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Current and Recent Advanced Strategies for Combating Biofilms

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Abstract: Biofilms are matrix-enclosed microbial aggregates that adhere to a biological or nonbiological surface. Biofilm formation is a significant problem in the medical, food, and marine industries and can lead to substantial economic and health problems. The complex microbial community of a biofilm is highly resistant to antibiotics and sanitizers and confers persistent survival that is a challenge to overcome. There are several conventional approaches to combating biofilms, physical and/or mechanical removal, chemical removal, and the use of antimicrobials, sanitizers, or disinfectants to kill biofilm organisms. However, biofilms are highly resistant to these approaches as opposed to planktonic cells. Thus, novel approaches other than the conventional methods are urgently needed. In this review, we discuss current and new advanced antibiofilm strategies that are superior to the conventional method in terms of addressing the biofilm problem for the improvement of healthcare, food safety, and in industrial processes.

Keywords: approach, biofilm, food, marine, medical

Introduction

Biofilm is a community of microorganism adhering to biotic or abiotic surfaces embedded by a self-produced extra-polymeric matrix facilitating the survival in an adverse environment. Biofilms are ubiquitous, occurring in aquatic and industrial water systems as well as a large number of environments and medical devices relevant to public health (Donlan and Costerton 2002). Biofilms can also avidly colonize the surfaces of a wide variety of household items such as toilet bowls, sinks, toys, cutting boards, and countertops in kitchen and bathroom. A microbial biofilm was first reported in 1943 (Zobell 1943), but it is still a concern and poses serious problems in a wide range of areas, especially in the food (Flint and others 1997; Veran 2002), marine (Dobretsov and others 2006), environmental (Maukonen and others 2003), and biomedical fields (Sihorkar and Vyas 2001). Globally, microbial biofilms are a daily challenge faced by the food industry and society. Many outbreaks of pathogens have been attributed to biofilms, and it is estimated that biofilms account for up to 80% of microbial infections (Epstein and others 2011b).

The prevalence of biofilms is a significant problem in food and the food industry. Major foodborne pathogens such as *Escherichia coli*, *Listeria monocytogenes, Salmonella* spp., and *Campylobacter jejuni* can form a biofilm and remain a significant food safety challenge for the food industry (Brandal 2006; Murphy and others 2006; Gandhi and Chikindas 2007). In food processing environments, a variety of microorganisms colonize food and food contact surfaces, survive, grow, and sometimes form multispecies biofilm communities. Once developed, biofilms are a significant potential source of contamination of food products; biofilms may lead to spoilage of food and/or substantial risks for consumer health after consumption. Many outbreaks that are associated with the consumption of fresh produce, such as lettuce, onions, spinach, and tomatoes, have been linked to surface colonization by a biofilm-forming pathogen (Beuchat 2002; Brandal 2006; Zhang and others 2008).

Probably, the worst reputation among biofilms belongs to those affecting the medical and healthcare industries because biofilm-associated organisms are responsible for more than 60% of all microbial infections in humans (Shunmugaperumal 2010). Biofilm-related organisms play a role in many life-threatening infectious diseases like cystic fibrosis (severe lung infection), bacterial endocarditis (infection of the inner surface of the heart and its valves), otitis media (acute ear infection) most common in children in the United States, urinary tract infection, and Legionnaire's disease (acute respiratory infection). Most of the microorganisms have the potential to adhere to, and form a biofilm in, different organs and surfaces in hospital settings, such as on lung tissue, teeth, implants, and urinary catheters (Costerton 1985; Donlan 2009). Biofilms are often responsible for chronic illness and hospital-acquired (nosocomial) infections. In most cases, biofilm-related infections are not responsive to conventional antimicrobials and persistently reoccur. Biofilm-related persistent infections may lead to a life-threatening disease.

At water and sewage treatment facilities, biofilms (biofouling) are also problematic: they cause metal corrosion, increased risk of contamination of products, decreased quality of water, and reduced efficacy of heat exchange (Coester and Cloete 2005; Palmer and others 2007; Vu and others 2009). Marine fouling, which is

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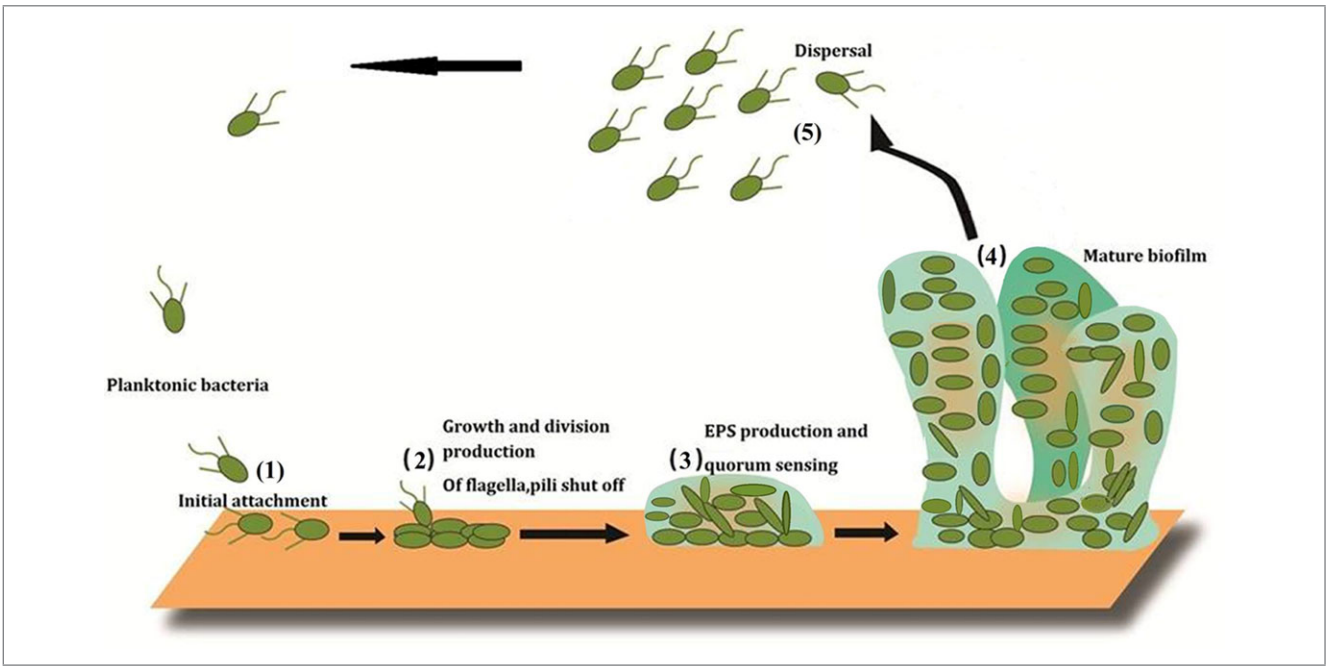

Figure 1–Representation of hypothetical developmental model of biofilm. Biofilm formation comprises 5 distinct stages identified as 1) initial attachment, 2) growth division and production of flagella, pili shut off, 3) EPS production and quorum sensing, 4) Mature biofilm, and 5) dispersal (adapted and modified from Mizan and others 2015).

precipitated by the aggregation of microbial biofilm on ship hulls followed by progressively larger marine organisms, can increase the fuel cost of seafaring vessels by up to 40% (Christie and Dalley 1987).

Concerning the severe adverse impact of biofilms on many human activities, various approaches to prevent and remove a biofilm have been utilized for many years. Traditional physical and chemical methods, such as flushing, chlorination, and ultraviolet disinfection, are used to control and remove biofilms. However, due to the lack of both effectiveness and safety of these strategies, the concerns have persisted (Srinivasan and others 2008), which have driven the search, development, and application of novel approaches for dispersing and/or inhibiting formation of biofilms. Recent advances in biofilm research have provided new insights into the mechanism of biofilm formation and led to exciting progress in the development of novel strategies for the prevention and inhibition of biofilms. Many new methods, such as inhibition of quorum sensing (QS), enzymatic disruption, bactericidal coating, nanotechnology, and bioelectric approach, have successfully been studied in an effort to find effective alternatives for the prevention and control of biofilms. In this review, we attempted to provide a comprehensive picture outlining current knowledge about new approaches to the prevention and control of biofilms, including biofilm formation, antibiofilm agents of different sources, their modes of action, specificity, safety, antimicrobial efficacy, and advantages and disadvantages with respect to potential applications. We also suggest a prospective research project on the prevention and control of biofilms.

The Process of Biofilm Development

Biofilm formation and maturation are sequential dynamic and complex processes, which depend on the substratum, the medium, intrinsic properties of the cells, signaling molecules, cellular metabolism, and genetic control (Donlan 2002; Renner and Weibel 2011). The process of biofilm formation begins with a conditioning layer of organic or inorganic matter on a surface. This conditioning layer alters the surface characteristics of substratum which eventually favors microorganisms to colonize on surface (Habash and Reid 1999). The biofilm formation process comprises several distinct steps: (i) initial reversible attachment of bacterial cells via weak interactions (such as van der Waals forces) with an abiotic or biotic surface (Bos and others 1999; Donlan 2002), (ii) irreversible attachment to the surface via hydrophilic/hydrophobic interactions by means of several attachment structures (flagella fimbriae, lipopolysaccharides, or adhesive proteins) (Bos and others 1999; Donlan 2002), (iii) then proliferation and production of a self-produced extracellular polysaccharide (EPS) matrix mainly composed of polysaccharides, proteins, and extracellular deoxyribonucleic acid (DNA) and ultimately the development of the biofilm architecture (Branda and others 2005; Flemming and others 2007), (iv) formation of a mature biofilm that contains water channels that effectively distribute nutrients and signaling molecules within the biofilm (Hall-Stoodley and others 2004; Dufour and others 2012), (v) the detachment of biofilm cells individually or in clumps due to intrinsic or extrinsic factors, and finally (vi) dispersion of the cells and colonization of other niches (Srey and others 2013). Figure 1 illustrates biofilm formation.

Antibiofilm Strategies (Natural)

Ancient cultures had experience with certain spices and herbs that could help to preserve foods and had medicinal effects. Accordingly, since the late 19th century, scientists have been testing experimentally whether some natural components possess antimicrobial properties (Cowan 1999). However, the antibiofilm activity of such compounds has not been validated rigorously. Recently, antibiofilm properties have been attributed to several natural compounds such as different plant extracts, essential oil (EO), and honey and these properties have been studied extensively.

Plant extracts

Several plant extracts and their active compounds were extensively investigated to eradicate the *Propionibacterium acne* biofilm (Coenye and others 2012). This study demonstrated that among 119 plant extracts, 5 (*Epimedium brevicornum*, *Malus pumila*, *Polygonum cuspidatum, Rhodiola crenulata,* and *Dolichos lablab)* showed a potent antibiofilm activity. These researchers also reported that extracts of *E. brevicornum* and *P. cuspidatum* and their active ingredients (icartin and resveratrol) exhibit a significant antibiofilm activity even when used at levels below the minimum inhibitory concentrations (MICs). Bark extracts of *Melia dubia* were assessed (Ravichandiran and others 2012) at the concentration of 30 mg/mL. These extracts showed strong suppression of hemolysis, swarming motility, hydrophobicity, and biofilm formation of *E. coli*. Similar results were also reported by Abraham and others (2011), concerning *Capparis spinosa* (caper bush) extract; at the concentration of 2 mg/mL, this extract significantly inhibited the biofilm formation and EPS production in *E. coli, Serratia marcescens, Pseudomonas aeruginosa,* and *P. mirabilis* (Issac and others 2011). Furthermore, established biofilm of all 3 microrganisms were dispersed. *Lagerstroemia speciosa* is a medicinal plant commonly found in Southeast Asia. The ability of *L. speciosa* fruit extracts was compared with biofilm formation by *P. aeruginosa* PAO1 (Singh and others 2012). These researchers demonstrated that *L. speciosa* fruit extracts significantly inhibited biofilm formation at the concentration of 10 mg/mL.

Green tea and Dandasa exhibit a good antibiofilm activity individually (Faraz and others 2012). The latter study showed that both Dandasa and green tea at the concentration of 6.2 and 12.5 mg/mL, respectively, had good antibiofilm effects on *Streptococcus mutans* and at the concentration of 12.5 and 3.1 mg/mL, respectively, on *E. coli*.

The possible strong inhibitory effect against biofilm was analyzed for fresh *Allium sativum* extract (fresh garlic extract [FGE]) (Harjai and others 2010). These authors reported that FGE reduced 6 log units *P. aeruginosa* biofilm. The *in vitro* screening of anti-*Staphylococcus epidermidis* biofilm activity of 45 aqueous extracts from 24 Caatinga (a Brazilian xeric shrubland) medicinal plant species was published (Trentin Dda and others 2011). The most promising extracts were isolated from *Bauhinia acuruana* branches, *Chamaecrista desvauxii* fruits, *B. acuruana* fruits, and *Pityrocarpa moniliformis* leaves, which reduced biofilm formation significantly even though they were tested at a 10-fold lower concentration. Moreover, those researchers also reported that *Commiphora leptophloes* and *Senna macranthera* fruit extracts reduced biofilms by 67.3% and 66.7%, respectively. Biofilm formation by *Mycobacterium smegmatis* was examined using various qualitative and quantitative techniques (Syed and others 2014). These researchers studied 5 plants (*Azadirachta indica*, *Hippophae rhamnoides*, *Juglans regia*, and *Vaccinium oxycoccus*) and spices to search for effective biofilm-controlling natural substances (antimicrobial activity). The test of efficacy of the plant extracts as antibiofilm agents revealed that the extract of *A. indica* (Neem) was most efficient at reducing and removing *M. smegmatis* biofilms. These findings might be extrapolated to other pathogenic biofilm-forming *Mycobacteria;* this notion may ensure effective *Mycobacterium* biofilm control.

The ability of casbane diterpene isolated from the extract of *Croton nepetaefolius* (native plant in Brazil) to inhibit biofilm formation of 2 Gram-positive species of bacteria (*Staphylococcus aureus* and *S. epidermidis*), 5 Gram-negative bacteria (*Pseudomonas fluorescens*, *P. aeruginosa, Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *E. coli*), and 3 yeast species (*Candida tropicalis, C*andida *albicans,* and *C. glabrata*) has been extensively evaluated (Carneiro and others 2011). The latter authors reported that biofilm formation was inhibited at MICs even when planktonic growth was not reduced. Another study showed that *Candida* biofilm formation was reduced significantly by *Boesenbergia pandurata* (finger root) oil (Taweechaisupapong and others 2010); biofilms were reduced by 63% to 98% when sub-MIC levels (from 4 to 32 μL/mL) were used. Recently, diverse plant extracts were screened against enterohemorrhagic *E. coli* (EHEC) O157:H7 biofilm (Lee and others 2013). This study showed that among 498 plant extracts, 16 inhibited biofilm formation of EHEC by more than 85% without the growth of planktonic cells.

Indeed, these findings indicate that different plant extracts have inhibitory effect on biofilms of many organisms. Further research is needed to study these extracts in detail and their potential as antibiofilm agents.

Honey

Honey is a natural product that is produced from the floral nectar by honey bees. Honey is widely popular and used for its antibacterial activity and wound-healing, antioxidant, and anti-inflammatory properties. It possesses antimicrobial properties against about 60 species of bacteria and fungi (Molan 2013). Recently, honey was reported as an effective agent for prevention of the formation of a biofilm (Maddocks and others 2012). It was demonstrated that honey is effective at inhibiting *Enterococcus* spp. biofilm formation and may serve as a possible therapeutic agent against biofilm-related enterococcal infections (Ng and others 2014). Honey can also reduce biofilm formation of EHEC O157:H7 (Lee 2011). The latter authors reported that low concentrations of honey reduced biofilm formation, QS, and virulence of *E. coli* O157:H7. A low concentration of honey reduced biofilm formation by inhibiting the expression of biofilm-related curling QS and virulence genes in bacteria without inhibiting the cell growth (Lee 2011). Honey at high concentrations can also inhibit biofilm formation and adhesion of bacteria due to its antibacterial properties (Lee 2011). Besides antimicrobial properties, it is also believed that honey prevents biofilm formation due to the bee defensin 1, which is an antimicrobial peptide that inhibits bacterial viability (Santangelo 2013). This peptide indirectly inhibits biofilm formation.

The detailed mechanisms of action underlying the antimicrobial effects of honey are still poorly understood (Nassar and others 2012). It would be beneficial if further research were conducted to identify the in-depth molecular mechanisms that drive honey's inhibition and prevention of biofilm formation. Exploring the actual antimicrobial mechanism of honey, we can apply honey to *in vivo* experiment. These research findings eventually could assist to establish honey as an antibiofilm agent. Thus, honey could be a potential cost-effective natural antibiofilm agent that also has no remarkable side effects like chemical drugs.

Essential oil

EOs are naturally plant-derived volatile substance. Due to their preservative and antimicrobial effects, EOs are promising and effective natural ingredients for food industry. These oils are popular and have been used widely since ancient time against a wide variety of pathogens (Hammer and others 1999). Most of the EOs exert their antimicrobial effect on microbial cell wall which leads to destruction of microrganisms. Moreover, it is also reported that EOs inactivate bacteria without developing antimicrobial resistance (Ohno and others 2003; Ali and others

2005). In particular, availability of many EOs, low mammalian toxicity, and quick degradation in environment make them safe antibiofilm agent (Isman 2000).

Cumin oil (Cuminum cyminum). Cumin oil is derived from a medicinal aromatic plant of the Apiaceae family and is used to flavor foods (cumin oil is added for fragrance) and for medical formulations (Iacobellis and others 2005). The fruits of this plant are known as cumin seeds, which possesses numerous medicinal properties. It acts as an astringent in digestive system. It has extensively been used as a remedy for mild digestive disorders, as a carminative and eupeptic, as an astringent in bronchopulmonary disorders and cough treatment, and as an analgesic. In traditional medicine, seeds of the Cumin plant have been used for hundreds of years. The efficacy of cumin seed EO against the biofilm formation by *K. pneumoniae* strains was examined (Safoura and others 2010). That study demonstrated that this EO reduced biofilm formation and increased the efficacy of the antibiotic ciprofloxacin.

Cinnamon oil. Cinnamon oil is a popular EO widely used in the food industry because of its special aroma (Chang and others 2001). There is evidence that cinnamon oil is effective against biofilm cultures of *S. mutans* and *Lactobacillus plantarum* (Filoche and others 2005). The activity of cinnamon oil against *S. epidermidis* was analyzed (Nuryastuti and others 2009). These researchers demonstrated that *S. epidermidis* strains (planktonic and biofilm forms) were susceptible to cinnamon oil. The antibacterial activity of *Cinnamomum cassia* EO was evaluated toward single- and mixed-species biofilms of enteropathogenic *E. coli* (EPEC) and *L. monocytogenes* attached to stainless steel (Oliveira and others 2012).

Oregano essential oil. Inhibitory activity of oregano EO on biofilm formation by staphylococci and *E. coli* was also assessed (Nebahat and others 2010). That study showed that oregano EO exerts antibacterial action on planktonic *S. aureus, S. lugdunensis, S. haemolyticus, S. sciuri,* and *E. coli* and was capable of preventing, or at least interfering with, biofilm formation. It also removed active biofilm even at the MIC (Nebahat and others 2010).

Vegetable oil. The antibiofilm effects of vegetable oil (Brazil nut oil) on commercially available dentifrice in terms of dental biofilm control were evaluated recently (Filogônio and others 2011). These researchers found that the addition of vegetable oil to a commercially available dentifrice improved dental biofilm control, suggesting that this oil may aid in the prevention and/or control of caries and periodontal diseases.

Tea tree essential oil. The antibacterial activity of the tea tree EO (TTO) combined with the conventional antibiotic ciprofloxacin (CIP) was evaluated against preformed *P. aeruginosa* biofilms (Coelo and others 2012). The results revealed that the synergistic effect of TTO with (CIP) reduced biofilm biomass considerably (by greater than 70%) and the number of cells (greater than 3 log reduction) at the minimum concentration (1.25 μg/mL) of CIP.

The effectiveness of 3 EOs (thymol, oregano, and cinnamon oils) at sublethal concentrations on biofilm formation of 3 biofilm-forming bacterial strains (*Acinetobacter*, *Sphingomonas,* and *Stenotrophomonas*) was determined (Sandra 2014). These authors found that at the MIC, 2 out of 3 strains showed inhibitory effects on bacterial biofilm formation. Among the 3 tested oils, thyme oil seemed to be a more efficient specific inhibitor of biofilm formation. Thyme oil effectively inhibited the development of a biofilm even at sublethal concentrations of 0.001% (w/v).

These data support the notion that natural products are a possible source of antibiofilm agents. Therefore, compounds obtained from natural sources and their different formulations could be a novel approach to combating biofilms.

Bacteriophages

Bacterial viruses or bacteriophages (phages) are presumed to be the predominant life form in the biosphere. Due to the emergence of antibiotic resistance, the application of phages to destruction of bacteria has elicited much attention recently along with reappraisal. Phages are currently considered a potential alternative or adjunct to antibiotics for bacterial infections, especially for biofilm inhibition or disruption. Phages possess many advantages over antibiotics and chemical agents. Phage isolation is fast and simple and production is relatively inexpensive; phages are highly specific against a host or host range and thus do not affect the normal microflora where they can be applied. Phages are also environmentally friendly; they self-replicate at the target site as long as the host bacterium persists and so far no adverse effects have ever been observed (Pires and others 2011).

Phages have been tested as potential antibiofilm agents. For example, T4 phage can effectively infect and replicate within *E. coli* biofilms and can disrupt the biofilm matrix by destroying bacterial cells (Meng and others 2011). One of the first studies designed to evaluate the interaction of phages with biofilms was reported by Doolittle and others (1995). The interaction between a phage and biofilm is a sequential and dynamic process. The most important step in phage infection is the adsorption of phages to the receptors of the target bacteria. The EPS matrix by which bacteria are protected in a biofilm offers a potential challenge for phages because EPS needs to be penetrated so that phages can reach and attach to the specific host receptors. However, phages can penetrate the EPS matrix via diffusion or with the help of phage-derived enzymes (such as polysaccharide depolymerase). These enzymes are well capable of destroying the biofilm architecture so that the phages can easily attach to lipopolysaccharides, outer membrane proteins, or other receptors necessary for their replication (Hughes and others 1998a, b). There is convincing evidence that phage-induced depolymerases can affect biofilms (Donlan 2009). Genetically engineered phages have been developed that express a biofilm-degrading enzyme during infection. The dispersion (dspB) gene was cloned into an *E. coli*-specific phage (T7), to produce an engineered enzymatic phage that showed more efficacy at removal of biofilms than did a noncloned phage (Lu and Collins 2007).

Despite the enormous advantages of phage use, there are still some drawbacks such as the release of a large amount of bacterial-membrane-bound endotoxins; some phages may encode toxins; there is a lack of pharmacokinetic data; and conversion of lytic phages to lysogenic phages (prophages) is also a problem. Some of the aforementioned concerns have been successfully resolved via different approaches. To address the endotoxin release issue, production of a recombinant phage from a *P. aeruginosa* filamentous phage was designed that reduced the rapid release of membrane-bound endotoxins significantly as well as the mortality rate in experimental animals (Hagen and others 2004). The combination approach, such as antibiotics and bacteriophages, has been suggested as a potential strategy for control of biofilms. Phage PhilBB-PF 7A showed 63% to 91% activity in terms of removal of biomass of *P. fluorescens* (Sillankorva and others 2008). The combined approach of impregnation of hospital settings with phages and incorporation of phages into the hydrogel coating of medical devices increased its efficacy against *S. epidermidis* (Del Pozo and others 2007).

Table 1–Natural quorum sensing inhibitor compounds known to inhibit biofilm formation.

Source	Compound	Effective against	References
Macroalga (*Delisea pulchra)*	Furanone	Inhibit biofilm formation in *A. hydrophila*	Ponnusamy and others 2010
Macroalga (*Delisea pulchra)*	Furanone	Inhibit biofilm formation in *E. coli*	Ren and others 2001
Garlic extract (Allium sativum)	*Ajoene*	Inhibit biofilm formation in *P. aeruginosa* PAO1	Bjarnsholt and others 2005
Citrus extract	Naringin	Decrease biofilm formation in *Y. enterocolitica*	Truchado and others 2012
Penicillium sp.	Patulin/Clavin	Inhibit biofilm formation in *P. aeruginosa*	Rasmussen and others 2005a
Penicillium sp.	*Penicillic acid*	Inhibit biofilm formation in *P. aeruginosa*	Rasmussen and others 2005a
Vanilla bean extract	*Vanillin*	Inhibit biofilm formation in *A. hydrophila*	Ponnusamy and others 2009
Sweet basil	Rosmarinic acid	Inhibit biofilm formation in *P. aeruginosa*	Annapoorani and others 2012
Plant extract (Sabucuschinesis)	Urosolic acid	Inhibit biofilm formation in *E. coli*	Ren and others 2005
Fruit extract (*Termanilia chebula* betz)	Ellagic acid(Benzoic acid)	Reduce biofilm formation in *B. cepacia*	Huber and others 2003
Green tea	Epigallocatechin Gallate	Inhibit biofilm formation in *S. aureus* & *B. capacia*	Huber and others 2003; Blanco and others 2005
Cinnamomum zeylanicum	Cynnamaldheyde	Inhibit biofilm formation in *P. aeruginosa*	Niu and others 2006
Grapefruit juice and extract (*Psoralea corylifolia* L.)	Furocoumarin/Psoralen	Inhibit biofilm formation in *E. coli*	Girennavar and others 2008
Ellagitannin-rich extract (Pomegranate)	Urolithin	Inhibit biofilm formation in *Y. enterocolitica*	Giménez-Bastida and others 2012
Curcuma longa	Curcumin	Inhibit biofilm formation in *P. aeruginosa, E. coli, P. Mirabilis,* and *S. marcescens*	Packiavathy and others 2014
Musaceae extract (*Musa paradiciaca)*	Musaceae	Inhibit biofilm formation in *P. aeruginosa* PAO1	Musthafa and others 2010
Piper betle extract	Piper betle	Inhibit biofilm formation in *P. aeruginosa*	Siddiqui and others 2012
Cuminum cyminum	*Cuminum cyminum*	Inhibit biofilm formation in *P. aeruginosa*	Packiavathy and others 2012

These studies indicate that some of the strongest impediments, such as higher antibiotic resistance, the presence of biofilm extracellular matrix that hampers the control of biofilm by antimicrobial agents, might be overcome via phage use. However, the narrow host range, bacterial resistance to phages, and phage-encoded virulence genes that can incorporate into the host bacterial genome are major limitations of phage use. The immune system may also decrease phage efficacy, and inappropriately obtained phage preparations can contain endotoxin. Phage mixtures or engineered phages may be an effective alternative helping to overcome these obstacles (Donlan 2009). Undoubtedly, phages, after extensive studies and proper selection, should become one of the most effective antibiofilm agents.

Quorum Quenching

QS is a delicate cellular process through which bacteria produce and recognize signal molecules and through which they coordinate their behavior in a cell density–dependent manner (Waters and Bassler 2005). They implement QS by secreting small extracellular signaling molecules acting as an autoinducer to start genetic programs. Three main QS system can be distinguished: the acetyl homoserine lactone (AHL) QS system in Gram-negative bacteria, the autoinducing peptide (AIP) QS system in Gram-positive bacteria, and the autoinducer 2 (AI-2) QS system in both Gram-negative and Gram-positive bacteria. Multiple reports have discussed the importance of QS in bacterial biofilm formation (Novick and Geisinger 2008; Ahmed and others 2009; Estrela and others 2009; Coenye 2010; Zhao and others 2010). It is reported that QS is linked to control of bacterial swarming and the maturation of biofilm architecture (Hooshangi and Bentley 2008; Ueda and Wood 2009). Still, much remains to be elucidated regarding the role of QS in biofilm formation, maintenance, and dispersal.

Inhibition of QS is a promising approach to the prevention of biofilms without significant planktonic cell death. QS inhibitors (QSIs) have been suggested as novel antibiofilm agents. There are several established quorum-quenching strategies through which a QS mechanism can be interrupted such as inhibition of signal synthesis or direct degradation of a signaling molecule, inhibition of binding of the signaling molecule to its receptor, and/or inhibition of binding of the signal transduction cascade. Most prokaryotes, as well as some eukaryotes such as certain traditional medicinal plants, can produce QS-inhibiting compounds (some natural QSI compounds that inhibit biofilm formation are listed in Table 1). A wide variety of molecules capable of disrupting the QS system have been identified and their mechanisms were revealed. These compounds have extensively been analyzed to combat biofilm. Over the last few years, a large number of quorum-quenching enzymes have been identified in various Gram-negative and Gram-positive bacteria; this is a new milestone in quorum-quenching research. These enzymes are often classified into 3 groups: (i) AHL acylase, (ii) AHL lactonases, and (iii) oxidoreductases. The mechanism of action of these enzymes is known: 4 potential cleavage sites in the AHLs are likely cut off after catabolic digestion of carbon and nitrogen sources.

The quorum-quenching approach leads to the dissociation of the biofilm architecture but not to killing of the biofilm microorganism. Nonetheless, QSIs have the potential to increase the sensitivity of biofilm-forming bacteria to antibiotics. Therefore, a combination of QSI compounds and antibiotics to handle biofilm has been suggested. To achieve this goal, several attempts have been made to kill the microorganisms released from the biofilm by treatment with standard antibiotics. As a consequence, many researchers have combined quorum quenching with antibiotic treatments and demonstrated in animal studies that these methods work well. For example, in a pulmonary model of chronic lung infection, the quorum-quenching agent furanone was administered to mice preinfected with *P. aeruginosa* 2 d before. Attenuation of expression of virulence factors and much better removal of the

bacteria by the immune system were observed (Hentzer and others 2003). Extracted from the fungas genus *Penicillium,* patulin (Wagner and others 2004) and penicillic acid (Abraham 2005) were identified as QSI compounds. These compounds were tested in a mouse model and showed results similar to those of furanone (Rasmussen and others 2005b). All 3 QSI compounds (furanone, patulin, and penicillic acid) increased sensitivity of *P. aeruginosa* to the antibiotic tobramycin.

These observations demonstrated that many quorum-quenching compounds enhance the sensitivity of pathogens to antibiotics, even though the quorum quenchers cannot cause complete removal of the biofilms. In animal models, this combined strategy has already been validated and such a combination is attractive and holds great promise for biofilm control.

New Surfaces for Prevention of Biofilm

The design of new surfaces is an emerging approach to prevent biofilm formation in the medical, food, and marine industries. Many innovative techniques could be applied to create new surfaces, for example, via new surface materials, surface modifications, new coating, and paint.

New surface materials and surface modifications

Selection of surface materials that do not favor the attachment of microorganisms is a promising approach to the prevention of biofilm. Many studies have been conducted to search for the materials that do not enhance or even suppress biofilm formation. Different surface materials have been ranked and categorized according to biofilm formation in general (Rogers and others 1994). However, modification of a surface yields another potential way to prevent biofilm. Numerous studies have been conducted, especially in the medical fields, to prevent biofilm formation via incorporation of biocides into surface materials, or to coat surfaces with biocides. The effectiveness of phosphorylcholine (PC)-containing polymers with nonfouling characteristics at enhancing the properties of medical instruments was also assessed (Lewis 2000).

New coating and paint

In marine and biomedical industries, the development of new coating materials and paints containing biocides or antibiotics is urgently needed. A variety of coating agents and paints were developed since the mid-1880s. Among these, arsenic, copper oxide, mercury oxide, and zinc oxide have widely been used as effective antifoulants (Yebra and others 2004). TBT (tributyltin)-based products and their derivatives are the most promising antifouling paints. In marine coating agents, the use of organotins and other toxic biocides is prohibited. As a consequence, safer methods of biofouling control based on environmentally friendly compounds are actively studied. Based on copper oxides and organic biocides, substitute products are produced (Konstantinou and Albanis 2004). The most widely used booster biocides were also reviewed (Yebra and others 2004).

In terms of medical devices, the surface is the common site of microbial adhesion. Many authors reported inhibition of biofilm formation on such devices *in vitro* via coating with silver (Klueh and others 2000; Jiang and others 2004; Stobie and others 2008). Furanones were assessed as a novel antimicrobial agent (Khan and Husain 2002). To biomaterial surfaces, furanone was applied via physical adsorption, and this coating prevents *S. epidermidis* biofilm formation significantly (Baveja and others 2004). Furanone also inhibits biofilm formation when covalently bonded to Silastic Tenckhoff catheters (Hume and others 2004). The latter authors demonstrated that catheters coated with furanone reduced infections.

Resistance of biofilms to biocides and antimicrobial agents is mainly achieved through a cell-to-cell communication (QS) process. Thus, blockage of cell–cell communication can be a novel approach to inhibition of biofilm formation. For example, recently, a peptide termed as "RNA III-inhibiting peptide" (RIP) was described that may prevent biofilm formation by *S. aureus* (Balaban and others 1998, 2000, 2001, 2003; Gov and others 2001). The process by which RIP inhibits QS involves inhibition of the phosphorylation of a protein called "target of RNA III" activating protein (TRAP) (Balaban and others 2001). These findings suggest that medical devices coated with RIP could be used to prevent biofilm formation.

Another alternative method is to coat surfaces with nontoxic materials so that the adhesion of microbes is greatly reduced. This effect is mainly due to a combination of hydrophobicity, low surface free energy, and microroughness (TsiBouklis and others 1999; Yebra and others 2004; Roosjen and others 2006). The development of nonstick surfaces in medical, food, and marine industries has been studied in recent years. Currently, in the food industry, application of biocides to keep the processing units clean is mostly avoided. The most attractive nontoxic alternative is nonstick and fouling-release materials. A nonstick fouling-release coating is mainly based on 2 components (silicones and fluoropolymers). Many studies (reviewed by Yebra and others 2004) have been conducted to clarify coating properties to prevent adhesion of microbes. Fluoropolymers can form nonporous surfaces with very low surface free energy and good nonstick characteristics (Berry and others 2000; Brady 2001; Brady and Aronson 2003).

Application of "Theta" surface concept has been proposed to control biofilm (Baier 2006). Numerous studies have been conducted on reduction of bioadhesion on a wide variety of surfaces possessing critical surface tension (CST) in the range of "Theta" surface concept (that is, 20 to 30 mN/m). Theta surface for easy release along with application of photodynamic therapy (PDT) was also investigated (Mang and others 2012). Their experiment revealed that treatment with radio frequency glow discharge (RFGD) resulted in increased CST, higher surface wettability, and surface energy rendering it more favorable for biofilm formation. Indeed "Theta" surface characteristics of nature on to materials could be copied to attain biomass easy release.

Recent progress in paints and surface coating agents has ensured long-term antimicrobial activity through the incorporation of nanomaterials. Nanocrystal line zirconium oxide (zirconia) is an attractive alternative material for implants because of its strong resistance to wear and biocorrosion as well as biocompatible properties. Nanocrystalline silicon carbide is a suitable material for artificial heart valves, mainly due to its light weight, high strength, and inertness. If nanomaterials can be manufactured at a low cost, they could serve as fouling-resistant coating agents for routine applications such as piping for domestic and industrial water systems.

A wide variety of microbe-resistant surfaces have been suggested to prevent biofilm formation beforehand, but the actual strategies rely either on a release of biocidal compounds or on preventing adhesion. Actually, traditional methods rely on the design of coatings that release antimicrobial agents such as antibiotics, quaternary ammonium salts, and silver ions into the surrounding aqueous environment. These agents have extensively been incorporated into a variety of engineering polymers and other materials (Banerjee and others 2010). Another approach is based on the use of surface chemical functional groups that inhibit protein adsorption

as a means to prevent bacterial colonization. Polyethylene glycol (PEG) is a commonly studied agent for such surface modifications (Prime and Whitesides 1991; Park and others 1998). Both of these techniques seem to be transient. Materials that persistently prevent microbial adhesion via surface chemistry only are a challenge. Even though microorganisms fail to attach directly to a substrate, other microbial nonspecific substances such as proteins and surfactants produced by bacteria on the surface eventually mask the underlying chemical functionality (Gristina 1987; Neu 1996; Bos and others 2000). The reservoir of leaching antimicrobial agents is usually finite and subject to depletion.

To minimize these challenges to the prevention of microbial surface attachment, new findings on the interactions governing microbial aggregation on nanopost substrates were reported (Epstein and others 2011a). These researchers investigated the effectiveness of a combinatorial approach to the attachment and biofilm formation behavior by altering the symmetries, dimensions, and pitch (center to center distance) of a nanopost. Eventually, they concluded that high-aspect-ratio (HAR) nanoarrays mimicking an extremely compliant flat surface show promise for different uses with respect to control and inhibition of biofilm formation.

Nanotechnology

Nanotechnology is the engineering of a functional system that mainly deals with the manipulations on the scale of individual atoms and molecules and with tolerance of less than 100 nm. Recently, nanotechnologies have become a promising tool for biofilm prevention and control.

Silver is an antimicrobial nontoxic metal. The antibiofilm effectiveness of silver nanoparticles (AgNPs) against *P. aeruginosa* and *S. epidermidis* strains was evaluated (Kalishwaralal and others 2010). Silver nanoparticles in that study were synthesized with *Bacillus licheniformis* and silver nitrate ($AgNO_3$). The average diameter of the resulting silver particles was 50 nm. They reported that treatment with silver nitrate ($AgNO_3$)-containing nanoparticles (100 nm) was reduced by 98% established (formed 24 h beforehand) biofilm. Treatment with AgNPs prevented biofilm formation without affecting bacterial viability (Kalishwaralal and others 2010). Next, AgNPs dose-dependent efficacy against *S. aureus* and *P. aeruginosa* biofilm was also demonstrated (Mohanty and others 2012). Silver nanoparticles with an average particle size of 20 nm were prepared in 1% soluble starch. Brief incubation of biofilms (24 h) with 1 or 2 μM (AgNPs) showed greater than 50% or 85% reduction of biofilm formation, respectively. Longer term (48 h) treatment with silver nanoparticles yielded 65% and 85% biofilm inhibition, respectively. The effectiveness of silver nanoparticles against *C. albicans* and *C. glabrata* biofilm formation was also evaluated (Jena and others 2012). The biomass of adherent *C. glabrata* cells was reduced by more than 90% when silver nanoparticles were added to a culture of *Candida*- adherent cells at the concentration of 3.3 μg/mL. Moreover, 54 μg/mL AgNPs treatment effectively disrupted (by 97%) mature biofilms; in terms of *C. albicans*, biofilm biomass was reduced by 85% with the silver nanoparticle treatment at concentrations greater than 6.7 μg/mL. With the treatment at higher doses (5 μg/mL) of CS-AgNPs, *S. aureus* biofilm formation was inhibited by 65% (Jena and others 2012). Recently, titanium dioxide (TiO_2) and ethylenediaminetetraacetic acid (EDTA) nanoparticles were developed and tested for their effects on biofilms of several strains of *C. albicans* (Haghighi and others 2013). These researchers found that TiO_2 nanoparticles can be considered a new alternative for the prevention of fungal biofilm, and therefore, can prevent adhesion of microorganisms. These authors suggested that TiO_2 can be used to coat medical insertion materials.

Nanotechnology was also applied to antimicrobial sprays (silicon compounds and organic quaternary ammonium salts) (He and others 2012). These authors developed nanoparticles with different electrically charged surfaces that through physical attraction bind to microorganisms and ultimately eliminate them. These electric charge–carrying nanoparticles are extremely effective at both prevention and removal of biofilm. Nitric oxide–containing silica nanoparticles were developed by Hetrick and others (2009). These silica nanoparticles exhibited activity against biofilms of *S. aureus, S. epidermidis, P. aeruginosa, E. coli,* and *C. albicans*. Apart from the dispersal of biofilm, the addition of nanoparticles to catheters prevented colonization. Similar results were also reported by Rai and others (2012). These authors applied silver nanoparticles to the inhibition of *S. aureus, Enterococcus* spp., *E. coli, P. aeruginosa,* and *C. albicans* biofilms. The superparamagnetic iron oxide (γ-Fe_2O_3) nanoparticles were also used against a biofilm (Taylor and Webster 2009). These researchers observed that the generated hydroxyl radicals can depolymerize polysaccharides, cause breaks in DNA, and inactivate enzymes that can compromise the EPS matrix of the biofilm architecture. The authors also pointed out that these nanoparticles can effectively disrupt cell membranes, causing death of planktonic cells.

Micro- and nanoemulsion

Microemulsions are a clear, thermodynamically stable isotropic emulsion of oil, water, and a surfactant frequently formulated with a cosurfactant. The aqueous phase of a microemulsion may contain salt(s) and/or other ingredients, and the oil phase usually is a complex mixture of different hydrocarbons and olefins. On the other hand, nanoemulsions are an oil-in-water (o/w) emulsion with mean droplet diameters ranging from 50 to 1000 nm. Generally, the average droplet size of a nanoemulsion is between 100 and 500 nm. Nanoemulsions are a unique class of disinfectants manufactured by emulsifying a water-immiscible liquid phase into an aqueous phase under high pressure. Both micro- and nanoemulsions usually are in the range of less than 100 nm size. Nanoemulsions are formed by mechanical shear; in contrast, microemulsion phases are formed via self-assembly. In recent years, researchers have shown that some micro- and nanoemulsions have antibiofilm properties (Al-Adham and others 2003). They investigated the effectiveness of 2 fine emulsions designated BCTP (nanoemulsions of soybean phosphate emulsified with Triton X-100, named as BCTP by the authors Hamouda and others 1999) and TEOP (microemulsions of ethyl oleate with Tween 80 as an emulsifier and n-pentanol coemulsified with Triton X-100, designated as TEOP by the authors Teixeira and others 2007) to reduce preformed biofilms of 5 bacteria: *Salmonella* spp., *E. coli* O157:H7, *P. aeruginosa, S. aureus,* and *L. monocytogenes*. With the exception of the *Listeria* biofilms, biofilms formed by the other 4 bacterial species were inhibited by both BCTP and TEOP. The actual mechanism of higher resistance of *L. monocytogenes* biofilms to the fine emulsions requires further research. The bactericidal activity of TEOP is due mainly to n-pentanol; it is a kind of alcohol, and the bactericidal properties of alcohols have been known for a long time. It has been proven that an alcohol disrupts bacterial cell membrane, leading to leakage of ions and metabolites. On the other hand, tributyl phosphate possesses surfactant properties.

Control of Biofilms with Enzymes

DNA, proteins, and EPSes constitute the biofilm matrix, and recent studies revealed that effective disruption of the

biofilm architecture could be achieved by various enzymes. Matrix-degrading enzymes such as deoxyribonucleases, glycosidases, and proteases play a significant role in the dispersal of a mature biofilm (Kaplan 2010).

Deoxyribonuclease 1 (DNase 1)

Effects of DNases on the biofilm of the Gram-positive (*S. aureus* and *Streptococcus pyogenes*) and Gram-negative (*Acinetobacter baumannii*, *Haemophilus influenza*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa)* bacteria were extensively studied (TeTz and others 2009). These researchers demonstrated that DNase 1 at the concentration of 5.0 μg/mL reduced the 24 h active biofilm biomass by approximately 40% among all tested organisms. Moreover, they also reported synergistic effects of DNase 1 and antibiotics: antibiotics (azithromycin, rifampin, levofloxacin, ampicillin, and cefotaxime) and DNase 1 (5 μg/mL) reduced the biofilm biomass significantly (TeTz and others 2009).

Biofilm degradation of clinical isolates of *Streptococcus pneumoniae* by a DNase was also evaluated (Hall-Stoodley and others 2008). These authors reported that DNase treatment resulted in significant degradation of a biofilm (by 66.7% to 95%), even though the biofilms were grown for 6 d. Moreover, the DNase suppression of biofilm formation by *S. aureus* and *P. aeruginosa* was studied (Eckhart and others 2007). The latter researchers reported that biofilm formation was effectively reduced by DNase treatment. Furthermore, bovine DNase 1 was reported to suppress biofilm formation by *P. aeruginosa*, *Streptococcus intermedius*, and *S. mutans* (Whitchurch and others 2002; Petersen and others 2005).

Lysostaphin (LS)

LS is a naturally occurring enzyme that can effectively invade into biofilms (Belyansky and others 2011a, b). The activity of LS toward biofilms was investigated (Walencka and others 2005). These authors studied the antibiofilm effects of this enzyme on clinical and reference strains of *S. aureus* and *S. epidermidis*. The researchers concluded that LS is capable of eradicating biofilms of all *S. aureus* and *S. epidermidis* strains effectively. Moreover, their study revealed that the combination of LS and oxacillin increased the sensitivity of the biofilm-growing bacteria to an antibiotic. LS's high potential antibiofilm effectiveness against *S. aureus* strains was also evaluated (Kokai-Kun and others 2009). These investigators devised a murine model to develop suitable strategy against a multiorgan biofilm infection on a medical device. They observed that treatment with LS (15 mg/kg) in combination with nafcillin (50 mg/kg) eradicated established *S. aureus*, including methicillin-resistant *S. aureus* (MRSA) biofilms from implanted catheters. Moreover, they also found that a single beforehand treatment of catheters with LS (10 mg/kg) completely protected from further biofilm infection. In addition, a synergistic effect of LS and an antibiotic in terms of the eradication of an *S. aureus* biofilm was evaluated (Aguinaga and others 2011). These authors observed the highest synergistic effect of LS and doxycycline against MRSA and methicillin-sensitive *S. aureus* (MSSA) (Aguinaga and others 2011).

α-Amylase

Potential use of commercially available α-amylase compounds from various biological sources was investigated for inhibition and removal of *S. aureus* biofilms (Craigen and others 2011). The latter investigators reported that amylases at doses of 10, 20, and 100 μg/mL reduced biofilm by 72%, 89%, and 90%, respectively, and matrix formation was also inhibited by 82%. In a time course experiment, biofilms were reduced by 79% and 89% within 5 and 30 min, respectively (Craigen and others 2011). Their data demonstrated that α-amylase compounds can disperse, as well as inhibit, biofilm of *S. aureus.* These authors suggested that α-amylase compounds could be used in the near future to control *S. aureus* biofilm infection.

Lyase

Coadministration of a lyase with an antibiotic was tested to inhibit and eradicate biofilms (Alkawash and others 2006). These researchers assessed a combined treatment with alginate lyase (20 μg/mL) and gentamycin (64 μg/mL) of a biofilm of 2 mucoid *P. aeruginosa* strains. Their results revealed that the combined treatment caused liquefaction of the biofilm matrix and complete eradication of the biofilm structure and living bacteria within 96 h. The viable counts were also reduced by 2 to 3 $\log_{10}$ units for both strains when the combined therapy was used (Alkawash and others 2006).

Lactonase

Lactonase as a potential antibiofilm enzyme was also examined (Kiran and others 2011). These authors found that treatment with 1 unit of lactonase significantly reduced biofilm formation by 4 *P. aeruginosa* strains. In addition, lactonase treatment at 0.3 U/mL disrupted biofilm structure and increases sensitivity to antibiotics (ciprofloxacin and gentamycin). Furthermore, lactonase treatment also downregulated virulence factors such as pyocyanin (by 85% to 93%), protease activity (by 86% to 96%), elastase activity (by 69% to 91%), and pyochelin secretion (by 40% to 90%).

Recently, in the food industry, enzymes, and detergents have been used synergistically to increase disinfectant potency. The synergistic effect of proteolytic enzymes with surfactants increases the wettability of biofilms, and therefore, enhances the cleaning efficacy. Formulations containing several enzymes seem to be a novel effective biofilm control strategy. Mainly proteases and polysaccharide-hydrolyzing enzymes may be useful for this purpose (Shia and Xinha 2009). However, the specific mode of action of different enzymes and the difficulty of identifying enzymes that are effective against all the different types of biofilms make this method rather complicated for practical application to biofilm control. Moreover, the use of enzymes for biofilm control is still limited due to the high cost of the enzyme-based detergents and these are mostly patent-protected. Moreover, another factor that limits their current uses is the low commercial accessibility of different enzymes (Simões and others 2010). Nevertheless, a combination of different enzymes and antimicrobials/disinfectants is a promising, highly effective method for removing and controlling biofilms.

Photodynamic Therapy

PDT is a medical treatment that utilizes a certain drug, called a photosensitizer or photosensitizing agent, and light at a particular wavelength. During PDT, photosensitizers are exposed to light at a specific wave length and produce a form of oxygen that destroys nearby cells. The application of PDT to microbial inactivation was first reported more than 100 y ago when Oscar Raab reported the lethal effect of acridine hydrochloride and visible light on *Paramecium caudatum*.

Recent research on antimicrobial PDT was focused on many areas such as investigation of the photophysical and photochemical properties, development of more effective and clinically compatible nontoxic photosensitizers, exploration of novel delivery methodologies, and preclinical and clinical examination of PDT

Table 2–Selected recent antimicrobial photodynamic therapy (APDT) studies on biofilm.

Biofilm organism	Photosensitizer	Efficacy (reduction of CFU)	References
P. aeruginosa Methicillin-resistant *S. aureus*	MB	>6 log	Biel and others 2011
C. albicans	MB	1 to 2 log	Rossoni and others 2014
P. aeruginosa	TMP	Significant	Collins and others 2010
Candida spp.	ZnPc	0.33 to 0.85 log	Junqueira and others 2012
Enterococcus faecalis	MB	1.9 log	Meire and others 2012
S. mutans	TBO	≥5log	Teixeira and others 2012
Enterococcus faecalis	TB	≥5log	Kishen and others 2010
Enterococcus faecalis	TBO	Total elimination	Zand and others 2014
A. israelii	MB	80%	Fimple and others 2008
F. nucleatum			
P. gingivalis			
P. intermedia (poly species)			
S. mutans	TBO	2 to 5 log	Zanin and others 2005
S. mutans	TBO	S. m & S. Sobr 1 log	
S. Sang ≥ 3 log			Zanin and others 2006
S. Sobrinus			
S. sanguinis (monospecies)			

TMP = 5-,10-,15-,20-tetrakis(1-methyl-pyridino)-21H, 23H-porphine, tetra-p-tosylate salt; ZnPc: cationic nanoemulsion of zinc 2-,9-,16-,23-tetrakis(phenylthio)-29H, 31H-phthalocyanine; TBO: toluidine blue O; TB: toluidine blue; MB: methylene blue.

applications. The photodynamic approach has emerged as an innovative alternative to antimicrobial regimens and mechanical means for eradicating biofilms, and their application to biofilms has shown superior results compared to the conventional approaches (selected recent PDT studies on biofilms are listed in Table 2). It is reported that PDT against *Streptococci* species in a dental biofilm ensures total destruction of the microorganisms (Spinei 2013). One-time photomechanical wave treatment (laser light at 666 nm) in a biofilm of *Actinomyces viscosus* in the oral cavity increased penetration of methylene blue by up to 75% (Donnelly and others 2007). Another team found that multispecies oral biofilms treated with helium/neon laser light in the presence of toluidine blue are inactivated by 95% (Soukos and others 2000). One major obstacle for biofilm treatment with PDT is slime production and growth phases: both are properties of biofilms that reduce photodynamic inactivation of many pathogens such as *S. epidermidis* and *S. aureus*. This hindrance can be resolved partially through the use of polylysine-based cationic photosensitizers, which are currently being investigated (O'Neil and others 2002).

Biosurfactants

Microbial surfactants or biosurfactants (BSs) are a low-molecular-weight heterogeneous group of amphiphilic surface-active compounds (produced by microbes) containing a hydrophilic moiety (polar or nonpolar) and a hydrophobic moiety (lipid or fatty acid), either on the cell surface or when secreted extracellularly. Most BSs consist of a variety of complex molecules of different chemical compositions such as glycolipids, lipopeptides, lipoproteins, fatty acids, phospholipids, and neutral lipids. BSs have the potential to reduce the surface and interfacial tensions between individual molecules on the surface and at the interface, respectively, both in aqueous solutions and in hydrocarbon mixtures. These surface and interfacial tension-reducing properties of surfactants result in excellent detergency, emulsification, foaming, and dispersing characteristics. BSs have attracted immense renewed interest due to their versatile applications in the petroleum industry, food processing, agriculture, environmental remediation, and the cosmetic and pharmaceutical industries. BSs offer several advantages over synthetic surfactants: BSs are diverse, biodegradable, and have low toxicity and the potential for highly selective, specialized functions; BSs are also effective at extreme levels of pH, temperature, and salinity (Muthusamy and others 2008; Banat and others 2010).

The strong dispersal, high antimicrobial, and antiadhesive properties of BSs make them suitable agents for eradication of biofilms. BSs can modify the surface characteristics of bacterial cells and reduce their adhesive properties (Ahimou and others 2000). In addition, BSs produced by many bacteria have been proven to interfere with biofilm development and cell-to-cell communication (Dusane and others 2008, 2010; Rivardo and others 2009). In recent years, the use of BSs as alternatives to antibiofilm agents has extensively been explored (Dusane and others 2008, 2010; Rivardo and others 2009). Many studies have confirmed that under certain testing conditions, BSs can be more effective than other conventional biofilm inhibition and or disruption strategies (Epstein and others 2011b). Studies revealed that BSs derived from probiotic bacteria *L. acidophilus* inhibit and disperse a *Staphylococci* biofilm (Walencka and others 2008). Moreover, BSs obtained from probiotic lactobacilli have been shown to reduce adhesion of pathogenic bacteria to different food and biomedical surfaces such as glass, Silicon Robber, surgical implants, and voice prostheses (Velraeds and others 1996; Busscher and others 1997; Gan and others 2002; Rodrigues and others 2004). It is presumed that when a BS is applied to a substratum surface, it modifies its hydrophobicity, interfering with the microbial adhesion and adsorption process of microorganisms (Rodrigues and others 2006). Coating of catheters and other medical instruments with BSs may be used as a preventive strategy to slow down the onset of biofilm growth of pathogenic bacteria on wounds, hospital settings, and inert surfaces in the hospital environment (Busscher and others 1997; Gan and others 2002; Rodrigues and others 2004; Singh and others 2007; Walencka and others 2008). Lipopeptide BSs that are derived from *Paenibacillus polymyxa* have been shown to be effective against many biofilm-forming bacteria such as *B*acillus *subtilis*, *Micrococcus luteus*, *P. aeruginosa*, *S. aureus*, and *Streptococcus bovis* (Quinin and others 2012). The strong effectiveness of BSs against biofilms points to a future role in biofilm prevention and control strategies. Selected BSs with antibiofilm activities are listed in Table 3.

Bacteriocin

Bacteriocins are ribosomally synthesized antimicrobial peptides that are produced by all prokaryotic lineages and are generally active against closely related species. It is generally a peptide or protein of varying sized chains of amino acids. They act as narrow-spectrum antibiotics (Himsley 1980). These compounds are of immense interest for the preservation of food and the enhancement

Table 3–Selected biosurfactants with antibiofilm activities.

Source	Biosurfactant class	Name	Effectiveness	References
Providencia rettgeri	Lipopeptide	NS	Inhibit biofilm formation of *P. aeruginosa*	Padmavathi and Pandian 2014
Bacilus flexus	Lipopeptide	NS	Inhibit biofilm formation of *P. aeruginosa*	Padmavathi and Pandian 2014
Lactobacillus jensenii	NS	NS	Inhibit biofilm formation of *A. baumannii*, *E. coli*, *and S. aureus*	Sambanthamoorthy and others 2014
Lactobacillus rhamnosus	NS	NS	Inhibit biofilm formation of *A. baumannii*, *E. coli*, *and S. aureus*	Sambanthamoorthy and others 2014
Lysinibacillus fusiformis S9	Glycolipid	NS	Inhibit biofilm formation of *E. coli and Streptococcus mutants*	Pradhan and others 2014
Bacillus sp. strain SW9	Lipopeptide	NS	Inhibit biofilm formation in a wide range of bacteria	Wu and others 2013
Bacillus tequilensis	Lipopeptide	NS	Biofilm inhibition of *E. coli* and *Streptococcus mutans*	Pradhan and others 2013
Brevibacterium casei	Glycolipids	NS	Inhibit mixed pathogenic biofilm bacteria	Kiran and others 2010
Lactobacillus paracasei A20	NS	NS	Biofilm inhibition for a range of bacteria, yeasts, and filamentous fungi	Gudina and others 2010
P. aeruginosa	Glycolipid	Rhamnolipid	Inhibit biofilms in *S. aureus Candida tropicalis*	Rodrigue and others 2006
Bacillus subtilis	Lipopeptide	NS	Biofilm inhibition of *S. entrica* on urethral catheter	Mireles and others 2001
Pseudomonas putida	Lipopeptide	Putisolvin I and II	Biofilm inhibition of *Pseudomonas* spp	Kuiper and others 2004

NS = Not specified.

of human health. In the food industry, 2 bacteriocins (nisin and pediocin PA-1) are currently being used.

Due to their antibiofilm properties, bacteriocins have been well studied (García-Almendárez and others 2008; Simões and others 2010). Treatment with a bacteriocin is a promising method for the reduction of bacterial attachment and biofilm formation (Mahdavi and others 2007). The latter authors demonstrated that 4×10^3 IU/mL nisin is effective against biofilm of *S. Enteritidis* (87%), *L. monocytogenes* (57%), and *S. aureus* (30% reduction). Bacteriocin produced by *Lactobacillus sakei 1* can control *L. monocytogenes* biofilm formation (Winkelströter and others 2011). It was also reported that spray-dried crude bacteriocin fermentate (CBF) of *Lactococcus lactis UQ2*, or *Lactococcus lactis UQ2* reduced by greater than 5 log the number of planktonic and sessile cells of *L. monocytogenes* (García-Almendárez and others 2008). Synergistic effects of bacteriocin and other antibiofilm agents such as electrolyzed water (EW) were also investigated (Arevalos-Sa'nchez and others 2012). These researchers demonstrated that nisin, when combined with EW, had bactericidal properties at neutral pH and was effective at reducing a *L. monocytogenes* population in a biofilm on stainless steel and glass. Broad-spectrum bacteriocins could also be used in combination with a biocide for disinfection directed at both planktonic and biofilm organisms. Inactivation of sessile (24 h biofilms) *Staphylococci* increased remarkably when biocides were applied in combination with enterocin As-48 at a concentration of 50 mg/L, indicating that the selected combination of enterocin As-48 and biocides holds promise for use against planktonic and sessile *S. aureus* (Gómez and others 2013).

The behavior and modes of action of 3 bacteriocins with different structures (nisin A, lacticin Q, and nakacin ISK-1) were extensively studied in relation to MRSA planktonic and biofilm cells (Okuda and others 2013). Among the bacteriocins tested, nisin A showed the highest bactericidal activity planktonic cells and biofilm cells. Lacticin Q also showed bactericidal activity against both planktonic cells and biofilm cells but its activity against was significantly lower than that of nisin A. Their results suggest that bacteriocins that form stable pores on biofilm cells are highly effective for the treatment of biofilm infections. A purified recombinant ColA-43864 bacteriocin gene was highly effective at killing *E. coli, Citrobacter* species, and *K. pneumoniae* cells in a planktonic and biofilm state (Shanks and others 2012).

The effects of a subinhibitory concentration of bacteriocin on microbial adhesion and biofilm formation were evaluated recently (Pimentel-Filho and others 2014). These authors reported that a subinhibitory concentration of the bacteriocins bovicin HC5 and nisin reduces cell adhesion; they concluded that this is probably due to changes in the hydrophobicity of the bacterial cell as well as surfaces. Moreover, they found that expression of some important biofilm-associated genes (icaD, fnbA, clfB, and rnaIII) is also affected by bacteriocin treatment. Their result indicates that treatment of food contact surfaces with bacteriocin can be an innovative and powerful strategy for the prevention of biofilms in the food industry.

Despite powerful antimicrobial and antibiofilm properties, application of bacteriocins to the biomedical and food industries has been hampered by the slow development of a reliable bacteriocin delivery system (Yamakami and others 2013). However, a liposome-encapsulated delivery system of bacteriocin could overcome this challenge (Yamakami and others 2013). The latter investigators demonstrated that the concentration of liposome-encapsulated nisin required for the efficacious inhibition of glucan biofilm synthesis by *S. mutans* is 4-fold lower than that of naked nisin. Their findings suggest that the nisin–liposome complex may play a role in preventive medicine as an antibiofilm agent targeting glucan-biofilm synthesis. These studies also suggest that bacteriocins can be effective in the control of surface-attached (biofilm state) pathogenic bacteria.

Bioelectric Approach

An electric current has a tremendous potential for antimicrobial activity (a long-standing one), which was demonstrated as early as 1919 (Anderson and Finkelstein 1919). Various other studies thereafter have documented the lethal effects of an electric current and electrochemical potential to microorganisms (Rosenberg and others 1965; Spadaro and others 1974; Thibodeau and others 1978; Francolini and others 2004). An electric current has the capacity to lower the dose of an antibiotic which is required to inactivate biofilm organism (Costerton and others 1994; Jass and others 1995; Wellman and others 1996; Liu and others

1997; Stewart and others 1999). This effectiveness of antimicrobials by the electric current is now termed as the bioelectric effect. These bioelectric effects are caused by pH modifications, production, and transportation of antimicrobial agents into the biofilm via electrophoresis, production of biocide ions, and hyperoxygenation (Khoury and others 1992; Costerton and others 1994; Stewart and others 1999). This hyperoxygenation is caused mainly by the hydrolysis of water that produces oxygen, improves oxygen tension, and improves the MICs of some bacteria (Stoodley and others 1997; Anderl and others 2003; Borriello and others 2004). It was demonstrated that the presence of a strong electric field with a low current density increases industrial effectiveness of a biocide against *P. aeruginosa* (Arrizubieta and others 2004). It has also been shown that without antimicrobial combination, electric current has no potential antibiofilm activity (Blenkinsopp and others 1992; Costerton and others 1994; Jass and others 1995). However, recently, only electric current for long duration (1, 2, 4, and 7 d) (Del Pozo and others 2009b;c) resulted in up to a 6 log reduction in *S. epidermidis* biofilms and 5 log reduction in *S. aureus* biofilms after 2 d at 2000 μA of electrical treatment. Moreover, the biofilm reductions amounted to up to 5 log for a *P. aeruginosa* biofilm after 7 d.

It has been presumed that the mechanism of the electricidal effect may be due to the ability of the electric current to create hydrated ions, which transport water throughout surfaces producing a detachment force (Poortinga and others 2000). Another possible mode of action includes the effective disruption of the bacterial membrane and the charged EPS biofilm matrix (Blenkinsopp and others 1992; Costerton and others 1994; Jass and others 1995; Liu and others 1997) along with enhancement of the electrostatic repulsive forces between the bacteria and the colonized surface (Jucker and others 1996). Furthermore, Del Pozo and others (2009a) assessed the feasibility of the electricidal approach as a possible *in vitro* treatment. They acknowledged the enormous potential for their future research work regarding the treatment of human biofilm–related infections associated with orthopedic hardware. These authors also commented on limitations of the study in terms of treatment duration, current strength, and bacterial species and on the method of surgical insertion of insulated wire electrodes into the patient. The electrodes might provide extra surfaces for possible bacterial attachment; as a result, this innovative work may benefit patients by offering a minimally invasive approach requiring redesigned hardware, such as administration of bioelectric or electrical treatments to patients *in vivo.* Indeed, this is a breakthrough biofilm control strategy with implications in different fields except healthcare industries.

Further research should be conducted to develop cost-effective electrified devices that will inhibit initial surface colonization and to treat active biofilms.

Ultrasonic Treatment

Ultrasound is an oscillating sound in the form of waves with frequencies greater than 20 kHz, beyond the upper limit of the human hearing range. The frequency range of ultrasound devices varies from 1 kHz to several GHz. Higher frequency ultrasound waves (more than 1 MHz) are used for medical imaging and physical therapy; low-frequency ultrasound waves (less than 500 kHz) are not attenuated and produce heating. Ultrasound waves of low frequency can significantly enhance the bactericidal activity of an antibiotic in both planktonic and biofilm forms (Pickering and others 2003). Acoustic energy offers numerous key advantages over other methods in combating biofilms. The most important one is that this method can mechanically disrupt the existing biofilm encasing, and another advantage is that low-frequency ultrasound (70 kHz) with low acoustic intensity also increases the transport of oxygen and nutrients to the cells, thereby destroying pathogens in biofilms (Carmen and others 2004a).

Numerous goals were expected to be achieved with the application of acoustic energy to the prevention and control of biofilms, for example, abrogation of the 2 vital steps in biofilm formation, such as adhesion of planktonic microorganisms to surfaces and the ensuing strong attachment to substrates (An and others 2000), thus microstreaming a situation that ultimately disrupts autoinducer gradients and abolishes the signaling. Ultrasound is also presumed to improve oxygen and nutrient transport to cells within biofilms and to planktonic cells (Pitt and Ross 2003; Jayaraman and Wood 2008), facilitating production of stable cavitation in biofilms to ensure more effective penetration and transport of antimicrobials and sanitizers through their EPS encasing.

Ultrasonic energy that is used to remove microbial biofilms is subdivided into 2 categories with respect to the effects it has. One produces cavitation; these are the wave frequencies of $\geq$100 kHz generated at acoustic power intensity of 0.5×10^3 to 2×10^3 mW/cm^2. The other one does not cause cavitation due to the lower power intensity (Leighton 1997). The high acoustic power intensity levels are more appropriate for the dispersal of an existing biofilm rather than preventing biofilm formation. *In vitro* ultrasonication has been found to significantly increase transport of antibiotics (gentamycin) across biofilms, enhancing the destruction of *E. coli* and *P. aeruginosa* within the biofilm matrix (Carmen and others 2004b).

Removal of a biofilm was found to strongly rely on the intensity of acoustic energy and to a far lesser extent on the frequency (Pitt 2005). That author found that coupling the acoustic energy with a convective fluid flow sharply improved biofilm removal (at acoustic intensity of 27 W/cm^2), up to 80% of biomass removal in 2 min and close to 100% removal when intense ultrasonication was coupled with gas bubbles in the fluid. Augmenting antibiotic application with ultrasound is a promising combination approach to control biofilms. It has been reported that sonication of *E. coli* or *P. aeruginosa* biofilms with microbubbles enhances antibiotic efficacy (Rediske and others 2000). For instance, in an *in vivo* rabbit model, ultrasound-targeted (0.08 MHz) microbubble destruction of biofilm enhances the effects of vancomycin (He and others 2011).

Recently piezoelectric elements (fine-tune vibration energy devices) have been developed (Hazan and others 2006). Such studies demonstrated that this device can be attached to the outer surface of catheters that spread low acoustic waves (approximately 0.2 mW/cm^2) throughout the catheters and adjacent aqueous environment causing vibration of pathogen with same tune. The effect of vibrating the coat of catheters results in extreme inhibition of biofilm formation for a wide variety of microrganisms for a long period (more than 48 h). By applying this device, one of the most advanced steps toward clinical application of acoustic energy was demonstrated in a rabbit model (Hazan and others 2006), where catheters utilizing piezoelectric elements were noninfected, whereas all control showed urinary tract infections. This latest innovative acoustic method can be considered an effective feasible means to prevent biofilms on medical insertion materials.

Electrolyzed Water

For more than 100 y, the electrolyzed water technology was an important, versatile, and evolving method in commercial development. In the pursuit of prevention and control of biofilms, this technology is time-tested and clinically proven, thus deserving serious consideration. EW has enormous advantages over other conventional cleaning agents, such as effective disinfection, easy operation, relatively low cost, and environmental friendliness. The most attractive advantage of EW is its safety, and it is also not corrosive toward the skin, mucous membranes, or other organic materials. A combination of EW and other physical and chemical measures is also possible. Production of EW is very simple: it requires only water and salt (sodium chloride). Preparation of EW involves sequential reactions in a cell containing electrodes that are inert and positively and negatively charged, separated by a membrane through which highly diluted salt water passes. When electrodes are subjected to a direct current voltage current, 2 types of water with different properties are generated. The anode generates acidic or oxidizing water (pH < 2.5), and the cathode produces alkaline or reducing water.

As a disinfectant, electrolyzed oxidizing water (EOW) has been tested and used in the food industry and other fields. Electrolyzed acid solution water has a high oxidation potential and contains hypochlorous acid (HOCl) that possesses strong antimicrobial properties. Owing to these strong antimicrobial characteristics, during a cleaning procedure, EOW treatment could be an effective means of destroying of biofilm-forming bacteria on equipment surfaces (Kim and others 2001). The latter investigators found that a 300 s EOW treatment on stainless steel surfaces significantly reduced the population of biofilm-forming bacteria from 1.9×10^{10} colony forming units (CFU) per 82.5 cm^2 to below the detection level.

Alkali-based compounds are usually used to eradicate EPS produced by biofilm bacteria (Antoniou and Frank 2005). It was suggested that alkaline ion water (AW) has a strong ability to remove a remarkable amount of a biofilm by disintegrating glucans in the artificial biofilm of *S. mutans* (Gyo and others 2009). Even in the presence of organic matter, the sequential treatment with alkaline EW for 30 s followed by acidic EW resulted in a 4 to 5 log reduction of *L. monocytogenes* biofilms (Ayebah and others 2006). An electrolyzed basic solution has a strong reducing potential. Basic EW (BEW) has the ability to effectively remove an established *S. aureus* biofilm (Sun JL and others 2012). The application of 100 to 800 n-alkyl (50% Cl_4, 40% Cl_2, and 10% Cl_6) dimethyl dichlorobenzyl ammonium chloride (benzalkonium chloride [BAC]) reduced the number of biofilm-forming bacteria of the *Listeria* genus 100- to 1000-fold in 30 s (Frank and Koffi 1990).

Selected Other Promising Antibiofilm Agents

Other antibiofilm agents that can effectively disperse the biofilm architecture or inhibit biofilm formation have been the subject of immense interest.

Capsular polysaccharides

Capsular polysaccharides of *E. coli* CFTO73 exhibit antibiofilm properties (Valle and others 2006). These researchers demonstrated that the sterilized supernatant of this organism inhibits an *E. coli* (strain MG1655F) biofilm without affecting planktonic viability. Further studies revealed that K2 serotype group II capsule polysaccharide reduced the biofilm of MG1655F as well as other biofilm-forming bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, and *Enterococcus faecalis*). Next, the gene responsible for expression of the K2 serotype group II capsule polysaccharide (KPSD) was mutated and the researchers observed an enhanced biofilm phenotype. A similar study also revealed that *B. subtilis* biofilms lost their architecture after 6 d of incubation (Kolodkin-Gal and others 2010). The latter team demonstrated that an 8-d-old filter-sterilized supernatant is capable of preventing biofilms of *B. subtilis* (Kolodkin-Gal and others 2010). Further investigation confirmed that D-tyrosine, L-leucine, D-tryptophan, and D-methionine account for the prevention of immature biofilms and dissociation of existing biofilms. Furthermore, cultures of *S. aureus* and *P. aeruginosa* cannot form biofilms in the presence of D-tyrosine and the D-amino acid mixture (D-tyrosine, D-leucine, D-tryptophan, and D-methionine) (Kolodkin-Gal and others 2010). These findings suggest that incorporation of these secreted molecules in a targeted delivery system may play a promising role in preventing biofilm formation in food packaging and on artificial medical devices.

Molsidomine

The half-life of molsidomine is 1 to 2 h in plasma at pH 7.4 (Rosenkranz and others 1996). The molsidomine molecule has the ability to dislodge a biofilm. It is effective at dispersing biofilms formed by *S. enterica*, *S. Typhimurium* 14028, a cocktail of the 6 *Salmonella* outbreak strains, and *E. coli* O157:H7 (Marvasi and others 2014). The latter researchers found that molsidomine was especially effective toward polypropylene and most effective at 22 °C, inducing dispersal of about 50% of biofilms formed by *Salmonella* 14028 and the cocktail of 6 *Salmonella* outbreak strains after incubation for 6 h; up to 75% biofilm dispersal was observed after a 24 h treatment of *E. coli O157:H7* biofilms with molsidomine. These researchers also observed biofilm dispersal by molsidomine on polystyrene wells, which were dislodged after 24 h of contact time at room temperature. They reported that molsidomine can induce some dispersal even when biofilms are treated with the compound at 4 °C. The strongest dispersal was observed in response to incubation with 10 picomolar molsidomine. Due to its high potency at very low concentrations, this compound is a promising candidate for commercial use in biofilm dispersal.

Diethylamine nonoate diethylammonium

This is a class of molecules that easily dissociate in a pH-dependent manner with a half-life of 16 min at 22 to 25 °C, pH 7.4, and they have the ability to liberate 1.5 moles of NO per mole of the parent compound (Maragos and others 1991; Keefer and others 1996). After a 6 h treatment, diethylamine nonoate diethylammonium was the most potent dispersing agent for biofilms on polypropylene. Effective removal of biofilms formed by *Salmonella* ATCC 14028 on polypropylene was also observed. Similar results were observed with biofilms formed by *E. coli* O157:H7. Dispersal of a biofilm formed on polystyrene by the cocktail of 6 salmonella outbreak strains was also noted.

A catheter lock solution

The effectiveness of a catheter lock solution (CLS) against planktonic and biofilm-associated cells was evaluated by Steczko and others (2009). They observed that the novel CLS has synergistic effects against *S. aureus*, *E. coli*, and *P. aeruginosa*. In addition, this solution effectively inactivated *E. coli* and *P. aeruginosa* planktonic cells in 0.5 and 2 h, respectively. Moreover, the CLS destroyed biofilms in an hour. CLS treatment has interesting future implications for both biofilm prevention and treatment. However, these

authors acknowledged that the CLS may be ineffective against resistant bacterial strains.

Chitosan, terpinen, and povidone iodine (PVP)

The effectiveness of chitosan against 3 (upper, middle, and lower) biofilm layers was evaluated within a mature biofilm structure (Del Paz and others 2011). High-molecular-weight chitosan caused biofilm reduction of 21.4%, 7.5%, and 1.2% in the upper, middle, and lower layers of the biofilm, respectively. On the other hand, low-molecular-weight chitosan reduced mature biofilms by 93.6% to 96.7% in each biofilm layer. Moreover, the efficacy of chitosan against established biofilms of *Bacillus cereus*, *L. monocytogenes*, *P. fluorescens*, *S. aureus*, and *Salmonella enterica* was also investigated (Orgaz and others 2011). In the presence of 1%, 1.1%, and 1.01% chitosan, the *Listeria* biofilm matrix was reduced by >6, 4, and 2.5 log units, respectively. In case of *P. fluorescens* at the same amount (1%, 1.1%, and 1.01%) of chitosan exhibited 5, 1.5, and 1 log unit reductions, respectively. For *Salmonella* and *Bacillus* species with 1% chitosan, a > 3 log unit reduction was not observed. Chitosan exhibited the lowest antibiofilm effectiveness (1 to 2 log unit reduction) against *S. aureus*.

The antibiofilm effectiveness of terpinen-4-01-loaded lipid nanoparticles against *C. albicans* biofilms was studied by Sun LM and others (2012). At 10 μg/mL, this compound removed preformed biofilms.

The antibiofilm effectiveness of povidone iodine (PVP-1) was assessed recently (Hosaka and others 2012) against both *Porphyromonas gingivalis* and *Fusobacterium nucleatum* biofilms. PVP-1 at the concentration of 7% for 5 min causes a 6 log unit reduction of *P. gingivalis* (72-h-formed biofilm) viable counts. PVP-1 at concentrations 2% to 5% reduces biofilms by 2 log units. *F. nucleatum* biofilms are effectively reduced (by greater than 4 log) after 30 s of treatment with 5% PVP-1 (Hosaka and others 2012).

Gallium

Gallium is a chemical substance that is found in nature as the gallium (III) salt. Because of the chemical similarity of Galium to iron, it can substitute for iron in many biological systems and inhibit Fe-dependent cellular processes. A "Trojan" horse strategy was explored by using the transition metal gallium to disrupt *P. aeruginosa* iron metabolism and to exploit the iron stress in *in vivo* environments (Kaneko and others 2007). These authors demonstrated that gallium can inhibit *P. aeruginosa* growth and biofilm formation (Kaneko and others 2007). Iron salts such as ferric ammonium citrate were utilized to treat established *P. aeruginosa* biofilms, and the researchers observed that iron salts not only inhibited biofilm formation but also disrupted existing biofilms (Musk and others 2005). FDA has approved gallium nitrate for clinical use in the treatment of hypercalcemia associated with tumor metastasis to the bone (Warrella and others 1984); this method has been reported to interfere with biofilm formation (Rhoads and others 2008). More recently, gallium was evaluated as an antibiofilm agent against biofilms consisting of the major cystic fibrosis pathogen, *P. aeruginosa* (Kaneko and others 2007). Several patents were filed regarding the use of gallium against oral biofilms (Park and others 2010). More research is necessary on the pharmacokinetics of gallium (Bernstein and others 2000).

Lactoferrin

Lactoferrin is a protein with chelating properties that is found in most body fluids but is concentrated in milk (Valenti and others 2004). Lactoferrin has been found to be effective at killing both planktonic and biofilm bacteria. Lactoferrin has been studied *in vitro* regarding the prevention of *P. aeruginosa* biofilm formation by inhibiting bacterial adhesion to the surface (Singh and others 2002). Iron (III)-binding properties of lactoferrin have a bacteriostatic effect on bacteria by depriving the cells of this essential micronutrient (Weinberg 2001). Lactoferrin has also been reported to act on polysaccharide components of the outer membrane of Gram-negative bacteria causing membrane permeabilization and death. Owing to its diverse nature, lactoferrin is a broad-spectrum agent and has been studied *in vitro* regarding the control of biofilms consisting of periodontal pathogens (Wakabayashi and others 2009), cystic fibrosis-associated pathogens (O'May and others 2009), and atopic skin-associated pathogens (Leitch and Willcox 1999). Lactoferrin has been used effectively in the clinic as part of a comprehensive regimen for the management of biofilm-associated chronic rhinosinusitis (Psaltis and others 2008) and biofilm-associated ischemic wounds (Wolcott and Rhoads 2008).

Xylitol

Xylitol is an alcoholic sugar found in a limited number of fruits and vegetables (Granstrom and others 2007). Most of the studies concerning the antimicrobial properties of xylitol have focused on oral biofilms (Burt 2006). For instance, xylitol prevented biofilm growth in a 6-species oral biofilm model (Badet and others 2008). Xylitol was recently reported to be moderately active against wound-colonizing *P. aeruginosa* biofilms *in vitro* (Amons and others 2009).

Dispersin B

Dispersin B is a naturally occurring N-acetylglucosaminidase synthesized by the periodontal bacteria *Aggregatibacter actinomycetemcomitans* (Ramasubbu and others 2005). It has been proven to inhibit biofilm formation (Chaignon and others 2007; Izano and others 2007a). Dispersin B dissociated a bacterial biofilm by targeting the EPS and degrading the biofilm community structure. In particular, it hydrolyzed glycosidic bonds in the polysaccharide of the EPS thereby destroying the biofilm framework (Kaplan and Fine 2002; Kaplan and others 2003a;b, 2004; Izano and others 2007b).

Future Research Prospects

The development of potential antibiofilm strategies is of immense interest and now constitutes an important field of investigation. The molecular mechanisms underlying microbial biofilm formation and behavior have only begun to be understood. We urge researchers to focus further studies on all alternative approaches to inhibit and disperse biofilms. These emerging novel antibiofilm strategies are still in the nascent phase of development, and more research is urgently needed to validate these approaches, which may eventually lead to effective prevention and control of biofilms.

Conclusion

Indeed, biofilms are the dominant lifestyle of microorganisms in all environments, either natural or manmade, and remain a serious concern in the healthcare, food, and marine industries. The development of effective strategies to combat biofilms is a challenging task. Numerous innovative antibiofilm approaches have been published, but it is difficult to reliably compare all these strategies. Some of the emerging novel approaches, such as natural substances, bacteriophages, quorum quenching, nanotechnology,

bacteriocin, BSs, and various enzymes, are promising and may help to find antibiofilm strategies that are superior to the conventional ones. Moreover, a combination of these novel techniques with conventional methods (antibiotics, disinfectants, and physical methods) is expected to solve the "biofilm problem" in the near future.

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