses. Because the complex structure of biofilm with EPS matrix inhibits antibiotics from reaching bacteria, microorganisms associated with biofilm are more resistant to antimicrobials (Tasneem *et al.,* 2018). Antimicrobial agents are compounds that bacterial biofilms can confer resistance to, such as antibiotics, antivirals, medicines, personal care products, disinfectants, heavy metals, pesticides, and medications (Flores-Vargas, 2021). Antimicrobial agents are grouped into classes based on their mechanisms of action (Bello and Bello, 2021). Inhibitors of cell wall production, depolarizers of the cell membrane, inhibitors of protein synthesis, inhibitors of nucleic acid synthesis, and inhibitors of metabolic pathways in bacteria are the primary classes. Table 3 gives examples of drugs from each of these groups. The genes acquired through horizontal gene transfer are one of the mechanisms of antimicrobial resistance. The lysis of heterogeneous species' cells results in the accumulation of genetic elements within the biofilm. This creates an excellent atmosphere for resistance to gain a foothold (Goel *et al.,* 2021).

When compared to their planktonic counterparts, bacterial cells in biofilms are 10 to 1,000 times

Table 1. Bacteria that create biofilms on medical devices

Medical devices	Biofilm-forming bacteria
Contact lenses	*P. aeruginosa, S. aureus, S. epidermidis, S. saprophyticus, Klebsiella* spp.
Central venous catheters	Coagulase-negative Staphylococci, *S. aureus,* Enteric Gram-negative bacteria
Urinary catheters	*S. aureus, E. faecalis, P. aeruginosa*
Peritoneal dialysis catheters	*S. epidermidis, P. acnes, S. wameri, S. lugdunensis, R. mucilaginosa*
Mechanical heart valves	*Streptococcus* spp., *S. aureus, S. epidermidis,* Gram-negative *Bacillus, Enterococcus*
Cerebrospinal fluid shunts	*S. aureus, E. faecalis, S. epidermidis, E. faecium*
Breast implants	*S. epidermidis,* Coagulase-negative *Staphylococci, Propionibacterium acnes*
Orthopaedic implants	*S. aureus, S. epidermidis, P. aeruginosa, E. coli, S. haemolyticus*
Dental implants	Gram-positive cocci, *Actinomyces* spp., Gram-negative anaerobic oral bacteria
Voice prostheses	*S. aureus, P. aeruginosa, Klebsiella* spp., *Enterobacter* spp., *R. dentocariosa,* and *Proteus* spp.
Cardiac pacemakers	*S. aureus, S. epidermidis*
Biliary stents	*Pseudomonas, Citrobacter, Klebsiella, Staphylococcus, Enterococcus, Aeromonas, Proteus, Enterobacter*

Source: Muhammad *et al.* (2020)

Table 2. Bacterial species involved in biofilm-associated infections and their adherent surfaces

Bacteria species	Infection/Diseases	Surface
Streptococcus mutans	Dental caries, Endocarditis	Tooth surface, Vascular grafts
E. faecalis	Endocarditis, Root canal infection	Heart valves, Urinary catheters, Tooth, Central venous catheters
K. pneumoniae	Pneumonia, Respiratory tract infection, Urinary tract infection, Pyogenic liver abscess	Lungs, Liver
P. aeruginosa	Nosocomial infection, Otitis media, Cystic fibrosis	Central venous Catheters, Middle ear, Prostheses, Lungs, Contact lenses
Staphylococcus spp. (*S. aureus; S. epidermidis*)	Nosocomial infections, Chronic wounds, Endocarditis, Mucoloskeletal Infections, Otitis media	Sutures, Central venous catheters, Arteriovenous shunts Prostheses, Surfaces/ deep skin Prostheses, Heart valves bones, Middle ear
E. coli	Bacterial prostatitis, Urinary tract infection, Otitis media	Prostheses, Urinary tract Urinary catheters, Middle ear
Haemophilus influenzae	Otitis media	Middle ear
Burkholderia cepacia	Cystic fibrosis	Lungs
M. tuberculosis	Tuberculosis	Lungs

Source: Sharma *et al.* (2019)

Table 3. Antimicrobial groups based on mechanism of action

Mechanism of action	Antimicrobial group
Inhibit Cell Wall Synthesis	β-Lactams
	Carbapenems
	Cephalosporins
	Monobactams
	Penicillins
	Glycopeptides
Depolarize Cell Membrane	Lipopeptides
Inhibit Protein Synthesis	Bind to 30S Ribosomal Subunit
	Aminoglycosides
	Tetracyclines
	Bind to 50S Ribosomal Subunit
	Chloramphenicol
	Lincosamides
	Macrolides
	Oxazolidinones
	Streptogramins
Inhibit Nucleic Acid Synthesis	Quinolones
	Fluoroquinolones
Inhibit Metabolic Pathways	Sulfonamides
	Trimethoprim

Source: Reygaert (2018)

less susceptible to certain antimicrobial agents. This reduced susceptibility is due to a combination of factors, including (i) poor antibiotic penetration into the polysaccharide matrix; (ii) the arbitrary presence of cells with a resistant phenotype (known as "persisters"); and (iii) the presence of non-growing cells or cells that triggered stress responses under unfavorable chemical conditions within the biofilm matrix. These defensive mechanisms work in tandem with those that cause traditional resistance, which is caused by the presence of antibiotic resistance genes (ARGs) in bacterial genomes or extrachromosomal elements, resulting in enhanced biofilm resistance to antimicrobial chemicals. Antimicrobial resistance mechanisms are divided into four categories: (1) drug uptake limitation; (2) drug target modification; (3) drug inactivation; and (4) active drug efflux (Reygaert, 2018).

Biofilms have increased survival and tolerance to environmental and chemical stresses (e.g., antibiotics), owing in part to the extracellular polysaccharide matrix's protection (Hoiby *et al.,* 2010). Resistance to antibiotics is now the most important cause of ineffective therapy of biofilm-associated bacterial infections, and it is one of the most significant, challenging, and urgent threats to global public health resulting from either genomic mutation or transmission of resistance-conferring genes between bacterial species (Bowler *et al.,* 2020). Difficulty in the diffusion of antibiotics into the biofilm and electrostatic charge of the EPS, which attract oppositely charged antibiotics; slower growth rate; variations in phenotype acquired by bacteria forming biofilms; and inactivation of antibiotics by enzymes secreted by bacteria are the causes of increased antibiotic resistance in bacteria (Sharma *et al.,* 2019).

Bacteria in biofilms are resistant to antibiotics due to the following factors: (a) a polymeric matrix that restricts antibiotic diffusion, (b) antibiotic interaction with a polymeric matrix that reduces antibiotic activity, and (c) enzyme-mediated resistance such as -lactamase. (d) alterations in metabolic activity within the biofilm, (e) genetic modifications on target cells or obscuring target sites, (f) antibiotic extrusion via efflux pumps, and (g) the existence of outer membrane structure, as seen in Gram-negative bacteria. Antibiotic resistance and biofilm bacterium survival rely on these mechanisms (Abebe, 2020). Biofilms contain a large population of persister cells that can withstand antibiotic treatment (Srinivasan *et al.,* 2021).

These Gram-negative bacteria form biofilms that confer resistance to antibiotics: *Acinetobacter baumannii, E. coli, K. pneumoniae, P. aeruginosa, Klebsiella aerogenes, Burkholderia cepacia, Serratia marcescens, Proteus mirabilis*, while the Gram-positive bacteria that confer resistance to antibiotics include *Corynebacterium striatum, Streptococcus agalactiae, E. faecalis, E. faecium* (Folliero *et al.,* 2021). Because most bacterial pathogens form biofilms, biofilm resistance to antibiotics is a serious concern for human health, as many chronic infections are linked to biofilm growth on natural surfaces (e.g., teeth, lungs) or foreign-body devices (e.g., pacemakers, catheters, and prosthetic heart valves) (Balcazar *et al.,* 2015).

Biofilms in oil and gas industries

Biofilms are discovered in the petroleum sector in places where water accumulates or remains long enough for a micro niche to form that is conducive for growth, at which point the bacteria can become irrevocably attached. Biofilms cause severe operational challenges in oil and gas/biofuel pipelines and storage tanks owing to microbial invasion. Bio-corrosion, also known as microbiologically induced corrosion (MIC), is the interaction of biofilms, microorganisms and their metabolites, abiotic corrosion products, and the metal surface. Bio-corrosion is responsible for almost 40% of all internal corrosion occurrences in pipelines used in the oil industry (de Oliveira *et al.,* 2021). The presence of microbial activities that accelerate the rate of anodic and cathodic reactions may be responsible for the rise in corrosion rates caused by MIC.

The adhesion of planktonic microbes to a metal surface initiates the microbiologically influenced corrosion process, which leads to the production of complex biofilms. Bacteria catalyze multiple slow electrochemical processes at the cell's metallic surface interfere during the formation of the biofilm and through their metabolic activities (Vishwakarma, 2020). Sulfate-reducing and oxidizing bacteria, iron, and CO_2-reducing bacteria, and iron and manganese oxidizing bacteria are the bacteria most commonly connected with metals in pipeline systems while sulfate-reducing bacteria (SRB) are the most common bacteria that cause internal pipeline corrosion (Akpan *et al.,* 2015). Examples of Sulfate reducers associated with pipelines are *Desulfomicrobium*, *Desulfovibrio*, *Desulfohalobium*, *Desulfococcus*, *Desulfosarcina*, *Desulfobacter*, *Desulfobacterium* and *Desulfobulbus.* These common iron reducers are associated in pipelines *Desulfuromusa*, *Pelobacter*, *Malonomonas,* and *Desulfuromonas.*

Other microorganisms isolated from oil pipelines include *B. subtilis, B. cereus, Klebsiella oxytoca, P. aeruginosa, Halomonas subglaciescola, Serratia marcescen, S. epidermidis,* and *S. aureus* (Akpan *et al.,* 2015). The biofilm colonizes the pipeline surfaces, forming a complex microbial structure that breaks down even the thick-walled carbon steel (CS) pipe. Leaks and catastrophic pipeline failure ensue as a result of this. This degrades the quality of oil, gas, and biofuel pipelines and storage tanks, resulting in significant financial losses around the world (Vishwakarma, 2020).

Beneficial effects of bacterial biofilms

Biofilms in agriculture

Bacterial biofilms can develop on the surfaces of leaves, roots, and stems of plants. Because of their ability to colonize plant surfaces, biofilm-forming rhizobacteria can operate as bio-control agents. *Bacillus, Pseudomonas, Streptomyces, Serratia*, and *Stenotrophomonas* are examples of rhizobacteria (Arrebola *et al.,* 2019). Bio-control agent biofilms may protect the host by the following mechanism: (a) Prevent stress tolerance (b) Inhibit plant pathogens by producing antimicrobial agents (c) Produce antagonism and thus clear pathogens and exert competition for nutrients (d) In multi-species biofilms, metabolites, and enzymes could hinder pathogen growth or the host could benefit through cooperation (e) Plant physiology could be directly influenced by biofilm bacteria, such as the activation of plant defenses and/or the encouragement of plant development (Yadav, 2017).

Bacillus is a genus of plant-associated microorganisms that are used for both bio-control and plant growth promotion. *Pseudomonas* spp., which are found in the roots, can operate as bio-control agents. They may make a variety of antagonistic chemicals, such as cyclic lipopeptides, pyrrolni-

Table 4. Bacteria that create biofilms on plants and their mechanisms

Biofilm forming bacteria	Biofilms on host	Effects of biofilms on host
Bacillus atrophaeus	Biofilms on tomato, sugar beet plants	Biofilms produce surfactin and fengycin which are antimicrobial agents and induced systemic resistance
B. amyloliquefaciens SQR9	Cucumber roots	The bacteria biofilms produce the antimicrobial agent bacillomycin
B. subtilis	Wheat seeds	The bacteria biofilms on wheat seeds prevent fungal mycelial growth
B. subtilis 3610	Tomato roots	*Bacillus* biofilms produce surfactin which is an antimicrobial agent
B. subtilis Bs916	Rice stem	*Bacillus* biofilm produces the antimicrobial agent fengycin
B. subtilis UMAF6614	Melon phylloplane	*Bacillus* biofilms produce bacillomycin and fengycin which are antimicrobial agents
B. subtilis 6051	*Arabidopsis thaliana*	Biofilms of *Bacillus subtilis* protect *Arabidopsis thaliana* by producing the antimicrobial agent surfactin
B. amyloliquefaciens SQR9	Maize roots	The biofilm bacteria showed promoted plant growth (PGP) activity
B. amyloliquefaciens SQY162	Tobacco roots	In tobacco plant roots *Bacillus* biofilms produce surfactin and induce systematic resistance
Paenibacillus polymyxa	*Arabidopsis thaliana*	The biofilms give mechanical protection to plants and clear pathogens from the site
P. polymyxa A26	Wheat seeds	The biofilms clear pathogens from the site
P. polymyxa B5	*Arabidopsis thaliana*	The biofilms clear pathogens from the site of infection
Pseudomonas corrugata CCR04 and CCR80	Pepper roots	The biofilm of pseudomonas inhibits other pathogens by competitive colonization.
Pseudomonas chlororaphis-PA23	Canola roots, wheat roots	In roots, pseudomonas biofilms produce pyrrolnitrin which prevents fungal pathogen infection.
Pseudomonas putida 06909	Citrus roots	*P. putida* biofilms protect the root by preventing mycelial attachment.
P. putida KT2440	Corn roots, *Arabidopsis thaliana*	The biofilms of *P. putida* induce systematic resistance and enhance plant growth
Pichia kudriavzevii	Pear fruit	*P. kudriavzevii* biofilms activate antioxidation system
Kloeckera apiculate	Citrus fruit	Biofilms clear pathogens and give mechanical protection to plant
Pseudoalteromonas tunicate	Green macroalga, *Ulvalactuca*	Produces anti-fouling compounds that inhibit colonization

Source: Yadav (2017)

trin, and phenazines, to stop plant pathogens from multiplying (Arrebola *et al.,* 2019). For example, *P. putida* 06909 attaches to and colonizes the hyphae of the citrus root rotting fungus *Phytophthora parasitica* by feeding on its exudates, and then forms a biofilm around the citrus roots, preventing the fungus from spreading further and *B. polymyxa* forms a peanut rhizosphere biofilm Plant-pathogen biofilm development has also been observed in plants.

Dickeya dadantii, for example, is a gram-negative bacterium that causes soft rot diseases in a variety of plant species. Due to the synthesis of degradative enzymes, the bacteria invade and build biofilms on chicory leaves, causing illness (Pandin *et al.,* 2016). On the wheat rhizosphere, *P. chlororaphis* develops biofilms that defend against fungal disease. By producing biofilms, *P. putida* protects citrus roots from phytopathogen infection. The bacteria colonize the *P. parasitica* mycelium and feed on its exudates, eventually forming a biofilm around the citrus roots that prevents disease growth (Yadav, 2017). Table 4 shows the biofilm-forming bacteria on plants and their modes of action.

Biofilms in bioremediation

By employing potential microorganisms, bioremediation is an environmentally acceptable way of detoxification of dangerous contaminants from soil, water, and air (Prasad and Prasad, 2012). Furthermore, biofilm-mediated remediation approaches are more effective at transforming toxic wastes because pollutants are more bioavailable to degrading organisms and degrading microbes are more adaptable to varied harmful chemicals. It can be accomplished by adding limited nutrients and electrons to polluted areas (bio-stimulation) or by adding microbes to polluted sites (bio-augmentation) to speed up the transformation process (Mangwani *et al.,* 2016). Depending on the area where waste material is processed, microbial bioremediation can be *in situ* or *ex-situ*. *In situ* bioremediation treats the polluted sample at its original location, eliminating the need to transport the waste material to another location for treatment, whereas ex-situ treatment transports the sample to a new location for treatment (Vogt and Richnow, 2013).

Biofilm-mediated remediation is an environmentally acceptable method of removing contaminants from contaminated locations. Biofilms are cost-effective for bioremediation because they absorb, immobilize, tolerate contaminants, have a possibility of survival and adaptation, and can digest a variety of pollutants via catabolic pathways. Biofilm-forming bacteria can be effectively used in the remediation process because their cells are enclosed in an EPS matrix that protects them from a variety of environmental threats (Mangwani *et al.,* 2016). Biofilms also provide an important environment for intercellular gene transfer, cellular communication with QS, cohesion, and metabolite diffusion, as well as bacterial chemotaxis (Santos *et al.,* 2018). Diverse species of aerobic and anaerobic bacteria can be found in biofilm-mediated cleanup, and they frequently employ the breakdown of pollutants as an energy source.

Oil spills, persistent organic pollutants (such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and polychlorinated ethenes), heavy metals, dyes, explosives, pesticides, and pharmaceutical products are all examples of environmental pollutants that can be removed using bacterial biofilms-mediated remediation. As a result, biofilm-mediated bioremediation is used in the industry for contaminated soil and groundwater cleanup. These contaminants can be remedied by *Pseudomonas, Dehalococcides, Arthrobacter, Bacillus, Alcanivorax, Cycloclasticus, Burkholderia,* and *Rhodococcus* (Yoshikawa *et al.,* 2017).

Biofilms in corrosion prevention and control

Corrosion is now widely recognized as a major issue in the drinking water distribution system, as well as the medical, marine, and food processing industries (Guo *et al.,* 2018). Corrosion can be accelerated by both chemical and biological processes (Kip and van Veen, 2015). Microbes' activity on the surfaces of metallic items, can either impede or accelerate corrosion. Because of their efficiency, low cost, and environmentally favorable behavior, beneficial bacterial biofilms are utilized to prevent corrosion (Guo *et al.,* 2018). Possible mechanisms of microbial inhibition of corrosion include: (1) Removal of corrosive cathodic agents by bacterial activity is one possible method of microbial corrosion inhibition (such as oxygen respiration under aerobic conditions) (2) Antimicrobials released by bacteria suppress the growth of corrosion-causing microorganisms (3) the production of a protective coating on the metal surface, such as passive metal oxides or secreting sticky corrosion inhibitors, and (4) the formation of biofilms that act as a diffusion barrier to prevent metals from dissolving (Zuo, 2007; Guo *et al.,* 2018).

Under aerobic conditions, biofilm-forming bacteria inhibit corrosion by removing oxygen; under anaerobic conditions, chemoorganotrophic bacteria inhibit corrosion by removing corrosion products and destroying the ecological environment for SRB. The biofilm matrix acts as a transport barrier, preventing corrosive chemicals (such as oxygen, chloride, and others) from penetrating and minimizing their contact with the metal surface, hence lowering corrosion. *B. brevis, B. subtilis, B. mycoides, Pseudomonas cichorii, P. fragi*, facultative anaerobic *E. coli* form biofilms to protect corrosion. The degree of corrosion inhibition was determined by the nature of the biofilms (i.e., good biofilm former or bad biofilm former) (Zuo, 2007). Although both aerobic and anaerobic biofilms can reduce corrosion rates on many materials' surfaces, aerobic biofilms suppress metal corrosion more effectively, suggesting that oxygen consumption can further improve corrosion protection (Kip and van Veen, 2015). The application of bacterial biofilms to corrosion prevention and control is a relatively emerging field that demands special attention.

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