Chapter 15
Approaches Towards Microbial Biofilm Disruption by Natural Bioactive Agents

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Abstract Biofilms formed by microbes are the aggregates of bacterial masses that are fixed in the matrix produced by itself comprising of extracellular polymeric substances (EPS). Microbial biofilms pose serious threat to the hospital-based infections as well as other types of infections. This is because biofilm provides highly enhanced protection and tolerance to the pathogens towards antimicrobial compounds. Moreover, the pathogen also survives the immune response of the host. This leads to extremely intractable, prolonged infections resulting in high tolls of morbidity and mortality. The fact that around 80% of human diseases are biofilm-based; the scientists have started to explore effective remedies to precisely aim at the disruption of biofilm, thus, diffusing the cells of microbes into their more susceptible planktonic type of life. With the advent of the significance of biofilm disruption to combat serious infections, various antibiofilm agents have been investigated for their efficacy. This includes some primary metabolites including complex carbohydrates, peptides and fats and various categories of secondary metabolites. Many enzymatic biofilm dispersal agents have also attracted the attention of those working in the given area. Other dispersal compounds include anti-matrix molecules, dispersal signals and sequestration molecules. These antibiofilm agents have shown high effectiveness in inhibiting clinically relevant pathogens. These biofilm dispersal agents will pave a way for a new approach towards future drug development for the treatment of clinically severe infections.

Keywords Biofilm · Pathogens · Quorum sensing · Dispersal agent · Enzymatic · Natural compounds

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15.1 Introduction

Microbial biofilms are the masses of cells which are fixed in a matrix produced by itself in extracellular polymeric substances (EPS). These biofilms pose serious damage to human health by causing various lethal infections which are strong and show resistance to antibiotics and immune system of host. This makes the treatments of pathogens challenging and expensive.

In the environment under natural conditions, microorganisms frequently grow on various biotic and abiotic planes in the form of multi-cellular populations which are recognized as biofilms. Microbes can form biofilms by their capability of attachment to household items like showers, taps and water tanks besides living in the form of biofilms in nature (Mullis and Falkinham 2013; Rozej et al. 2014; Xu et al. 2014). *Pseudomonas aeruginosa* is the most common organism which forms biofilm in the household system (Mullis and Falkinham 2013). Bacterial biofilm populations can comprise of multiple cell layers and also in the form of mushroom-like assemblies as in case of *Staphylococcus epidermidis* and various *Pseudomonas* species. The morphology of biofilms vary from dry, flat and wrinkled colonies on agar plates as in case of *Bacillus subtilis* and *Vibrio cholerae* (Bester et al. 2010; Lopez et al. 2010; Seper et al. 2014) to small yellow air balloons as in *Myxococcus xanthus* (Dubey 2003; Jiyoung et al. 2009). *Anoxybacillus flavithermus* is capable of forming biofilms in silica and is a danger in processing of food (Saw et al. 2008). But the common character of all microbial biofilms is a matrix surrounding the cells, a slimy layer on the surface of each bacterial cell that provides defence to the cells and provides food and water to bacteria (Bester et al. 2010; Lopez et al. 2010). The matrix comprises of complex polysaccharides, proteins, phospholipides and extracellular DNA (Christner et al. 2010, 2012; Linnes et al. 2013; Reichhardt et al. 2014; Becker et al. 2014). Occasionally, the matrix appears in different colours (pink, brown or blackish) so as to prevent DNA impairment by solar radiations (Xu et al. 2014). Nutrients and other materials can be pooled amongst the microbial cells by passive diffusion through the porous matrix. In this manner, they work as cell communication machineries, and this phenomenon is known as quorum sensing (Banat et al. 2014). The most significant consequence of the matrix is the defence against external stimuli and mechanical destruction. Moreover, the matrix also guards the cells from chemical compounds for instance antibiotics, antimicrobial agents and disinfectants. Additionally, the biofilm also safeguards bacterial cells against shearing forces, physical and chemical forces and inadequate availability of nutrient (Taylor et al. 2014; Banat et al. 2014). The cells present in the matrix share mutual benefit from one another and help in each other's progression and survival (Xu et al. 2014; Bester et al. 2010).

15.2 Development of Biofilms as a Threat to Human Health

In the early 1940s, antibiotics have been introduced to human medicine and now emerged as a major threat to public health at an alarming rate. This problem is amplified by pathogenic bacteria existing most commonly in biofilm form, creating additional bacterial tolerance to antimicrobial agents and has been considered as primary cause of chronic infection, transforming bacteria into antibiotic-resistant form in biofilm formulation (Bowler 2018). Biofilm-associated bacteria are 1000 times more resistant to antibiotics than their planktonic counterparts and are often insensitive towards host immune system (Olson et al. 2002). The resistant mechanism attributed by biofilm bacteria due to resistant capsules, enzyme-mediated resistance, heterogeneity in metabolism and growth rate, metabolic state of the organisms in the biofilm, genetic adaptation and most important effective quorum sensing and other membrane modification (Singh et al. 2017).

The mono-species of bacteria which have the capacity to synthesize a biofilm in clinical surroundings are accountable for biofilm-related diseases, in both animals and humans (Dubey 2003). These are most frequently seen not only close to oral areas like dental caries and periodontitis, or in infections of respiratory tract in cystic fibrosis patients (Lambiase et al. 2009; Hall-Stoodley et al. 2004), but also found on the exteriors of implanted medical devices. Amongst clinical pathogens, the most dangerous are gram-positive, coagulase-negative staphylococci (e.g. *S. epidermidis*) and coagulase-positive *S. aureus* that make biofilms on abiotic surfaces (Rohde et al. 2006; Mack et al. 2006; Moretro et al. 2003; Rupp and Archer 1994; Mack et al. 1992). Microbes have enhanced pathogenicity in the form of biofilm and frequently cause infections on artificial biomedical parts such as surgical pins or hip joints (McCann et al. 2008). Infections can occur in cardiac pacemakers with a trailing endocarditis and intravenous catheters because of extracellular matrix of human and coating of the implant by serum being rich in nutrient and provide environment for the growth (Hall-Stoodley et al. 2004; McCann et al. 2008). This is the reason that the disease and death of hospital acquired, nosocomial infections are increasing each year (Rohde et al. 2006; Rupp and Archer 1994). Majority of the cells associated with material-associated infections are due to *S. epidermidis* cells, as they are skin inhabitant where wounds and implants are easily accessible (Gotz 2002). Another deleterious effect of biofilm synthesis on abiotic planes is the infection of edible items during production of food and its processing. Meat and milk can be contaminated through multi-resistant staphylococcal species on coming in contact with the biofilm-coated surface (Moretro et al. 2003; Mettler and Carpentier 1998).

The approaches for the inhibition and elimination of these biofilms are very restricted. This is because of the increasing antibiotic resistance of microbial species, but also because of the increased resistance of biofilm-based structure of microbes against antimicrobial agents and disinfectants (Mack et al. 2006; Gotz 2002; Ganeshnarayan et al. 2008). The extracellular polymeric compounds of the matrix are responsible for providing the protection to the microbes against external forces which comprise of polysaccharides, proteins, lipids, nucleic acids and humic acids. These matrix

components also result in the characteristic mushroom-like biofilm assembly (Flemming and Wingender 2010). Considering the complicated structural organization and regulatory network resulting in the formation of biofilm, the understanding of the complexity of the matrix in detail and also the innovative strategies are essential to disperse established microbial biofilms and prevent their occurrence on abiotic surfaces to improve the management of medical patient and food safety. Up to now, novel targets for the screening of antimicrobial agents and vaccine development for the prevention of biofilms are promising (Gotz 2004). The present chapter reviews the latest developments in the area of biofilm disruption taking into consideration various agents which have shown potential towards this.

Biofilms can be developed on almost every moist surface which is most often unwanted as they cause serious complications in various sectors, including the food division. They are recognized as the preferential microbial lifestyle due to the numerous advantages offered by them for the embedded cells. Biofilm cells show strong resistance to stress conditions, mainly to antimicrobials, since their multifarious and compact structure hinders the permeation of antimicrobials and the contact with the cells present deep in the biofilm. The increased resistance to the presently employed control strategies accentuates the urgent requirement of new alternative and/or complementary eradication approaches. To this direction, the use of enzymes is an interesting alternative antibiofilm approach due to their capability to degrade crucial components of the biofilm matrix, cause cell lysis, promote biofilm disruption and interrupt the cell-to-cell signalling events monitoring biofilm formation and maintenance. This review provides an overview of the enzymes used for biofilm control, their targets and examples of effective applications (Meireles et al. 2016).

15.3 Natural Antibiofilm Agents

15.3.1 Fatty Acids as Antibiofilm Agent

15.3.1.1 Myriads Role of Fatty Acid Inhibitor (FAI) in Biofilm Formulation

The mode of growth of microorganisms could be manipulated either by preventing foaming of biofilms or by disrupting the existing ones. In this context, extensive literature are available that have detailed signal (extracellular) accountable for biofilm scattering coupled with an array of factors that have been shown to arouse biofilm disturbance. For example, addition of chemicals, rapid reduction in oxygen and increased concentration of organic carbon resulted in cell cluster disaggregation in *P. aeruginosa* (Chen and Stewart 2000). In this section, emphasis will be primarily upon the different fatty acids as inhibitor molecules to augment the vulnerability of biofilm cells by weakening the normal biological processes that maintain biofilm integrity,

and also critically discussed process in controlling bacterial virulence, alteration in cellular phenotype and promote biofilm tolerance (Table 15.1).

15.3.1.2 FAI Interactions with Quorum Sensing (QS)

Owing to its importance in nutrition, fatty acids (FA) are representative of quorum sensing chemicals capable of modulating virulence-associated behaviour of bacterial population. Bacterial pathogen component actively up-regulated the expression of QS system to induce virulence factors that promote biofilm formation and infectious diseases. Recently, it was documented that chains of monounsaturated fatty acids such as palmitoleic acid and myristoleic acids significantly diminished biofilm synthesis of *Acinetobacter baumannii* with drastically reduced motility (Nicol et al. 2018). This might be due to fatty acids involvement in down-regulation of LuxIR-type quorum sensing (QS) communication system, thus consequently reduced the *N*-acyl-homoserine lactone production (AHL). Another medium-chain fatty acid derivative, *cis*-2 decenoic acid (C2DA), showed lethality towards multiple bacterial strains, including gram-positive, gram-negative and yeast strains by inducing dispersion of biofilms, although this biofilm inhibition was only demonstrated in *P. aeruginosa* (Stoodley et al. 2011). Furthermore, combination of antibiotic (linezolid) with C2DA resulted in 16% inhibition of biofilm either individually or in combination with daptomycin and vancomycin. The easy incorporation of linezolid and C2DA into the plasma membrane of bacterial cells and also due to cis-conformation increased membrane permeability are the fundamental mechanisms for enhancement of antibiofilm activity of these compounds (Jennings et al. 2012). Global gene expression analysis lyngbyoic acid (LA)-treated *P. aeruginosa* revealed that LA down-regulates gene controlled by quorum sensing (Kwan et al. 2011). Another study conducted by Zhen Cai et al. clearly revealed that fatty acid diffusible signal factor (DSF) binds and allosterically activates histidine kinase RpfC of phytopathogenic bacterium *Xanthomonas campestris* to regulate quorum sensing and virulence. The role of fatty acid or its derivative is directly or indirectly involved in inhibitor of biofilm formulation as well as virulence, but précised mode is doubtful in various studies hence extensive effort is required for better understanding.

15.3.1.3 Fatty Acid as a Signalling Molecule

In pathogenicity research, fatty acid molecules have already been identified as signalling as well as inducer molecules, ranging from yeast to gram-positive and gram-negative bacteria which regulate a wide range of cellular functions (Marques et al. 2014). In addition to this, signals generated by fatty acid are engrossed in intra-species, inter-species and cross-kingdom communication. These signals are known to regulate motility, virulence, polymer production, biofilm development, biofilm dispersion, bacterial growth and persistence. The different fatty acids as signalling molecules in various organisms have been summarized in Table 15.1. More than 50

Table 15.1 List of fatty acid signalling molecules reported in various microorganisms with their known functions

S. no	Compound name	Bacterial species	Function	References
1	cis-11-methyl-2-dodecenoic acid (DSF)	*Xanthomonas campestris, Xanthomonas oryzae, Stenotrophomonas maltophilia, Burkholderia multivorans*	Virulence, biofilm formation, floc Disaggregation, microcolony formation, tolerance to antibiotics, detoxi fication, hyphal growth inhibition	Wang et al. (2004), Deng et al. (2010), Tang et al. (1991), Barber et al. (1997), He et al. (2010), Huang and Wong (2007)
2	cis-2-dodecenoic acid (BDSF)	*Burkholderia cenocepacia, Burkholderia lata Burkholderia stabilis Burkholderia vietnamiensis Burkholderia dolorosa Burkholderia ambifaria Burkholderia anthina Burkholderia pyrrocinia B. multivorans, X. oryzae*	Virulence, hyphal growth inhibition	Deng et al. (2010), He et al. (2010), Boon et al. (2008)
3	cis-2-decenoic acid (cis-DA)	*Pseudomonas aeruginosa*	Biofilm formation, biofilm dispersion, persister cell formation, persister cell awakening, tolerance to antimicrobials	Davies and Marques (2009), Marques et al. (2014), Sepehr et al. (2014), Jennings et al. (2012), Rahmani-Badi et al. (2014)
4	cis-2-tetradecenoic acid	*Xylella fastidiosa*	Virulence and aggregation	Beaulieu et al. (2013)
5	trans-2-decenoic acid (SDSF)	*Streptococcus mutans*	Hyphal growth inhibition	Vílchez et al. (2010)
6	cis-11-methyldodeca-2,5-dienoic acid (CDSF)	*B. multivorans, B. stabilis B. anthina, B. pyrrocinia, X. oryzae*	Hyphal growth inhibition	Deng et al. (2010), He et al. (2010)

(continued)

Table 15.1 (continued)

S. no	Compound name	Bacterial species	Function	References
7	12-methyltetradecanoic acid	*Xylella fastidiosa*	Virulence, biofilm formation, motility	Colnaghi Simionato et al. (2007), Chatterjee et al. (2008)
8	3-hydroxypalmitic acid	*Ralstonia solanacearum*	Virulence	Flavier et al. (1997)
9	3-(3-hydroxy alkanoyloxy) alkanoic acids (HAAs), and phospholipids (e.g. phos-phatidylethanolamine)	*P. aeruginosa*	Biosurfactant	Desai and Banat (1997), Lang and Wullbrandt (1999)

molecules including autoinducer-1 (AI-1) also known as *N*-acylhomoserine lactones (*N*-AHL), autoinducer-2 (AI-2) a furanosyl borate, PQS, oligopeptides (5–10 amino acid cyclic thiolactone) known as autoinducer peptides (AIP) and short-chain fatty acids which are typically unsaturated at the number 2 carbon in a *cis* configuration have been identified till date (Parsek and Greenberg 2005; Ryan and Dow 2011; Kalia et al. 2015). Signals of fatty acids are known to increase aggregative behaviour and biofilm formation capability. For example, isolation from *Xylella fastidiosa,* i.e. 12-Me-C14, has been reported to eradicate swarming motility and to subside biofilm formation by capability of *X. fastidiosa* and in *P. aeruginosa*. Apart from bacteria, fungi like *C. albicans* are also documented for the induction of fatty acid signals which reported the production of farnesoic acid that inhibits the formation of germ tube by regulating the morphological transition from a yeast-form to a hyphal-form (Estrela and Abraham 2010).

Biofilm dispersion autoinducers have been reported the experience of involvement of the fatty acid. Like in *P. aeruginosa* commonly known fatty acid signal, *cis*-2-decenoic acid (C10:Δ2) is identified and has been established as a biofilm dispersion auto inducer (Davies and Marques 2009). Moreover, *Propionibacterium acnes*, *Actimomyces naeslundii*, *Lactobacillus casei* and *Streptococcus mutans* when cultured as single or mixed species are seen to be susceptive towards *cis*-DA which induces biofilm dispersion in them. However, an entire comprehensive signalling mechanism of the *cis*-DA system is yet to be elucidated.

15.3.1.4 Fatty Acid Regulation in Biofilm Dispersion

Studies in the past decades related to the effect of fatty acids and its derivatives on the biofilm resulted in concluding on the intracellular mechanisms involved in some species of bacteria. *P. aeruginosa* produces C*is*-2-decenoic acid in batch cultures, and biofilm cultures induce a dispersion response in biofilms formed by a range

of gram-negative and gram-positive bacteria and yeast, as well as in *P. aeruginosa* (Huang and Wong 2007; Chatterjee et al. 2008). It is also well cited that DSF has also played roles for the regulation of pathogenicity in *X. campestris* (Barber et al. 1997) together with sysnthesis of extracellular proteases and exopolysaccharide production, flagellum synthesis, aggregative behaviour, biofilm formation and resistance to toxins. Along with *cis*-2-decenoic acid and DSF, small chain-monounsaturated fatty acids and BDSF have activity across a wide array of bacteria as extracellular signals. If microorganisms depend on the degradation of extracellular polymers produced by neighbouring microorganisms of other species as well as their own species will relieve the cells from the biofilm matrix during a dispersion response. Cross-kingdom activity has been proposed previously for fatty acid messengers from evidence that DSF is recognized by *C. albicans* binding to the receptor of farnesoic acid, leading to an arrest in filamentation. The application of a dispersion inducer mainly fatty acid prior to, or in combination with, treatment by antimicrobial agents provides a novel mechanism for enhancing the activity of these treatments through the disruption of existing biofilms; in this context, broad-spectrum activity of *cis*-2-decenoic acid suggests that this and other short-chain *cis*-2-monounsaturated fatty acids likely have deep evolutionary roots. It is interesting that fatty acid communication has been found to be present in many plant and animal species, and the connection to cell dispersion in these systems may be an interesting area for future investigation.

15.3.2 Enzymes as Antibiofilm Agent

15.3.2.1 Amylases and Cellulases

Amylase and cellulase enzyme complex was produced from *Penicillium janthinellum*, a mutant EU2D-21 under submerged fermentation. Good specific enzyme activities were found after eight days of incubation at 30 °C. This enzyme complex was evaluated for its capability to target and disrupt the biofilms of different bacteria. It was seen that it disrupted biofilms of *Escherichia coli* (85.5%), *Salmonella enterica* (79.72%), *P. aeruginosa* (88.76%) and *Staphyloccus aureus* (87.42%) within 1 h of incubation at 50 °C. The exopolysaccharide matrix of the biofilm and bacteria from the cell surface were detached by the enzyme complex as shown by the scanning electron microscopy (SEM), quantitative analysis of biofilm removal assay and crystal violet assay (Nagraj and Gokhale 2018).

15.3.2.2 Proteases

The cell surface proteins in *Staphylococcus aureus* strains promote the development of biofilm. Proteinase-mediated biofilm dispersion was investigated in the present study in different isolates of *S. aureus*. It was shown by microtitre plate-based biofilm assay demonstrated that Proteinase K (2 μg/mL) markedly checked the development

of biofilm in *bap*-positive *S. aureus* as well as other *S. aureus* strains but not in weak biofilm-producing strains, that is, *bap*-mutant M556 and SA392. However, there was no effect of Proteinase K treatment on the planktonic growth of *S. aureus*. It was indicated by the results of the study that Bap might also play role in eDNA retention in the matrix of biofilm that supports biofilm stability. A synergistic response in antibiotic efficiency was seen against all biofilm forming *S. aureus* strains when a combination of Proteinase K was applied in combination with antibiotics (Mukherji et al. 2015; Shukla and Rao 2017).

The treatment of biofilms with broadly specific proteases, such as Proteinase K and trypsin results into biofilm disassembly (Boles and Horswill 2008; Mootz et al. 2013). The serine proteases Proteinase K (from *Tritirachium album*) and trypsin have often been utilized as efficient biofilm disruption agents that hamper bacterial adherence and biofilm formation in *S. aureus* (Gilan and Sivan 2013) presumably through degradation of surface structures (Boles and Horswill 2008; Gilan and Sivan 2013; Loughran et al. 2014). Shukla and Rao (2013) also reported that biofilms formed by *S. aureus* with the help of Bap proteins were vulnerable to Proteinase K-mediated detachment and dispersion. Biofilm assays done in 96-well-plates showed that Proteinase K obstructed both biofilm adherence and progression in Bap expressing *S. aureus* cultures.

A number of proteases have been recognized as an antibiofilm disrupting agents, with varying degrees of success (Loughran et al. 2014; Craik et al. 2011). Serine proteases, in particular, have been effective at disrupting the matrix. This is not entirely surprising, as serine proteases have been produced number of biofilm-forming microbes which likely aid in active dispersal and biofilm structural arrangement (Loughran et al. 2014; Marti et al. 2010; Chen et al. 2013).

15.3.2.3 Hydrolase

Glycoside hydrolases were evaluated for potential therapeutic effect on *P. aeruginosa,* and it was showed that glycoside hydrolases specifically target and degrade the exopolysaccharide constituent of the biofilm matrix. Bacterial biofilms present a significant clinical challenge because they are recalcitrant to existing therapeutic regimes. The main part of biofilm production in the opportunistic human pathogen *P. aeruginosa* is the biosynthesis of the exopolysaccharides Pel and Psl, which are responsible for the formation and maintenance of the structural biofilm scaffold and defence against antimicrobials and host defenses. Knowing that the glycoside hydrolases PelAh and PslGh encoded in the Pel and Psl biosynthetic operons, respectively, are utilized for in vivo exopolysaccharide processing, it was anticipated that these would provide specificity to target *P. aeruginosa* biofilms. Evaluating these enzymes as potential therapeutics, it was demonstrated that these glycoside hydrolases selectively target and degrade the exopolysaccharide component of the biofilm matrix. PelAh and PslGh restrict biofilm synthesis over a 24-h period with a half-maximal effective concentration (EC50) of 69.3 ± 1.2 and 4.1 ± 1.1 nM, respectively, and are capable of disrupting pre-existing biofilms in 1 h with EC50 of 35.7 ± 1.1 and

12.9 ± 1.1 nM, respectively. This treatment was effective against clinical and environmental isolates of *P. aeruginosa* and reduced biofilm biomass by 58–94%. These noncytotoxic enzymes potentiated antibiotics because the addition of either enzyme to a sublethal concentration of colistin reduced viable bacterial counts by 2.5 orders of magnitude when used either prophylactically or on established 24-h biofilms. In addition, PelAh was able to enhance neutrophil killing by ~50%. This work illustrates the feasibility and benefits of using bacterial exopolysaccharide biosynthetic glycoside hydrolases to develop novel antibiofilm therapeutics (Baker et al. 2016).

Aspergillus fumigatus and *P. aeruginosa* produce galactosaminogalactan and Pel, respectively, which are cationic heteropolysaccharides. These exopolysaccharides both contain 1,4-linked N-acetyl-D-galactosamine and play a vital function in biofilm formation by these microorganisms. Proteins comprising glycoside hydrolase domains have been identified recently as a part of the anabolic pathway of each exopolysaccharide. Recombinant hydrolase domains from these proteins degrade their respective polysaccharides under in vitro conditions. These glycoside hydrolases were shown to exhibit antibiofilm activity against varied microorganisms and may be useful as novel therapeutic agents for the degradation of biofilms and reducing virulence (Snarr et al. 2017).

In another study, a Psl-specific glycoside hydrolase (PslG) was covalently bound to numerous, chemically different planes using amine functionalization (APTMS) and glutaraldehyde (GDA) linking. Since bacterial colonization and biofilm synthesis on surfaces are typically facilitated by the accumulation of exopolysaccharides and conditioning protein layers. *P. aeruginosa* is a nosocomial opportunistic pathogen that employs strain-specific exopolysaccharides such as Psl, Pel or alginate for both initial surface attachment and biofilm formation. To generate surfaces that resist *P. aeruginosa* colonization, in situ quartz crystal microbalance (QCM) experiments and fluorescence microscopy confirmed a complete lack of Psl adsorption on the PslG-bound surfaces. Covalently bound PslG was also seen to markedly reduce *P. aeruginosa* surface adherence and biofilm formation over-extended growth periods (8 days). The PslG surfaces showed a ∼99.9% (∼3 − log) reduction in surface-associated bacteria compared to control surfaces or those treated with inactive enzyme. This work showed a non-eluting 'bioactive' surface that specifically targets a mechanism of cell adhesion, and that surface-bound glycoside hydrolase can significantly reduce surface colonization of bacteria through local, continuous enzymatic degradation of exopolysaccharide (Psl). These results have significant implications for the surface design of medical devices to keep bacteria in a planktonic state, and therefore, susceptible to antibiotics and antimicrobials (Asker et al. 2018).

Poly-β(1,6)-*N*-acetyl-D-glucosamine (PNAG) is a main constituent of biofilm of many pathogenic bacteria. The synthesis, modification and export of PNAG in *E. coli* and *Bordetella* species need the protein products encoded by the *pgaABCD* operon. PgaB is a two-domain periplasmic protein that contains an N-terminal deacetylase domain and a C-terminal PNAG-binding domain that is crucial for export. In the current study, it was shown that the C-terminal domains of *Bordetella bronchiseptica* PgaB (PgaB) and *E. coli* PgaB (PgaB) work as glycoside hydrolases. These enzymes

hydrolyze purified deacetylated PNAG (dPNAG) from *S. aureus*, degrade PNAG-dependent biofilms formed by *Bordetella pertussis*, *Staphylococcus carnosus*, *S. epidermidis* and *E. coli*, and potentiate bacterial killing by gentamicin. Furthermore, it was seen that PgaB was only able to hydrolyze PNAG produced in situ by the *E. coli* PgaCD synthase complex when an active deacetylase domain was present. Mass spectrometry analysis of the PgaB-hydrolyzed dPNAG substrate showed a GlcN-GlcNAc-GlcNAc motif at the new reducing end of detected fragments. This work magnifies the role of PgaB within the PNAG biosynthesis apparatus, defines a new glycoside hydrolase family GH153 and identifies PgaB as a possible therapeutic agent for treating PNAG-dependent biofilm infections. The work provides further insight into the mechanism of periplasmic PNAG modification, and suggests PgaB could be utilized as a therapeutic agent to eliminate biofilms (Little et al. 2018).

15.3.2.4 Lactonase

The tenacity of bacterial infection is often linked to quorum sensing-mediated biofilm synthesis. Thus, the interruption of this signalling circuit presents an attractive anti-virulence strategy. Quorum sensing is an important aspect of biofilm formation. Quorum-quenching lactonases have been shown to be effective in disrupting of quorum sensing circuits. The present study pronounces a method to degrade biofilm in a clinically significant *A. baumannii* S1 strain by the application of an engineered quorum-quenching lactonase. This treatment by engineered lactonase attained significant reduction in *A. baumannii* S1 biofilm (Tay et al. 2016).

15.3.2.5 Dispersin B

An artificial gene encoding dispersin B of *Aggregatibacter actinomycetemcomitans* was cloned and expressed in *E. coli* cells. Procedure for purification of recombinant dispersin B was established, and its in vitro activity was determined. The enzyme was used in experiments on disruption of the biofilms formed by various microorganisms. It exhibited high activity against *S. epidermidis* biofilms. The biofilms formed by *Burkholderia cenocepacia* and *Achromobacter xylosoxidans* were more resistant to the recombinant enzyme (Dobrynina et al. 2015).

15.3.2.6 Papain

Considering the proteolytic nature of papain and the biopolymer matrix structure of bacterial biofilms, the present study was aimed to evaluate the ability of papain to act as an inhibitor of biofilms in different concentrations. The effect of different concentrations of papain in biofilm production by several methicillin-resistant *S. epidermidis* (MRSE) and methicillin-resistant *Staphylococcus haemolyticus* (MRSHa)

isolates was explored. When papain was mixed into the culture, the biofilm formation by MRSE was restricted. However, the enzymatic action of papain exhibited more efficiency for MRSHa isolates. The experiment suggested that papain is able to affect the ability of cells to form biofilm, thus affecting the bacterial attachment (de Oliveira et al. 2014).

15.3.2.7 DNAses

DNases have been applied for degradation of mucous in cystic fibrosis patients, demonstrating its viable potential for human use as a therapy (Shak 1995; Sawicki et al. 2015; Shak et al. 1990). In vivo studies utilizing DNase have shown that degradation of eDNA can disrupt biofilms present in a mammalian host (Hymes et al. 2013; Conover et al. 2011).

15.3.2.8 Polysaccharide Depolymerase

In the current work, bacterial exopolysaccharide was degraded by a heat-stable polysaccharide depolymerase which was prepared from the phage infecting *Klebsiella*. Treatment at 75 °C for 10 min led to the complete inactivate of phage. However, there was no loss of phage enzyme activity after the treatment. The colony counting showed the phage enzyme could rapidly decrease the number of biofilm-associated bacteria. The rate of inhibition reached the maximum (80%) after 4 h of treatment. Enzyme pre-treatment could also enhance the fumigation effect of chlorine dioxide. Approximately, 92% of the BF bacteria were eliminated after treatment with the phage enzyme followed by 30 min of treatment with chlorine dioxide. According to the results of colonies counting and scanning electron microscopy, the phage enzyme could effectively decrease the bacterial adherence as well as the adhesion of extracellular polymeric substances in the BF. This study has demonstrated that the phage-borne polysaccharide depolymerase enzyme is valuable for eradicating the bacterial BF (Chai et al. 2014).

15.3.3 Inhibitors of Quorum Sensing

15.3.3.1 Implication of Quorum Sensing in Biofilm Development

The interactions and communication amongst bacteria done collaboratively for biofilm formation are known as quorum sensing (QS). This mechanism was elucidated initially by Professor Greenberg where he proposed different ways to intervene with pathogenic microflora and to moderate microbiome for better health approaches (Fuqua et al. 1994). Many species of bacteria are inhibitors of biofilm. This communication system includes induction of a specific set of bacterial genes that hold

potential of response to expanded volume of cell population (Platt and Fuqua 2010). Today, targeting the inter-bacterial interactions has become the foremost in healthcare researches, especially with multi-drug resistance amongst the pathogens (Haque et al. 2018; Golberg et al. 2013; Saurav et al. 2016).

Recently, the use of natural products to interfere with pathogenic bacterial quorum-sensing systems (Jamal et al. 2018; Hirakawa and Tomita 2013; Brackman and Coenye 2015; Rémy et al. 2018) has been proposed for development of new antimicrobial agents. Since this strategy does not require bacterial killing, it is proposed to reduce the development of resistant strains (Tang and Zhang 2014).

Autoinducers like acyl homoserine lactones (AHL) are synthesized and secreted by the phenomenon of quorum sensing (Newton and Fray 2004). Gram-positive bacteria secrete quorum-sensing peptides as a regulatory system consisting of two components, a membrane-bound histidine kinase receptor and an intracellular response regulator (Platt and Fuqua 2010). Besides these, gram-negative as well as gram-positive bacteria can use a common entity, borate furanosyl, an autoinducer-2 (IA-2) and (IA-3). The mechanism of quorum sensing is also reported for the control of growth of biofilm (Abee et al. 2011).

15.3.3.2 Bacterial QS Inhibitors

Quorum sensing affects the structure of biofilm, and lack of quorum sensing is related with development of thin biofilm as well as increased susceptibility to antibiotics (Shih and Huang 2002).

In aeromonads, the QS regulates biofilm formation, motility, multicellular synchronized social life and virulence (Talagrand-Reboul et al. 2017).

Numerous bacteria produce certain substances (Table 15.2) on their surface that prevents biofilm formation by others, such as **extracellular polysaccharides**, which are crucial in formation of biofilm in bacteria. They also prevent biofilm formation in adjacent cells (Rendueles et al. 2013).

Actinobacillus pleuropneumoniae serotype 5 which exhibits antibiofilm activity has extracellular polysaccharide which prevents intra- and inter-cellular communications amongst microbes, thereby exhibiting an example of natural anti-biolfilm phenomena (Karwacki et al. 2013).

Another study of Schertzer et al. exhibited similar activity of glycolipid and glycoprotein structure and QS signals to sense and stop external positive-charged compounds (Schertzer et al. 2009).

Similarly, the extracts of coral-associated bacteria have been shown to induce a decrement in *S. aureus* and *Serratia marcescens* biofilm development. Ethyl acetate extracts of ***Bacillus firmus***—a coral-associated bacterium–show antibiofilm activity against multi-drug resistant (MDR) *S. aureus* (Gowrishankar et al. 2012). A new natural compound, **4-phenylbutanoic acid**, extracted from *Bacillus pumilus,* demonstrated antagonistic activity against biofilms (Nithya et al. 2011).

Table 15.2 Bacterial antibiofilm agents

S. no.	Bacteria inhibiting biofilm formation in other bacteria	Bacteria inhibited	Mechanism of biofilm inhibition (antibiofilm agents)	Reference
1	*Actinobacillus pleuropneumoniae* serotype 5 EPS	Broad-spectrum, non-biocidal antibiofilm activity	Extracellular polysaccharides (EPS)	Karwacki et al. (2013)
2	*Bacillus pumilus* (marine bacteria)	Antagonistic activity against biofilms from a broad range of bacteria	4-phenylbutanoic acid	Nithya et al. (2011)
3	Ethyl acetate extract of *Bacillus firmus* (coral-associated bacteria)	Antibiofilm activity against MDR *S. aureus*		Gowrishankar et al. (2012)
4	*Streptococcus salivarius*, a harmless, non-biofilm inhabitant of human mouth	Inhibit dental biofilms (plaque) formation	Uses two enzymes—fructo-syltransferase (FTF) and exo-β-D-fructosidase (FruA), to inhibit EPS production and dental plaque formation	Ogawa et al. (2011)

Another example is seen with ***Streptococcus salivarius***, that exhibit antibiofilm activity by use of two enzymes, viz. fructosyltransferase (FTF) and exo-β-D-fructosidase (FruA), that decrease EPS production (Ogawa et al. 2011). However, excess production of FruA may have an involvement in development of sucrose-dependent biofilm in the oral cavity.

15.3.4 Inhibition of QS by Phytochemicals

Phytochemicals from fruits and vegetables hold the potential to hinder quorum sensing in human pathogens (Vattem et al. 2007). This is appealing, since the regular intake of such anti-QS associated food may be of therapeutic value in preventing the invasion of intestinal pathogens.

15.3.4.1 Polyphenols or Phenolic Compounds

Range from simple molecules to polymerized tannins, and have one aromatic ring with six carbons. These secondary metabolites affect biofilm development in microbes (Huber et al. 2003; Sarabhai et al. 2013).

15.3.4.2 Phenolic Acids

Are organic compounds which have one carboxylic functional group and phenolic hydroxyl group. In plants, the acid phenols are mostly in the form of esters of aliphatic alcohol or of quininic acid, glycosides or rosmarinic acid. Hydroxylation of the phenolic group has exhibited direct effect upon their antimicrobial activity. The mechanism for this phenolic damage involves inhibition of enzymes by oxidized compounds (Mason and Wasserman 1987).

15.3.4.3 Flavonoids

Belong to the polyphenol family. They have a structure with 15 carbon atoms, constituting two aromatic units, two C_6 rings joined by a C_3 chain (Heim et al. 2002); flavonoids are the compounds which give colours to flowers, fruits as well as leaves.

Flavanones, found enormously in citrus spps, show interference with quorum sensing and also affect other physiological process (Truchado et al. 2012). **Naringenin, quercetin** and **apigenin** are inhibitors of HAI-1 or Al-2-mediated bioluminescence production in *Vibrio harveyi*. Naringenin and **taxifolin** decrease the synthesis of pyocyanin and elastase in *P. aeruginosa* where growth of bacteria was unaffected. Naringenin and taxifolin also hindered the quorum sensing by affecting the gene expression in *P. aeruginosa* PAO1. Naringenin holds the potential of hindering the synthesis of quorum-sensing mediators such as N-(3-oxododecanoyl), lactone-1-homoserine (3-oxo-C12-HSL), acylhomoserine lactone and N-butanoyl-1-homoserine lactone (C4-HSL) (Vandeputte et al. 2011).

15.3.4.4 Quercetin, Sinensetin, Apigenin

Also inhibit the development of biofilms in *V. harveyi* BB120 as well as *E. coli* O157:H7 (Truchado et al. 2012; Vikram et al. 2010). In *P. aeruginosa* PAO1, flavonoids such as the catechin decrease virulence factors (pyocyanin and elastase) influenced by quorum signalling, leading to inhibition of biofilm development.

The HLA molecules are deteriorated by legume products including alfalfa, clover, lotus, peas and yam beans (Delalande et al. 2005; Gotz et al. 2007).

15.3.4.5 Terpenes

Are natural compounds having hydrocarbons of either cyclic or open-chain skeleton. The basic molecule is isoprene, i.e. C_5H_8. The term terpenoid refers to terpene skeleton substances with one or more functional groups (alcohol, aldehyde, ketone, acid, lactone, etc.). The terpenoid classification is based on the number of repetitions of the isoprene base unit: hemiterpens (C_{30}), tetratepens (C_{40}) and polyterpens.

Essential oils are plant extracts that mainly contain mono- and sesqui-terpenes. Essential oil from medicinal plants such as *Citrus reticulate* (Luciardi et al. 2016), *Eucalyptus radiate*, *Eucalyptus globulus* (Luis et al. 2016) and *Thymus vulgare* (Myszka et al. 2016) have shown anti-QS effects.

In addition, compounds such as vanillin in *Vanilla planifolia* (Ponnusamy et al. 2009), eriodictyol in *Eriodictyon californicum* (Vandeputte et al. 2011), methyl eugenol in *Cuminum cyminum* (Packiavathy et al. 2012), erucin in *Brassica oleracea* (Ganin et al. 2013), ajoene in *Allium sativum* (Jakobsen et al. 2012a) and naringin, naringenin, kaempferol, quercetin, rutin, neoeriocitrin in *Citrus sinensis* (Vikram et al. 2011) were found to cease quorum sensing.

15.3.5 Medicinal Plants as QS Inhibitors

Different parts and organs of various plants have been reported to possess medicinal values. Examples include *Glycyrrhiza glabra* (Bhargava et al. 2015), *Psoralea corylifolia* (Husain et al. 2018), *Piper bredemeyeri* (Olivero et al. 2011), *Bauhinia acuruana*, *Pityrocarpa moniliformis*, *Commiphora leptophloeos* (Trentina et al. 2011), *Cocos nucifera* (Viju et al. 2013) and *Terminalia catappa* (Taganna et al. 2011).

Rubus rosaefolius (Oliveira et al. 2016), *Centella asiatica* (Vasavi et al. 2016), *Areca catechu* (Koh and Tham 2011) and *Sclerocarya birrea* (Sarkar et al. 2014) have also been reported to have an inhibitory effect against QS signalling.

15.3.6 Plants as a Source of Anti-QS Drugs: Mechanism of Action

Anti-quorum sensing role of **essential oils** and their major constituents: amongst the essential oils, lavender, clove and rosemary have shown anti-QS activity. Recently, Luciardi et al. revealed essential oil from *Citrus reticulata* to possess antibiofilm and anti-quorum sensing activity against *P. aeruginosa*. At 0.1 mg/mL, this compound showed 41% decrease in biofilm cell viability and a 33% decrement in AHL production (Luciardi et al. 2016).

Thymus vulgare and its derivatives carvacrol and thymol exhibited antibiofilm and anti-quorum sensing activity against *Pseudomonas fluorescens* KM121, thus

revealing a tremendous decrease in AHL production and biofilms (Myszka et al. 2016).

Organic compounds extracted from the medicinal plants also act as inhibitory agents of quorum sensing (Thakur et al. 2016). **Organic compounds** from the medicinal plants (Table 15.3) have same chemical skeleton to those of quorum sensing signals or possess the potential to deteriote signalling receptor (LuxR/LasR) (Vattem et al. 2007; Al-Hussaini and Mahasneh 2009).

- Indeed, GABA (gaminobutyric acid), synthesised by some plants, promotes signal degradation by OHC8HSL HLA lactonase (ATTM) in *Agrobacterium tumefaciens*, thus hindering the process of quorum sensing-dependent infection (Chevrot et al. 2006).
- *Medicago truncatula* modulating AhyR, CVIR and LuxR activities in various microorganisms (Gao et al. 2003) and quorum sensing in *P. aeruginosa* and *Sinorhizobium meliloti* (Mathesius et al. 2003).
- *Curcuma longa* produces curcumin which hinders the expression of the virulence genes of *P. aeruginosa* PA01.
- Polyphenols like hydroxycinnamic acids, rutin and epicatechin found in the extracts of apples and its derivatives have shown a significant role in anti-quorum sensing activity; they also show synergistic activity against *Chromobacterium violaceum* (Fratianni et al. 2011, 2012).

The anti-quorum sensing activity has also been exhibited by various other medicinal plants like *Laurus nobilis*, *Rosmarinus officinalis* and *Pityriasis alba*. These plants had the potential to decrease the synthesis of violacein (Vattem et al. 2007; Al-Hussaini and Mahasneh 2009).

The **hydroalcoholic extracts** of *Berberi saristata* and *Camellia sinensis* exhibited anti-quorum sensing activity against *E. coli* (Thakur et al. 2016). In addition, the in silico studies affirm the anti-quorum activity of the phytomolecules present in these extracts (flavonoids, alkaloids and tannins) through the antagonistic activity of LuxS.

The extract fractionation permits screening of chemical molecules and moieties for a significant outcome. Vasavi et al. showed that the flavonoids-rich ethyl acetate fraction of *C. asiatica* decreased pyocyanin synthesis and biofilms development by inhibition of elastolitic and proteolytic activity in these bacteria (Vasavi et al. 2016).

The ethanol and ethyl acetate extracts from *Hypericum connatum* have been shown to possess an anti-quorum sensing effect against *C. violaceum*, which hinders in the synthesis of violacein (Fratianni et al. 2013).

Gallic acid containing polyphenolics such as epigallocatechin gallate, ellagic acid and tannic acid from medicinal plants, hold the potential of interfering and inhibiting the interaction amongst bacterial cells via AHL mediated signalling (Sarabhai et al. 2013). Similarly, grenades and berries are rich in ellagitannins such as punicalagin and ellagic acid (Larrosa et al. 2010). In the intestinal flora, the conversion of ellagitanins to ellagic acid is done by the micro-gut flora and then metabolized to form urolithin A and urolithin B. The resulting metabolites get easily assimilated in the human intestine, playing their vital roles. In recent studies, it has also been revealed

Table 15.3 Anti-quorum sensing activity of some medicinal plants extracts and essential oils

Plant species	Products	Major compound	Strains tested	Effects	Reference
Thymus vulgare	Essential oils	Carvacrol thymol	*Pseudomonas fluorescens* KM121	Significant diminution of AHL synthesis in 72 h. Its action suppresses bacterial motility and lowers the mRNA flagella gene expression	Myszka et al. (2016)
Piper bredemeyeri	Essential oil	ND	*Chromobacterium violaceum*	It inhibits violacein production	Olivero et al. (2011)
Citrus reticulate	Essential oil	Limonene	*Pseudomonas aeruginosa*	At 0.1 mg/ml concentration, inhibits biofilm cell viability (41%) and AHL production	Luciardi et al. (2016)
Eucalyptus radiate	Essential oil	Limonene; a-terpineol; a-terpinyl acetate; a-pinene	*Acinetobacter baumannii*	Inhibits quorum sensing-regulated bacterial pigment violacein	Luis et al. (2016)

(continued)

Table 15.3 (continued)

Plant species	Products	Major compound	Strains tested	Effects	Reference
Centella asiatica	Flavonoid-rich fraction	*Pseudomonas aeruginosa* PAO1 and *Chromobacterium violaceum* ATCC12472	ND	Hinders synthesis of violacein in *C. violaceum* at 400 mg/mL. Inhibits quorum sensing-regulated phenotypes such as pyocyanin production, elastolytic and proteolytic activities, swarming motility and biofilms formation in PAO1	Vasavi et al. (2016)
Cocos nucifera	Extracts from fibres	ND	*Pseudomonas sp., Alteromonas sp.,* and *Gallionella* sp.	Inhibits biofilm formation and EPS production	Viju et al. (2013)
Eucalyptus globules	Essential oils	1,8-cineole (eucalyptol); a-pinene; Aromadendrene; p-cymene	*Acinetobacter baumannii*	Inhibits QS-regulated bacterial pigment violacein	Myszka et al. (2016)
Terminalia chebula	Fruit extract	Ellagic acid (benzoic acid)	*Burkholderia cepacia*	Inhibits biofilm formation	Huber et al. (2003)
Rubus rosaefolius	Phenolic extracts	ND	*Chromobacterium violaceum, Aeromona hydrophila* and *Serratia marcescens*	Inhibits violacein production, swarming motility and biofilms formation	Oliveira et al. (2016)
Glycyrrhiza glabra	Methanol extract	Flavonoids	*Acinetobacter baumanni*	Decreases biofilm formation and motility. Reduction of virulence factors promoting QS	Bhargava et al. (2015)

that urolithin A and B hinder quorum-sensing activity and also lower the levels of AHL produced by entherocolitica entheropathogen.

15.3.7 Anti-QS Action of Isolated Molecules from Medicinal Plants

With the application of bio-guided fractional approach, numerous secondary metabolites extracted from medicinal value plants have been obtained which exhibit anti-quorum sensing activities. For example, furanones have been exhibited to inhibit AHL processes (Manefield et al. 2002). Numerous plant secondary metabolites imitate AHL of bacteria, hence influencing quorum sensing and biofilm development in bacteria (Teplitski et al. 2000).

Cinnamaldehyde (major constituent in essential oils) and their derivatives have been estimated to hinder quorum sensing and biofilm development (Brackman et al. 2008; Niu et al. 2006). Cinnamaldehyde has been shown to inhibit bioluminescence in *V. harveyi* BB170 and eugenol in *P. aeruginosa* and *C. violaceum* via inhibition of virulence factor production such as violacein, elastase, pyocyanin and biofilm (Zhou et al. 2013).

Iso-limonic acid and ichangin (molecules contained in bigaradier seed extracts) include **limonoids** that inhibit the growth of *V. harveyi* at a very low concentration and these mechanisms are related to the inhibition of HAI- and AI-2-mediated bioluminescence (Vikram et al. 2011).

Curcumin, obtained from *C. longa*, has anti-quorum sensing activity. This molecule can attenuate the QS dependent factors such as EPS production of several pathogenic strains such as *E. coli*, *P. aeruginosa*, *Proteus mirabilis* and *S. marcescens*. In addition, curcumin has been reported to reduce a few phenotypes concerned with QS inhibition including swimming, swarming and motility. Curcumin also inhibits biofilms and pyoacin formation in *P. aeruginosa* via modulation at gene expression related to QS signalling pathways (Rudrappa and Bais 2008).

Usnic acid, a lichen metabolite, has anatagonistic activity against biofilm of fungus and bacteria. QS inhibitors can increase the susceptibility of biofilms to antibiotics and do not pose threat to humans (Sun et al. 2013).

Garlic inhibits quorum sensing at the gene expression level. Ajoene is obtained after crushing the garlic, and it has sulphur. Ajoene also shows antagonistic activity against synthesis of rhamnolipid, which protects biofilms from white blood cells. The combination of ajoene and the antibiotic tobramycin has been shown to kill ~90% of biofilm bacteria. Garlic possesses anti-viral, anti-fungal and anti-protozoal properties and is useful for the cardiovascular and immune systems (Jakobsen et al. 2012b). The only drawback with these sulphur-containing compounds is that their activity is lost as soon as they get in contact with oxygen.

Hamamelitannin extracts from the willow bark, also hinders quorum sensing (Morgan 2015).

Bacterial extracts with anti-quorum sensing activity are also analyzed using a mass spectrometry-based metabolomics Global Natural Products Social Molecular Networking platform (GNPS; https://gnps.ucsd.edu/) for compound dereplication (Allard et al. 2016). In addition, an integrated biological, genomic and metabolomic approach using 16SrRNA sequences is being lately employed to discover anti-quorum sensing molecules from bacterial strains (Ong et al. 2019).

15.3.8 Conclusion

Biofilm-associated bacteria are less sensitive to antibiotics than free-living (planktonic) cells. Furthermore, with variations in the concentration of antibiotics throughout a biofilm, microbial cells are often exposed to levels below inhibitory concentrations and may develop resistance. This, as well as the irresponsible use of antibiotics, leads to the selection of pathogens that are difficult to eradicate. Biofilms comprising of multicellular, surface-adherent communities help the microorganisms to survive in various stress conditions which include antibiotics, lack of nutrient, heat shock and immune responses. In view of the increment in the numbers of old patients who require artificial medical devices such as knees and hips, there will be an extended need for novel agents and strategies to treat biofilm-related infections. Some strategies have been described recently which appears to play an important role in future antibiofilm therapies. Both established and novel experimental treatments targeted at various stages of wound healing that are specifically aimed at reducing and eliminating biofilm bacteria. Importantly, the highly tolerant nature of these bacterial communities suggests that most singular approaches could be circumvented and a multifaceted, combinatorial approach will be the most effective strategy for treating these complicated infections. The usage of enzyme complex as antibiofilm therapeutics to eradicate biofilms is feasible and beneficial. This can also be used as a promising strategy to improve treatment of multidrug-resistant bacterial infections. The biofilm-forming microorganisms pose great threat to frequent infections in immune-compromised patients and is problematic to eliminate from medical devices. There are several classes of antibiofilm agents which need proper investigation in terms of their efficiency in inhibiting the microbial growth, understanding of the complex matrix organization in biofilms to deduce the mechanism of their action. The potential biofilm inhibiting compounds include natural bioactive compounds, enzymes that disturb the biofilm structure and other nonenzymatic molecules (Fig. 15.1).

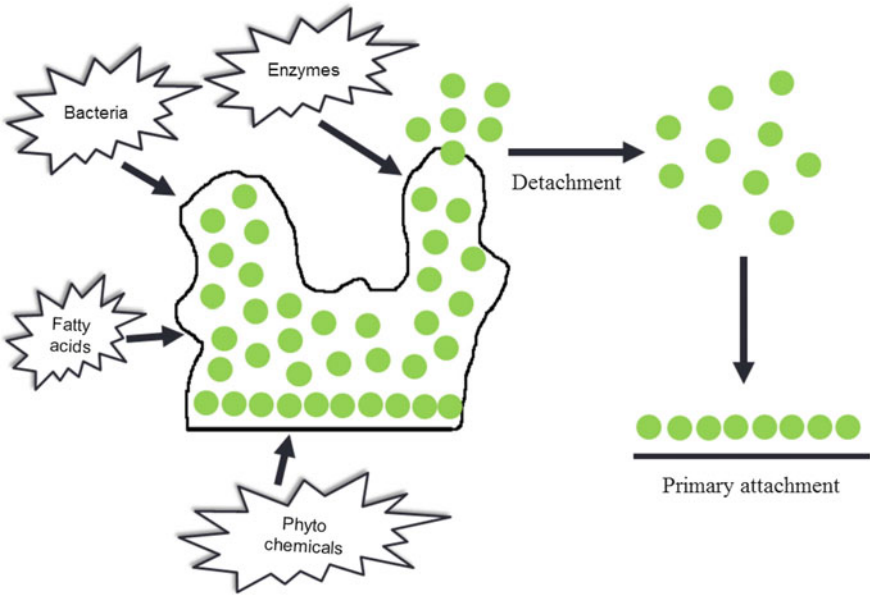

Fig. 15.1 Disruption of microbial biofilms by various antibiofilm agents

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