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MINI-REVIEW

# Regulation and controlling the motility properties of *Pseudomonas aeruginosa*

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## Abstract

Chronic infections caused by *Pseudomonas aeruginosa* have been a major concern as their spread and mortality continue to be on the rise. These infections are majorly attributed to biofilm formation via sequential steps where motility plays an essential role in initial attachment of bacterial cells onto biotic and abiotic surfaces, thereby contributing to multi-drug resistance among pathogens. Therefore, attenuating motility properties can be considered as highly potential for controlling *P. aeruginosa* biofilm formation. This strategy has employed the use of various natural and chemically synthesized compounds. The present review article explained the importance and regulation of different types of motilities properties. Furthermore, it also covered several important alternative approaches using anti-motility agents which could be helpful for controlling *P. aeruginosa* biofilm-associated infections. Further studies are required for in-depth understandings about the mechanisms of motilities controlling of these molecules at molecular levels.

**Keywords** Biofilm · Motility · Attenuation · Antibiofilm drugs · *Pseudomonas aeruginosa*

## Introduction

*Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen which has been known as a common cause of human infections ranging from acute pneumonia in immunocompromised patients to chronic bronchiectasis and ciliary dyskinesia in cystic fibrosis patients (Stover et al. 2000). Such variations in infectious levels are given by the bacterial existence in two different lifestyles—planktonic and surface-associating (sessile) (Furukawa et al. 2006). The acute infections which occur in the host within hours or days are caused by planktonic population, whereas the chronic infections which prolong for months or years are caused by sessile population (Turner et al. 2014). Such long-term infectious effect of sessile population is primarily due to the bacterial formation of biofilm upon attaching on a surface which is either biotic (e.g., damaged tissues and wounds) or abiotic (e.g., medical devices and processing equipment) (Maurice et al. 2018; Mulcahy et al. 2014). Biofilm formation is in fact one of the consequential resistant mechanisms intrinsically emerged by *P. aeruginosa* due to the overuse and misuse of conventional antibiotics as treatments against this bacterium (Hoiby et al. 2010; Mah and O'Toole 2001). Due to the extreme complications in physiology, behaviors, and metabolism, the biofilm population requires either potentiation of conventional antibiotics or innovative strategies which employ non-antibiotic novel antimicrobial agents (in individual form or in combination) and new different targets (e.g., biofilm extracellular matrix, biofilm cell-to-cell communication, or different virulence properties produced simultaneously with biofilm formation) (Roy et al. 2018; Valentini et al. 2018; Wu et al. 2015a).

Studies have shown that planktonic and sessile lifestyles of *P. aeruginosa* can be flexibly switched in response to the environment where the bacteria colonize, thereby forming a survival cycle in which planktonic state is exhibited at surface sensing, translocation, initially reversible attachment to the surface, and dispersal stages, whereas sessile state is exhibited

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at irreversible surface attachment, biofilm formation, and biofilm maturation stages (Ha and O'Toole 2015). This switch from planktonic to sessile lifestyles and *vice-versa* is the consequence of complicated signaling networks mainly driven by (1) the Gac/Rsm cascade, (2) the bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) global second messenger, and (3) the near-surface motility modes performed by flagella and type IV pili (T4P) (Gellatly and Hancock 2013). The binding of small RNAs (sRNAs) with their repressors in the Gac/Rsm cascade would modulate the level of c-di-GMP, which at high level would up-regulate the biofilm components such as Pel, Psl, and alginate exopolysaccharides and down-regulate the assembly and function of flagellar and T4P (Valentini and Filloux 2016). The flagella and T4P themselves also have a machinery which operates during planktonic lifestyle in accordance with environmental conditions; particularly, flagella perform swimming in liquid media, T4P perform twitching on solid media, and both flagella and T4P cooperatively perform swarming on semi-solid media (Maier and Wong 2015; Nirody et al. 2017). Studies have confirmed that the sequential cooperation between these two appendages launches proper attachment of micro-colonies onto the surface, thereby activating biofilm formation as well as numerous different pathways which contribute to bacterial pathogenesis and virulence (Conrad et al. 2011).

For the reasons mentioned above, the near-surface movements of *P. aeruginosa* (e.g., swimming, swarming, and twitching) have been regarded as a virulence property, along with formation of biofilm and synthesis of numerous other virulence compounds (Deziel et al. 2003). In the current situation when virulence properties have become a preferable target in order to reduce selective pressure for the bacterial resistant strains, extensive research for an effective anti-motility agent have been conducted among the naturally derived compounds, the chemically synthesized compounds and their combinations (Masak et al. 2014). Due to the complications in motility regulation and their interactions with other physiochemical and functional properties, the inhibition activities of most compounds were found varied towards swimming, swarming, and twitching modes (de la Fuente-Núñez et al. 2012). Furthermore, the molecular studies of these activities were mostly not yet reported. However, these findings have introduced the possibility of *P. aeruginosa* surface-associated motilities as a new target for the future of controlling *P. aeruginosa* biofilm formation as well as its related infections.

## The importance and their regulation of different *P. aeruginosa* motilities

*P. aeruginosa* population living in planktonic state causes acute infections to their host through rapid release of toxins and effectors, while the sessile state causes chronic infections through production of biofilm as well as numerous different extracellular virulence factors (Gellatly and Hancock 2013). Furthermore, the presence of biofilm structure also provides the enclosed bacterial community with a thousand-time protection from antibiotics pressure (Kostakioti et al. 2013). Opting for such significant adaptation and survival over a long-term, the bacterium has developed the flexibility in switching between the planktonic and surface-associated lifestyles and utilized the surface motilities (e.g., swimming, swarming and twitching) for their presence in different environmental reservoirs, resistance against the extreme conditions, and neutralization the immunological responses of the host (D'Argenio and Miller 2004; Kolter and Greenberg 2006).

*P. aeruginosa* pathogenesis firstly requires the search for an infection site using swimming motion augmented by a single polar flagellum (Conrad et al. 2011). Under hydrodynamic force and chemotaxis navigation, swimming enables the bacteria to sense and directionally translocate in low-agar environment (0.3–0.4% agar) despite the surface repulsion to reach and attach reversibly to the desirable surface (Yeung et al. 2009). A transition from reversible to irreversible attachment is then proceeded with swarming and twitching motions. Swarming is performed in more viscous or semi-solid environment (0.5–0.7% agar), thus in comparison to swimming, swarming requires multiple flagella, T4P, rhamnolipid biosurfactants, specific bacterial cell density, and nutrient availability (e.g., iron, copper, nitrogen and carbon sources) (Deziel et al. 2003; Patriquin et al. 2008; Shrout et al. 2006). Importantly, swarming was confirmed as the major motility performed during the early stage of biofilm establishment (Shrout et al. 2006). Overall, swimming and swarming motilities reflect the multi-roles of flagella in surface sensing, surface attachment, biofilm formation, effectors production, and defense against host immune response. On the other hand, in the absence of flagella or on the solid media (1% agar) where swimming and swarming are unfavorable, twitching is performed instead (Burrows 2012). This motility is based on the extension and retraction of a pili group known as T4P, which is a polar filament with approximately 6 nm in diameter (Petrov et al. 2013). T4P consists of a large number of major pilins (PilA) and a small number of minor pilins and non-pilin protein (PilY1) (Marko et al. 2018). The contact with a solid surface induces a signal which is then transduced from the contact point between the major pilin (PilA) of T4P and the PilJ methyl-accepting chemotaxis protein to the chemosensory signal transduction system ChpA (Jansari et al. 2016). On one hand, ChpA induces CyaB (an adenylate cyclase) and cyclic-AMP (c-AMP) production, resulting in (1) virulence factor regulator (Vfr) expression which would activate the virulence factors production by quorum sensing (QS) type II and III secretion system, and (2) pilY1 expression

which would activate SadC (a diguanylate cyclase) to produce c-di-GMP (Fulcher et al. 2010; Luo et al. 2015). The c-di-GMP not only regulates the T4P-mediated motility, and it is also involved in controlling the swarming and swimming types of motility either directly or individually by other signaling pathways. Apart from controlling these types of motility, c-di-GMP exhibits pleotropic functions, where it regulates several other functional properties such as virulence properties, cell cycle, and biofilm formation in *P. aeruginosa* (Lin Chua et al. 2017; Valentini and Filloux 2016). Hence, c-di-GMP plays an important role in the transition from the planktonic to biofilm stage of the *P. aeruginosa.* In addition, surface contact could also induce stress to bacterial periplasm and cell wall; thus, a recovery pathway carried out by sigma factor is activated, which negatively regulates the cAMP-Vfr pathway by producing exopolysaccharide (alginate) (Boucher et al. 2000).

Overall, in *P. aeruginosa*, it can be seen that swimming, swarming, and twitching are essentially involved in (1) sensing or searching for a desirable surface, (2) translocating the bacterial cells towards the surface, (3) allowing the bacterial cell-surface contact, (4) transitioning from planktonic into sessile lifestyle, and (5) attaching properly to the surface or transitioning from reversible to irreversible attachment so that biofilm formation can take place. Furthermore, throughout the stages of biofilm formation and development, swimming, swarming, and twitching continue to interact as well as regulate numerous determinants of the bacterial pathogenesis, thus having been categorized as virulence properties of the bacteria (Glessner et al. 1999). Playing such an important role in bacterial physiology and phenotypes, swimming, swarming, and twitching are regulated by the level of c-di-GMP global second messenger. Although in *P. aeruginosa*, numerous pairs of diguanylate cyclases (DGCs) and c-di-GMP phosphodiesterases (PDEs) are involved in synthesizing and degrading c-di-GMPs, respectively, four main regulatory pathways have been previously reported, one involves two components Gac and Rsm, one involves the Chp chemotaxis system (described above), one is designated as Wsp chemosensory pathway, and the remaining is designated as HptB pathway, which all intersect with each other to determine the lifestyle of *P. aeruginosa* (Francis et al. 2017; Hickman and Harwood 2008; Römling et al. 2013). In the Gac/Rsm regulatory system, the level of c-di-GMP is determined by a sRNA repressor named RsmA, which upon binding with two sRNAs (RsmY/RsmZ) would no longer suppress the diguanylate cyclase SadC to synthesize c-di-GMP. As a result, the c-di-GMP level is elevated, which favors the biofilm-forming lifestyle. In contrast, if RsmA is free from binding with RsmY/RsmZ, RsmA would suppress c-di-GMP synthesis of SadC, causing the bacteria to exist in planktonic lifestyle (Chang 2017). The c-di-GMP increases due to SadC activation that is also carried out by ChpA regulatory system which has been described in detail earlier (Fulcher et al. 2010; Luo et al. 2015). In the Wsp chemosensory pathway, the level of c-di-GMP is mediated by a pair of activator and anti-activator of flagellar gene expression known as FleQ and FleN, respectively. The binding of c-di-GMP to FleQ prevents its binding the pelA promoter, which terminates the extracellular polymeric substances (EPS)-encoded gene transcription, hence suppressing the biosynthesis of EPS (Hickman and Harwood 2008). The remaining c-di-GMP regulatory system is known as HptB pathway. Basically, this pathway employs a diguanylate cyclase named as HsbD to monitor the synthesis of c-di-GMP, flagella, and pili and influence chemotaxis, thereby regulating the bacterial swimming, swarming, and twitching motilities (Valentini and Filloux 2016). c-di-GMP is also involved in regulating the assembly and functions of appendages performing swimming, swarming, and twitching, which are flagella and T4P. Particularly, c-di-GMP is produced for flagellar assembly and then is decreased by its binding to FleQ and YcgR regulators for surface attachment (Ha and O'Toole 2015; Wolfe and Visick 2008). Similarly, c-di-GMP production also initiates T4P assembly and retraction to perform twitching translocation and adhesion (Leighton et al. 2015). The details of signaling pathways which involve in the regulation of different types of motility and formation of biofilm in *P. aeruginosa* have been demonstrated in Fig. 1. In addition, studies have found that the production of Psl exopolysaccharides from *P. aeruginosa* also plays a crucial role in initial attachment (e.g., crawling and walking) and regulates surface movements (Gibiansky et al. 2010; Zhao et al. 2013). The knowledge of regulatory network combining with the actions of *P. aeruginosa* surface-associated motilities throughout the bacterial lifestyle transition and biofilm formation provides a fundamental platform to develop an alternative therapeutic strategy to prevent the bacterial biofilm establishment as well as its related infections.

## Attenuation of motility properties in *P. aeruginosa*

The pathogenesis and virulence of *P. aeruginosa* to the host cells are primarily dependent on the initial contact to the cell surfaces by the help of surface-specific phenotypes and appendages. Current therapies against *P. aeruginosa* biofilm formation have shifted to suppressing the virulence properties produced alongside with biofilm development, as such approaches seem to effectively reduce the resistance selection pressure. Due to their essential roles in initial attachment stage of biofilm formation, attenuating motions is proposed as a potential approach for biofilm inhibition, beside inhibition of virulence properties, quenching of the QS signaling circuit, and disruption of mature biofilm (Caiazza et al. 2007; de la Fuente-Núñez et al. 2012; Kearns 2010; Khan et al. 2019a).

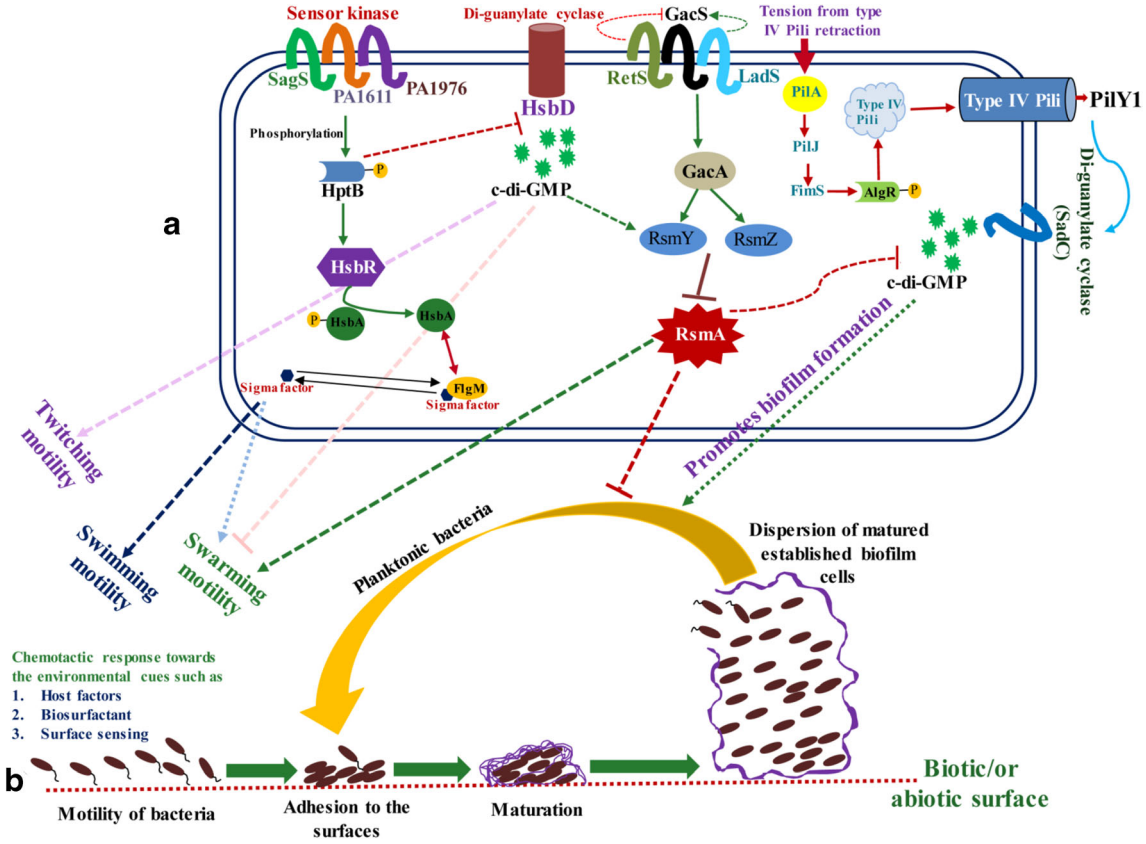



**Fig. 1** Signaling pathways involved in the regulation of biofilm formation and different types of motility in *Pseudomonas aeruginosa*. The information obtained from the literature (Chang 2017; Valentini and Filloux 2016; Valentini et al. 2016)

Though, QS interference poses as a strategy for biofilm inhibition by allowing the bacteria to modify their gene expression pattern in response to changes in the cell density and species composition of the microbial community and controls the activation of defense mechanisms (virulence factors) and biofilm formation (Oloketuyi and Khan 2017; Zhao et al. 2015). *P. aeruginosa* QS is regulated by three main cell-to-cell signaling systems—*las*, *rhl*, and PQS, which contain transcriptional regulators (LasR, RhlR, and PqsABCD), and cognate AHL synthases (LasI and RhlI) (Liu et al. 2019).

However, QS controls the cellular motility (swarming) in *P. aeruginosa*, mainly mediated by RhlR which activates expression of the *rhlAB* genes thereby contributing to biofilm formation (Shrout et al. 2006; Turkina and Vikstrom 2019; Wilhelm et al. 2007) and reported the role of *las* and *rhl* QS systems in twitching motility regulation and production of functional type IV pili in *P. aeruginosa* PAO1. Similarly, Blus-Kadosh et al. (2013) discussed the up-regulation of Rhl quorum sensing system in *P. aeruginosa* leading to hyper production of rhamnolipids thereby inducing swarming motility mediated by phosphate-specific transport system-PhoB.

Different molecules have been found to disrupt QS pathway/steps in *P. aeruginosa* by inhibiting or agonizing transcription factor/acyl homoserine lactones (AHLs) biosynthesis pathway, suppressing gene expression (*las*, *rhl*, and PQS), blocking of the LasR receptor and signal molecule degradation as discussed in several excellent articles (Fong et al. 2018; Khan et al. 2019b; Scoffone et al. 2019).

Despite the extreme complications in terms of activation, regulation, and interaction of motility-encoded genes as broadly discussed in the previous section, a wide range of effective anti-motility agents have been isolated from natural sources (e.g., plants, bacteria, and animals) or synthesized from chemical methods (O'May et al. 2012; Ulrey et al. 2014; Vadekeetil et al. 2016). Furthermore, these agents have been combined into conjugates with each other or with biocompatible nanomaterials to enhance the anti-motility effect, which shall be discussed in the following sections.

## Naturally derived products

A significant number of compounds inhibiting *P. aeruginosa* motilities have been discovered from natural resources (Table 1). The availability and abundance of these sources are the advantages for research and development of anti-motility strategies. For instance, sub-minimum inhibitory concentration (sub-MIC) of cinnamaldehyde present abundantly in cinnamon oil targeted the bacterial c-di-GMP level. As aforementioned, c-di-GMP plays an important role in assembling flagella—the swarming appendage. As a result, a

**Table 1** Naturally derived compounds inhibiting the motility of *P. aeruginosa*

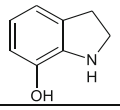
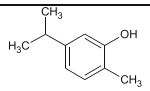
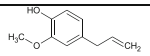
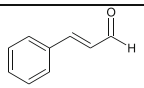
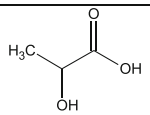
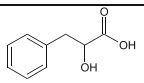
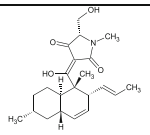
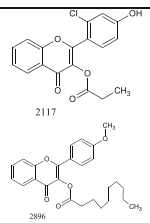
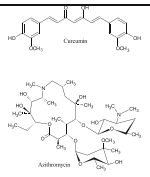
| Compound name | Chemical structure | Sources | Active concentrations | Types of motility inhibition | Other inhibitory activities | References |
|---|---|---|---|---|---|---|
| 7-Hydroxyindole |  | Conversion of indole by bacterial oxygenase | 0.5 mM | Swarming | It altered the gene expression of efflux pump and QS-regulated virulence factors without toxicity effects | (Lee et al. 2009) |
| Ginseng extract | NA | Plant | 0.25 % | Swarming | The extract reduced biofilm formation and eradicated the mature biofilm | (Wu et al. 2011) |
| Carvacrol |  | Plant | 0.9-7.9 mM | Swarming | The expression of *las* QS system was altered, followed by inhibition of pyocyanin and acyl-homoserine lactones production. | (Tapia-Rodriguez et al. 2019) |
| Eugenol |  | Plant | 0.2 mg/mL | Swarming | The presence of eugenol reduced the production of pyocyanin, QS signalling molecules, *las* and *rhl* systems and inhibited the expression of QS synthase genes | (Lou et al. 2019) |
| Ag-$TiO_2$, $TiO_2$-Ag, Ag-Cu and Cu-Ag nanocomposites | NA | Synthesized by plant flower extract | 3.12 and 6.25 μg/mL | Swarming | Biofilm architecture and production of pyocyanin were interfered by the presence of nanocomposites | (Alavi and Karimi 2018) |
| Cinnamaldehyde |  | Plant | 5.9 mM | Swarming | It also dispersed the mature biofilm by reducing c-di-GMP production. | (Topa et al. 2018) |
| Lactic acid |  | *Pediococcus acidilactici* strain M7 | Sub-MIC of 0.698 and 0.742 mg/mL | Swarming, swimming and twitching | Biofilm formation and production of several signalling molecules and QS-regulated virulence factors were inhibited. | (Kiymaci et al. 2018) |
| 3-Phenyllactic acid |  | *Lactobacillus* species | 1.2 mM | Swarming | 3-Phenyllactic acid inhibited the expression of virulence factors and QS signalling molecules by binding to QS receptors (RhlR and PqsR) | (Chatterjee et al. 2017) |
| Equisetin |  | Marine fungus *Fusarium* sp. Z10 | 300 μM | Swarming | Gene expression of QS systems (*las*, *rhl* and PQS) were downregulated, causing biofilm inhibition and virulence factors attenuation | (Zhang et al. 2018) |
| Flavone derivatives such as 2117 and 2896 |  | Natural phytochemical | 400 μM | Swarming and swimming | These QS inhibitors also targeted the bacterial biofilm developmental stages | (Manner and Fallarero 2018) |
| Curcumin, azithromycin and gentamicin alone and in |  | Plant | Sub-MICs (1/4× and 1/16× MIC) | Swarming and twitching | Expression of QS *las* and *rhl* systems and signalling molecules were significantly reduced | (Bahari et al. 2017) |

**Table 1** (continued)

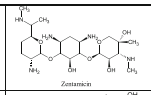
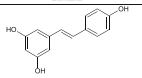
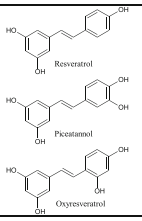
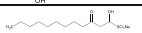
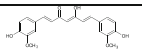
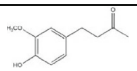
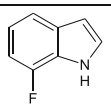
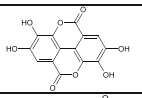
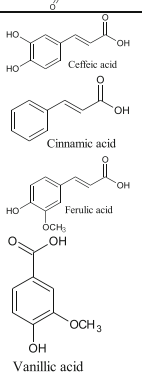
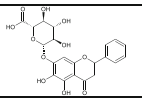
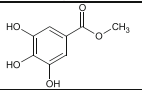
| | | | | | | |
|---|---|---|---|---|---|---|
| combination |  | | | | | |
| Resveramax™ (50% resveratrol, 1% sunflower lecithin and 1% grape seed oil) |  | Formulation of resveratrol | 10, 25 and 50 μg/mL | Swarming | QS interference through inhibition of pyocyanin production and biofilm formation | (Vasavi et al. 2017) |
| Stilbenoids (1-resveratrol, 2-piceatannol and 3-oxyresveratrol) |  | Phytoalexins in plant | 100-400 μM | Swarming | Decreased pyocyanin production and suppression of QS genes (*lasI*, *lasR*, *rhlI* and *rhlR*) | (Sheng et al. 2015) |
| Sodium houttuyfonate |  | *Houttuynia cordata* | 1 x minimum inhibitory concentration (MIC) (512 mg/mL) | Swimming, twitching and swarming | Downregulation of structural gene expression responsible for pili and flagella formation | (Wu et al. 2015b) |
| Curcumin |  | *Curcuma longa* L. | 50, 75 and 100 μg/mL | Swimming and swarming | Attenuation of QS factors and biofilm inhibition | (Packiavathy et al. 2014) |
| Zingerone |  | *Zingiber officinale*) | 10 mg/mL | Swimming, swarming and twitching | Increased susceptibility of *P. aeruginosa* to ciprofloxacin and biofilm inhibition | (Kumar et al. 2013) |
| 7-Fluoroindole |  | Commercial product | 1.0 mM | Swarming | It inhibits biofilm formation as well as suppressed the production of virulence factors (EPS, rhamnolipid, pyocyanin, pyoverdine and pyochelin) | (Lee et al. 2012) |
| Ellagic acid |  | *Camellia nitidissima* Chi | 0.1 mg/mL | Swarming and swimming | Inhibition of pyocyanin production | (Yang et al. 2017) |
| Caffeic acid, cinnamic acid, ferulic acid and vanillic acid |  | NA | 4 mmol/L | Swarming | Reduced pyocyanin production Decreased biofilm formation | (Ugurlu et al. 2016) |
| Tea polyphenols | NA | *Camellia sinensis* L. | 0.05 mg/mL | Swarming | Reduced QS related factors and biofilm inhibition | (Yin et al. 2015) |
| Baicalin |  | Commercial product | 256 μg/mL | Swarming and twitching | Repressed QS regulatory genes and related factors | (Luo et al. 2017) |
| Methyl gallate |  | NA | 16~256 μg/mL | Swarming | Biofilm inhibition and suppressed gene expression of QS signalling molecule. | (Hossain et al. 2017) |

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**Table 1** (continued)

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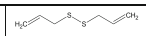
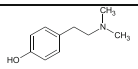
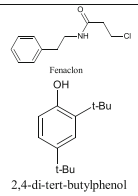
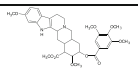
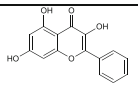
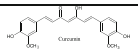
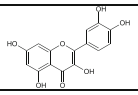
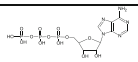
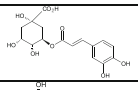
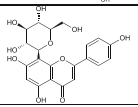
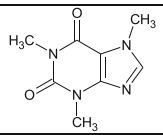
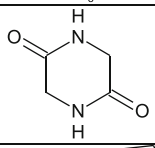
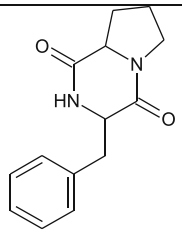
|---|---|---|---|---|---|---|
| Diallyl disulfide |  | Garlic oil | 1.28 mg/mL | Swarming | Inhibition of QS systems and virulence factors (elastase elastase and pyocyanin production) | (Li et al. 2018) |
| Hordenine |  | Sprouting barley extract | 1.0 mg/mL | Swarming and swimming | Downregulation of QS related genes | (Zhou et al. 2018) |
| Fenaclone and 2, 4-di-tert-butylphenol |  | *Diaporthe phaseolorum* SSP12 | 750 μg/mL | Swarming and swimming | Inhibition and modulation of virulence phenotypes-chitinase, Las protease, pyocyanin, EPS and rhamnolipids. | (Pattnaik et al. 2018) |
| Reserpine |  | *Rauwolfia serpentina* | $IC_{50}$ (400 μg/mL) and $IC_{80}$ (600 μg/mL) | Swimming and swarming | Suppressed QS related genes responsible for pyocyanin, rhamnolipids, proteases and elastases production. | (Parai et al. 2018) |
| 3, 5, 7-Trihydroxyflavone |  | *Alstonia scholaris* leaf | 100 μg/mL | Swimming | QS interference resulting to inhibition of biofilm and QS dependent phenotypes | (Abinaya and Gayathri 2019) |
| Glycoprotein DMBT1 (Deleted in Malignant Brain Tumors 1) | N/A | Tear fluid | 125 μg/mL | Twitching | NA | (Li et al. 2017) |
| Curcumin, ceftazidime and ciprofloxacin alone and in combination |  | Plant | Sub-MICs (1/4× and 1/16× MIC) | Swarming and twitching | Repressed expression of QS regulation genes-*lasR*, *lasI*, *rhlR* and *rhlI*) | (Roudashti et al. 2017) |
| Quercetin |  | Plant | 0.5 MIC | Twitching | Reduced biofilm formation | (Pejin et al. 2015) |
| D3112 protein gp05 | NA | Phage protein | | Twitching | NA | (Chung et al. 2014) |
| Adenosine triphosphate |  | Extracellular | 7.5 mM | Twitching | NA | (Nolan et al. 2015) |
| Chlorogenic acid |  | Plant | 1/2, 1/4, 1/8-MIC of 5.12 mg/mL | Swarming | Inhibition of virulence factors and biofilm formation | (Wang et al. 2019a) |
| Vitexin |  | *Vitex peduncularis* Wall | 110 μg/mL | Swarming | Antibiofilm agent | (Das et al. 2016a) |

**Table 1** (continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| Caffeine |  | Phytochemical | 0.3 mg/mL | swarming | AHL production was inhibited | (Norizan et al. 2013) |
| 2,5-Piperazinedione |  | Natural | 50 μg/mL | Swimming | It reduced QS dependent phenotypic factors | (Musthafa et al. 2012) |
| 3-Benzyl-hexahydro-pyrrolo[1, 2-a]pyrazine-1,4-dione |  | *Exiguobacterium indicum* | 1.0 mg/ mL (extract) | Swarming and swimming | Decreased virulence factors | (Singh et al. 2019) |
| $AiiA_{S1-5}$ | NA | *Altererythrobacter* sp. S1-5 | 0.2 μg | Swimming and swarming | It also inhibited the formation of biofilm as well as reduced the expression of virulence gene expression | (Wang et al. 2019b) |

*NA* not available

reduction in swarming motion was observed (Topa et al. 2018). Similar anti-swarming effect was also obtained from terrein compound isolated from *Aspergillus terreus*, in which the compound caused an elevation in c-di-GMP level to modulate the flagellar stator function (Kim et al. 2018).

On the other hand, 7-hydroxyindole produced by *Escherichia coli* suppressed the *P. aeruginosa* swarming by targeting the bacterial growth environment, including (1) carbon and nitrogen availability and (2) production of rhamnolipid biosurfactant (Lee et al. 2009). Numerous studies have confirmed the essentiality of these environmental factors to swarming motility, in which the sufficient carbon and nitrogen sources determine the synthesis of T4P that cooperates with flagella to perform swarming, while the presence of rhamnolipid conditions the surface tension, thus promoting migration of swarmer cells (Caiazza et al. 2005; Deziel et al. 2003). Targeting the rhamnolipid production at gene expression level in order to confer the bacterial swarming was also the mechanism employed by hordenine and baicalin extracted from sprouting barley and *Scutellaria baicalensis*, respectively (Luo et al. 2017; Zhou et al. 2018). As in *P. aeruginosa*, rhamnolipid is produced and regulated by QS system, and disrupting QS or quorum quenching also results in swarming inhibition. Plant phenolic compounds such as methyl gallate and tea polyphenols which played the quorum quenching role in *P. aeruginosa* were also found to suppress the swarming motility of this bacteria (Hossain et al. 2017; Yin et al. 2015). Likewise, diallyl disulfide isolated from garlic oil repressed all three QS systems in the bacteria, thereby down-regulating flagellar synthesis and flagellar-mediated motility, which are swimming and swarming (Li et al. 2018). Likewise, swimming and swarming of *P. aeruginosa* were also inhibited by the natural alkaloid (R)-norbgugaine from *Arisarum vulgare*, along with flagellar function, biofilm formation, and rhamnolipid synthesis (Majik et al. 2013). Recently, a wide range of secondary metabolites produced from non-pathogenic bacteria have been found to also exhibit anti-biofilm and quorum quenching activities to *P. aeruginosa* (Gutierrez-Barranquero et al. 2019; Zhao et al. 2019). Although their anti-motility performance was not noticed, disrupting the regulatory role of QS is expected to also result in an inhibitory effect in the bacterial motilities. Despite numerous evidences demonstrating for the major effects of QS inhibitors on *P. aeruginosa* swarming, such effect as well as its mechanism on the bacterial twitching motility remained limitedly known. What is more, the previous study conducted by Glessner et al. (1999) claimed that the two auto-inducers of *las* QS system—PAI-1 and PAI-2—were able to regulate twitching motility by involving in T4P assembly and retraction/extension function (Glessner et al. 1999). This finding was in contrast to another study conducted later on by Beatson et al. (2002) where the QS system only indirectly affected twitching motility by regulating alginate and virulence properties production, as well as biofilm formation. As twitching is also one of the motilities that performs initial attachment in biofilm formation process, such indirect link between twitching and QS could explain why twitching can be inhibited by several anti-biofilm or quorum-quenching agents (Bahari et al. 2017; Kiymaci et al. 2018; Luo et al. 2017). Also targeting the twitching motility of

*P. aeruginosa*, a phage protein named D3112 protein gp05 (Tip) has recently been found to be able to repress the expression of pilB which essentially involves in T4P synthesis and extension. This application of phage therapy has helped extending the variations in targets and treatments in controlling *P. aeruginosa* biofilm formation. Several other naturally derived products which have been identified as a drug for attenuating the different motility properties of *P. aeruginosa* are summarized in the Table 1.

## Chemically synthesized products

Several chemically-synthesized compounds have also exhibited high anti-motility activity against *P. aeruginosa*. Due to the currently high demand of treatments for the bacteria, the chemical-based synthesis of potent drugs might be helpful as an alternative approach. The advances in chemical methods also allow purposely, direct, and vast production of numerous molecules and compounds with competitive anti-motility function without performing complex extraction and characterization processes as seen for the natural compounds. Furthermore, the compounds which are chemically synthesized possess significant improvements in the bacterial motility inhibition and many other related phenotypes (Khan et al. 2019c). Table 2 represents several chemically synthesized compounds known to inhibit the swimming, swarming, and twitching motilities of *P. aeruginosa*.

Various chemically synthesized compounds have been reported to inhibit *P. aeruginosa* motilities by targeting the bacterial QS signaling system. For example, (z)-5-octylidenethiazolidine-2,4-dione and lipoic acid also significantly inhibited *P. aeruginosa* swarming (Cevik and Ulusoy 2015; Lidor et al. 2015). Another chemical named phenylalanine arginyl β-naphthylamide also targeted QS, swimming, and twitching, yet its role was previously known as an efflux inhibitor to *P. aeruginosa* (El-Shaer et al. 2016). Attenuating the flagellar functions, along with production of exopolysaccharides, rhamnolipid, and lipopolysaccharides, was the mechanism used by anteiso-C15:0—a branched-chain fatty acid to completely inhibit *P. aeruginosa* swarming motility, while it only partially affected swimming and twitching (Inoue et al. 2008). Anti-swarming activity of doxycycline antibiotic was by reducing rhamnolipid production (Husain and Ahmad 2013). However, the molecular basis of these anti-motility effects has yet to be elucidated. In contrast, in the case of the 2,5-piperazinedione compound, the compound was found to suppress the bacterial LasR system by competing with 3-oxo-C12-HSL ligand to bind to the glutamic acid receptor, thus causing down-regulation of all QS-related phenotypes, including swimming motility (Musthafa et al. 2012). In another study, de la Fuente-Núñez et al. (2014) have synthesized that the Peptide 1037 cationic peptide and its anti-biofilm activities were screened using microarray method. Results have shown that the peptide was able to directly down-regulate the expression of flagella-, QS-, and rhamnolipid-encoded genes, leading to significant reduction in both swarming and swimming motility. However, the positive correlation between rhamnolipid level and swarming motility may not always take place, as in a study by Oura et al. (2015), the inhibitory activity of 1-naphthol chemical against *P. aeruginosa* swarming was found dependent on expression of the flagella and pili-encoded genes instead of rhamnolipid (Oura et al. 2015). Overall, due to the advantages of time- and cost-saving, chemical methods should also be more extensively exploited in both phenotypic and molecular studies to add in the variation of anti-motility agents against *P. aeruginosa*.

## Combinatorial approaches

In recent years, with the attempt to potentiate the conventional antibiotics in terms of anti-motility activity, controlled release and stability at a lowered concentration over a long period of time, several combinations of the antibiotics with different antibiotic(s) or with non-antibiotic compound(s)/structure were applied to inhibit *P. aeruginosa* biofilm formation (Das et al. 2016b; Ferrer-Espada et al. 2019; Gupta et al. 2017). When forming a combination with non-antibiotics compound(s)/structure, the antibiotics can be loaded externally (a coating layer) or internally (encapsulation). Otherwise, combinations can also be constructed from natural compounds and chemically synthesized compounds, diversifying the inhibitory effect against *P. aeruginosa* motilities as well as numerous different virulence phenotypes (Tyers and Wright 2019). In general, the significances of combination approaches include (1) improving the performance of each individual compound and (2) actively reducing the potentials of resistance emergence in *P. aeruginosa*. For example, the clarithromycin antibiotic which was encapsulated into a lipid nanocarrier named liposome showed enhanced stability, and the most significant inhibitory activity against various types of *P. aeruginosa* motility was obtained from positively charged liposome (Alhajlan et al. 2013). Chitosan is a biopolymer that when combined with polypyrrole into nanocomposites has shown an increase in inhibitory action against *P. aeruginosa* swimming and swarming (Khan et al. 2019c). Similar effect was obtained in *P. aeruginosa* swarming, swimming, and twitching when treated with fucoidan-capped gold nanoparticles (Khan et al. 2019a). Recently, a report showed that newly synthesized chitosan oligosaccharide capped-gold nanoparticle attenuates the swimming and twitching motility properties of *P. aeruginosa* (Khan et al. 2019d).

Anti-swarming action of natural compound named vitexin was also significantly improved by conjugating with conventional antibiotics (azithromycin and gentamicin) (Das et al. 2016b). However, the number of available anti-motility conjugates as well as the knowledge about underlying mechanism

**Table 2** Chemically synthesized compounds inhibiting the motility of *P. aeruginosa*

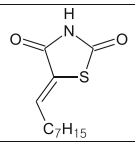
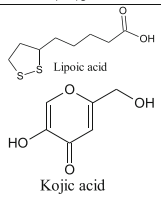
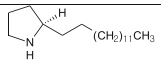
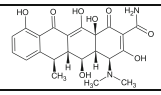
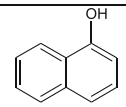
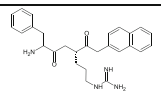
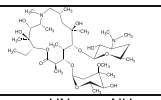
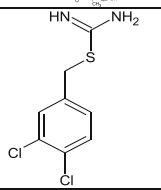
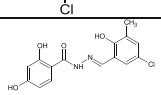
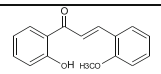
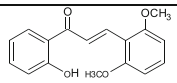
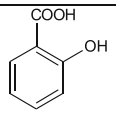
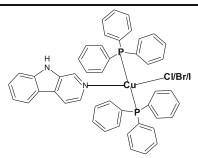
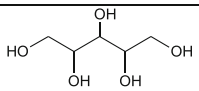
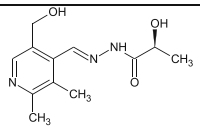
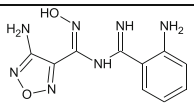
| Compound name | Chemical structure | Sources | Active concentrations | Types of motility inhibition | Other inhibitory activities | References |
|---|---|---|---|---|---|---|
| (z)-5-octylidenethiazolidine-2,4-dione |  | Chemically synthesized | 20 μM | Swarming | NA | (Lidor et al. 2015) |
| Lipoic and Kojic acid |   | Commercial | Lipoic acid-4mM and Kojic acid-2 mM | Swarming | NA | (Cevik and Ulusoy 2015) |
| (R)-norbgugaine |  | Chemical synthesis | 0.5 mM | Swarming | Inhibition of LasA protease, pyocyanin and rhamnolipid | (Majik et al. 2013) |
| Doxycycline |  | | 4.0 μg/mL | Swarming | Reduced EPS production and other virulence related factors | (Husain and Ahmad 2013) |
| Cationic peptide 1037 (9 amino acids length) | NA | Synthetic | 5-50 μg/mL | Swimming and swarming | Biofilm inhibition | (de la Fuente-Nunez et al. 2012) |
| 12-methyltetradecanoic acid (anteiso-C15:0) |  | Commercial | 10 μg/mL | Swimming and swarming | NA | (Inoue et al. 2008) |
| 1-naphthol |  | Commercial product | 500 μM | Swarming | Repression of biosynthesis of flagellar, pilus and pyochelin | (Oura et al. 2015) |
| Phenylalanine arginyl β-naphthylamide |  | Commercial product | 50 μg/mL | Swimming and twitching | Reduced QS signalling molecule and virulence factors | (El-Shaer et al. 2016) |
| Azithromycin |  | Antibiotic | 88.5 μg/mL | Swimming, swarming, and twitching | Inhibition of QS signalling | (Bala et al. 2011) |
| (S-3,4-dichlorobenzyl) isothiourea hydrochloride (A22) |  | Commercial product | 0.5 μg/mL | Swimming, swarming and twitching | Disruption of surface adhesion and biofilm formation | (Bonez et al. 2017) |
| Salicylidene acylhydrazide INP0341 |  | Commercial product | 10 μM (for swimming) and 5 μM (for swarming) | Swimming and swarming | Biofilm inhibition | (Uusitalo et al. 2017) |
| 3-(2-methoxyphenyl)-1-(2-hydroxyphenyl)-2-propen-1-on |  | Chemically synthesized | 100 μg/mL | Swimming | Repressed pyocyanin production | (Usjak et al. 2019) |

**Table 2** (Continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| 3-(2,6-dimethoxyphenyl)-1-(2-hydroxyphenyl)-2-propen-1-on |  | Chemically synthesized | 100 µg/mL | Swarming | Repressed pyocyanin production | (Usjak et al. 2019) |
| Salicylic acid |  | Commercial | 30 mM | Swimming and twitching | NA | (Bandara et al. 2006) |
| Norspermidine |  | Commercial | 4 mmol/L | Swimming | Inhibition of biofilm formation | (Qu et al. 2016) |
| Cationic peptide 103 | Amino acid sequences (KRFRIRVRV) | Chemically synthesized | 5-50 µg/mL | Swarming and swimming | Suppression of biofilm related genes | (de la Fuente-Nunez et al. 2012) |
| Phosphane copper (I) complexes of β-carboline |  | Chemically synthesized | ½-MIC | Swarming | Reduced EPS production | (Al-Shabib et al. 2019) |
| Esculentin-1a(1-21)NH2 | GIFSKLAGKKIKNLLISGLKG-$CONH_2$ | Synthetic | 1/8-MIC | Swimming, swarming and twitching | Suppressed pyoverdine and rhamnolipids. Downregulation of biofilm related genes | (Casciaro et al. 2019) |
| Xylitol |  | Synthetic | 200 mM | Swimming | Inhibition of EPS and biofilm formation | (Zhou et al. 2019) |
| Pyridoxal lactohydrazone |  | Synthetic | 32 and 8 µg/mL | Swarming and twitching | Inhibition of LasR protein, alginate and pyocyanin | (Heidari et al. 2017) |
| 2- Amino-N- [(4-amino-1,2,5-oxadiazol-3- yl) (hydroxyimino)methyl] benzene- 1 carboximidamide (PI3) |  | Synthetic | 16·74 µg/mL | Swarming | Sub-MIC of PI3 compound exhibits the inhibition of biofilm formation, reduction of proteolytic activity and production of exo-polysaccharide | (Das et al. 2016a) |

*NA* not available

against the bacterial motility of these combinations has remained lacking.

Overall, a wide variety of natural compounds have been able to inhibit *P. aeruginosa* motilities by different motility-regulating pathways. As motilities play a major role at initial stage of the bacterial biofilm formation, their suppression can be taken as a highly potential approach towards biofilm inhibition. The nanomaterials which are ranged from metallic to polymeric forms have been used for conjugating the motility inhibiting drugs which works either synergistically or act as a carrier for the drugs (Dos Santos Ramos et al. 2018). The anti-motility agents currently being used to attenuate *P. aeruginosa* motilities would provide an important insight and propose surface motility attenuation as a potential approach against *P. aeruginosa* biofilm formation.

## Concluding Remarks and Future Perspectives

In the current urge to combat biofilm-related infections caused by *P. aeruginosa* and prevent the risks of new resistance emergence, the virulence properties produced during biofilm formation and development have become attractive. The bacterial surface motility consisting of swimming, swarming, and twitching are the virulence properties which are essential for a multitude of functions: surface sensing and translocation, lifestyle switching, biofilm formation, biofilm maturation, and biofilm dispersal. Therefore, combating the bacterial motility can be considered as a promising strategy to prevent biofilm formation. The essential roles of *P. aeruginosa* motility were firstly described in the present review paper, followed by their performing machineries (e.g., flagella and pili) and regulation carried out by chemotaxis, QS as well as several signal transducing pathways. The focus of the present review includes (1) to highlight the importance of *P. aeruginosa* motilities and (2) to summarize the up-to-date compounds derived from natural resources or synthesized chemically, in individual form or in combination that have been used to attenuate the bacterial motilities. The targets of these anti-motility agents were highly varied from the flagella or pili assembly, QS, wetting agent (i.e., rhamnolipid) to the signaling molecules (e.g., c-di-GMP) and regulatory genes expression. Due to the complications in the motility induction and regulation, further studies are required to explore the molecular insight of these motility inhibitors, as well as their actions to motility-related phenotypes, thereby developing a powerful agent which can effectively suppress multiple targets for controlling *P. aeruginosa* biofilm formation.

Similar to many other anti-virulence approaches, full understandings about the potentials of anti-motility approach have remained in research. A number of compounds exhibiting inhibition activity towards *P. aeruginosa* motilities have not been elucidated for detailed mechanism. Furthermore, each type of motility may require different conditions to be suppressed. For example, inhibiting swarming motility is more complex than swimming motility, as beside flagellar assembly and functions, c-di-GMP level and QS, the targets are extended to (1) the unique differentiation and gene expression between the flagella tip and the central population (Tremblay and Deziel 2010); (2) the bacterial synthesis of biosurfactant (i.e., rhamnolipid) to reduce the surface tension; (3) the bacterial swarming disability by modifications in culture media using agar, salt, water, viscous agents, or sugar; and (4) nutrient source such as iron. For being regulated by a different machinery which is T4P, twitching motility, on the other hand, is known to be suppressed by different mechanisms, including reducing c-AMP production, interfering major pilin-encoded gene expression and availability of inorganic polyphosphate and iron. In addition, due to the well-known advantages of stability and longevity in combinatory approaches, more research on combining anti-motility-agent with other compounds or carriers is strongly recommended. Several positive outcomes discussed in the previous section are worth more attention. Furthermore, the anti-motility effect of these conjugates also has not been clarified to whether result from synergism between the two individuals or from only one individual. In case it is synergism that causes biofilm inhibition in *P. aeruginosa*, distribution and targets of each compound would require further studies. Finally, the natural resources of the motility inhibitors should be diverse and the new chemical compounds should continue being developed in order to maintain the availability of anti-motility agents over a long period of time.

## Outstanding questions

1. With the significant benefits of adopting combinatory approaches, can more anti-motility agents be incorporated/encapsulated into other compounds/carriers? Can their effects be studied at molecular level?
2. Is it possible that the inhibition to *P. aeruginosa* motility by using combinatory approaches is derived from synergism between compounds? If so, what is the mechanisms of such effects?
3. How do the individual compounds perform anti-motility action in *P. aeruginosa* differently when in combination with other compounds? Can this effect be studied at molecular and genetic level?
4. By conjugating nanocarriers with anti-motility agent(s), will there be any unexpected effects to other motilities caused by the nanocarriers? As a number of anti-motility agents were able to suppress only one/two motilities per application time.
5. How the c-di-GMP receptor/effector protein functions and how their presence benefits other related pathways?
6. How can *P. aeruginosa* differentiate which the surfaces they prefer attaching? What is the mechanism of chemical interaction between the biofilm components and other surface components?
7. How do the motility machineries behave differently on different surfaces?
8. How does T4P involve in regulating swarming motility? Does T4P presence play a crucial role in the bacterial swarming performance?

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This review paper does not contain any studies with human participants or animals.

## References


Abinaya M, Gayathri M (2019) Inhibition of biofilm formation, quorum sensing activity and molecular docking study of isolated 3, 5, 7-trihydroxyflavone from *Alstonia scholaris* leaf against *P. aeruginosa*. Bioorg Chem 87:291–301. https://doi.org/10.1016/j.bioorg.2019.03.050

Alavi M, Karimi N (2018) Antiplanktonic, antibiofilm, antiswarming motility and antiquorum sensing activities of green synthesized Ag-TiO2, TiO2-Ag, Ag-Cu and Cu-Ag nanocomposites against multi-drug-resistant bacteria. Artif Cells Nanomed Biotechnol 46: S399–S413. https://doi.org/10.1080/21691401.2018.1496923

Alhajlan M, Alhariri M, Omri A (2013) Efficacy and safety of liposomal clarithromycin and its effect on *Pseudomonas aeruginosa* virulence factors. Antimicrob Agents Chemother 57:2694–2704. https://doi.org/10.1128/AAC.00235-13

Al-Shabib NA, Husain FM, Khan RA, Khan MS, Alam MZ, Ansari FA, Laeeq S, Zubair M, Shahzad SA, Khan JM, Alsalme A, Ahmad I (2019) Interference of phosphane copper (I) complexes of beta-carboline with quorum sensing regulated virulence functions and biofilm in foodborne pathogenic bacteria: a first report. Saudi J Biol Sci 26:308–316. https://doi.org/10.1016/j.sjbs.2018.04.013

Bahari S, Zeighami H, Mirshahabi H, Roudashti S, Haghi F (2017) Inhibition of *Pseudomonas aeruginosa* quorum sensing by subinhibitory concentrations of curcumin with gentamicin and azithromycin. J Glob Antimicrob Resist 10:21–28. https://doi.org/10.1016/j.jgar.2017.03.006

Bala A, Kumar R, Harjai K (2011) Inhibition of quorum sensing in *Pseudomonas aeruginosa* by azithromycin and its effectiveness in urinary tract infections. J Med Microbiol 60:300–306. https://doi.org/10.1099/jmm.0.025387-0

Bandara MB, Zhu H, Sankaridurg PR, Willcox MD (2006) Salicylic acid reduces the production of several potential virulence factors of *Pseudomonas aeruginosa* associated with microbial keratitis. Invest Ophthalmol Vis Sci 47:4453–4460. https://doi.org/10.1167/iovs.06-0288

Beatson SA, Whitchurch CB, Semmler AB, Mattick JS (2002) Quorum sensing is not required for twitching motility in *Pseudomonas aeruginosa*. J Bacteriol 184:3598–3604

Blus-Kadosh I, Zilka A, Yerushalmi G, Banin E (2013) The effect of pstS and phoB on quorum sensing and swarming motility in *Pseudomonas aeruginosa*. PLoS One 8:e74444. https://doi.org/10.1371/journal.pone.0074444

Bonez PC, Rossi GG, Bandeira JR, Ramos AP, Mizdal CR, Agertt VA, Dalla Nora ESS, de Souza ME, Dos Santos Alves CF, Dos Santos FS, Gündel A, de Almeida VR, Santos RCV, de Campos MMA (2017) Anti-biofilm activity of A22 ((S-3,4-dichlorobenzyl) isothiourea hydrochloride) against *Pseudomonas aeruginosa*: influence on biofilm formation, motility and bioadhesion. Microb Pathog 111:6–13. https://doi.org/10.1016/j.micpath.2017.08.008

Boucher JC, Schurr MJ, Deretic V (2000) Dual regulation of mucoidy in *Pseudomonas aeruginosa* and sigma factor antagonism. Mol Microbiol 36:341–351

Burrows LL (2012) *Pseudomonas aeruginosa* twitching motility: type IV pili in action. Annu Rev Microbiol 66:493–520. https://doi.org/10.1146/annurev-micro-092611-150055

Caiazza NC, Shanks RM, O'Toole GA (2005) Rhamnolipids modulate swarming motility patterns of *Pseudomonas aeruginosa*. J Bacteriol 187:7351–7361. https://doi.org/10.1128/JB.187.21.7351-7361.2005

Caiazza NC, Merritt JH, Brothers KM, O'Toole GA (2007) Inverse regulation of biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. J Bacteriol 189:3603–3612. https://doi.org/10.1128/JB.01685-06

Casciaro B, Lin Q, Afonin S, Loffredo MR, de Turris V, Middel V, Ulrich AS, Di YP, Mangoni ML (2019) Inhibition of *Pseudomonas aeruginosa* biofilm formation and expression of virulence genes by selective epimerization in the peptide Esculentin-1a(1-21)NH2. FEBS J. https://doi.org/10.1111/febs.14940

Cevik K, Ulusoy S (2015) Inhibition of *Pseudomonas aeruginosa* biofilm formation by 2,2'-bipyridyl, lipoic, kojic and picolinic acids. Iran J Basic Med Sci 18:758–763

Chang CY (2017) Surface sensing for biofilm formation in *Pseudomonas aeruginosa*. Front Microbiol 8:2671. https://doi.org/10.3389/fmicb.2017.02671

Chatterjee M, D'Morris S, Paul V, Warrier S, Vasudevan AK, Vanuopadath M, Nair SS, Paul-Prasanth B, Mohan CG, Biswas R (2017) Mechanistic understanding of phenyllactic acid mediated inhibition of quorum sensing and biofilm development in *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 101:8223–8236. https://doi.org/10.1007/s00253-017-8546-4

Chung IY, Jang HJ, Bae HW, Cho YH (2014) A phage protein that inhibits the bacterial ATPase required for type IV pilus assembly. Proc Natl Acad Sci U S A 111:11503–11508. https://doi.org/10.1073/pnas.1403537111

Conrad JC, Gibiansky ML, Jin F, Gordon VD, Motto DA, Mathewson MA, Stopka WG, Zelasko DC, Shrout JD, Wong GC (2011) Flagella and pili-mediated near-surface single-cell motility mechanisms in *P. aeruginosa*. Biophys J 100:1608–1616. https://doi.org/10.1016/j.bpj.2011.02.020

D'Argenio DA, Miller SI (2004) Cyclic di-GMP as a bacterial second messenger. Microbiology 150:2497–2502. https://doi.org/10.1099/mic.0.27099-0

Das MC, Sandhu P, Gupta P, Rudrapaul P, De UC, Tribedi P, Akhter Y, Bhattacharjee S (2016a) Attenuation of *Pseudomonas aeruginosa* biofilm formation by vitexin: a combinatorial study with azithromycin and gentamicin. Sci Rep 6:23347. https://doi.org/10.1038/srep23347

Das MC, Paul S, Gupta P, Tribedi P, Sarkar S, Manna D, Bhattacharjee S (2016b) 3-Amino-4-aminoximidofurazan derivatives: small molecules possessing antimicrobial and antibiofilm activity against Staphylococcus aureus and *Pseudomonas aeruginosa*. J Appl Microbiol 120:842–859. https://doi.org/10.1111/jam.13063

de la Fuente-Núñez C, Korolik V, Bains M, Nguyen U, Breidenstein EB, Horsman S, Lewenza S, Burrows L, Hancock RE (2012) Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. Antimicrob Agents Chemother 56: 2696–2704. https://doi.org/10.1128/AAC.00064-12

de la Fuente-Núñez C, Reffuveille F, Haney EF, Straus SK, Hancock RE (2014) Broad-spectrum anti-biofilm peptide that targets a cellular stress response. PLoS Pathog 10:e1004152-e1004152. https://doi.org/10.1371/journal.ppat.1004152

Deziel E, Lepine F, Milot S, Villemur R (2003) rhlA is required for the production of a novel biosurfactant promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiology 149:2005–2013. https://doi.org/10.1099/mic.0.26154-0

Dos Santos Ramos MA, Da Silva PB, Spósito L, De Toledo LG, Bonifácio BV, Rodero CF, Dos Santos KC, Chorilli M, Bauab TM

(2018) Nanotechnology-based drug delivery systems for control of microbial biofilms: a review. Int J Nanomedicine 13:1179–1213. https://doi.org/10.2147/IJN.S146195

El-Shaer S, Shaaban M, Barwa R, Hassan R (2016) Control of quorum sensing and virulence factors of *Pseudomonas aeruginosa* using phenylalanine arginyl beta-naphthylamide. J Med Microbiol 65: 1194–1204. https://doi.org/10.1099/jmm.0.000327

Ferrer-Espada R, Shahrour H, Pitts B, Stewart PS, Sanchez-Gomez S, Martinez-de-Tejada G (2019) A permeability-increasing drug synergizes with bacterial efflux pump inhibitors and restores susceptibility to antibiotics in multi-drug resistant *Pseudomonas aeruginosa* strains. Sci Rep 9:3452. https://doi.org/10.1038/s41598-019-39659-4

Fong J, Mortensen KT, Nørskov A, Qvortrup K, Yang L, Tan CH, Nielsen TE, Givskov M (2018) Itaconimides as novel quorum sensing inhibitors of *Pseudomonas aeruginosa*. Front Cell Infect Microbiol 8: 443. https://doi.org/10.3389/fcimb.2018.00443

Francis VI, Stevenson EC, Porter SL (2017) Two-component systems required for virulence in *Pseudomonas aeruginosa*. FEMS Microbiol Lett:364. https://doi.org/10.1093/femsle/fnx104

Fulcher NB, Holliday PM, Klem E, Cann MJ, Wolfgang MC (2010) The *Pseudomonas aeruginosa* Chp chemosensory system regulates intracellular cAMP levels by modulating adenylate cyclase activity. Mol Microbiol 76:889–904. https://doi.org/10.1111/j.1365-2958.2010.07135.x

Furukawa S, Kuchma SL, O'Toole GA (2006) Keeping their options open: acute versus persistent infections. J Bacteriol 188:1211–1217. https://doi.org/10.1128/JB.188.4.1211-1217.2006

Gellatly SL, Hancock RE (2013) *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. Pathog Dis 67:159–173. https://doi.org/10.1111/2049-632X.12033

Gibiansky ML, Conrad JC, Jin F, Gordon VD, Motto DA, Mathewson MA, Stopka WG, Zelasko DC, Shrout JD, Wong GC (2010) Bacteria use type IV Pili to walk upright and detach from surfaces. Science 330:197–197. https://doi.org/10.1126/science.1194238

Glessner A, Smith RS, Iglewski BH, Robinson JB (1999) Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of twitching motility. J Bacteriol 181:1623–1629

Gupta P, Sarkar A, Sandhu P, Daware A, Das MC, Akhter Y, Bhattacharjee S (2017) Potentiation of antibiotic against *Pseudomonas aeruginosa* biofilm: a study with plumbagin and gentamicin. J Appl Microbiol 123:246–261. https://doi.org/10.1111/jam.13476

Gutierrez-Barranquero JA, Reen FJ, Parages ML, McCarthy R, Dobson ADW, O'Gara F (2019) Disruption of N-acyl-homoserine lactone-specific signalling and virulence in clinical pathogens by marine sponge bacteria. Microb Biotechnol 12:1049–1063. https://doi.org/10.1111/1751-7915.12867

Ha DG, O'Toole GA (2015) c-di-GMP and its effects on biofilm formation and dispersion: a *Pseudomonas aeruginosa*. Review Microbiol Spectr 3:MB-0003-2014. https://doi.org/10.1128/microbiolspec.MB-0003-2014

Heidari A, Noshiranzadeh N, Haghi F, Bikas R (2017) Inhibition of quorum sensing related virulence factors of *Pseudomonas aeruginosa* by pyridoxal lactohydrazone. Microb Pathog 112:103–110. https://doi.org/10.1016/j.micpath.2017.09.043

Hickman JW, Harwood CS (2008) Identification of FleQ from *Pseudomonas aeruginosa* as a c-di-GMP-responsive transcription factor. Mol Microbiol 69:376–389. https://doi.org/10.1111/j.1365-2958.2008.06281.x

Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35:322–332. https://doi.org/10.1016/j.ijantimicag.2009.12.011

Hossain MA, Lee SJ, Park NH, Mechesso AF, Birhanu BT, Kang J, Reza MA, Suh JW, Park SC (2017) Impact of phenolic compounds in the acyl homoserine lactone-mediated quorum sensing regulatory pathways. Sci Rep 7:10618. https://doi.org/10.1038/s41598-017-10997-5

Husain FM, Ahmad I (2013) Doxycycline interferes with quorum sensing-mediated virulence factors and biofilm formation in gram-negative bacteria World. J Microbiol Biotechnol 29:949–957. https://doi.org/10.1007/s11274-013-1252-1

Inoue T, Shingaki R, Fukui K (2008) Inhibition of swarming motility of *Pseudomonas aeruginosa* by branched-chain fatty acids. FEMS Microbiol Lett 281:81–86. https://doi.org/10.1111/j.1574-6968.2008.01089.x

Jansari VH, Potharla VY, Riddell GT, Bardy SL (2016) Twitching motility and cAMP levels: signal transduction through a single methyl-accepting chemotaxis protein. FEMS Microbiol Lett:363. https://doi.org/10.1093/femsle/fnw119

Kearns DB (2010) A field guide to bacterial swarming motility. Nat Rev Microbiol 8:634–644. https://doi.org/10.1038/nrmicro2405

Khan F, Manivasagan P, Lee JW, Pham DTN, Oh J, Kim YM (2019a) Fucoidan-stabilized gold nanoparticle-mediated biofilm inhibition, attenuation of virulence and motility properties in *Pseudomonas aeruginosa* PAO1. Mar Drugs 17. https://doi.org/10.3390/md17040208

Khan F, Javaid A, Kim YM (2019b) Functional diversity of quorum sensing receptors in pathogenic bacteria: interspecies, intraspecies and interkingdom level. Curr Drug Targets 20:655–667. https://doi.org/10.2174/1389450120666181123123333

Khan F, Manivasagan P, Pham DTN, Oh J, Kim SK, Kim YM (2019c) Antibiofilm and antivirulence properties of chitosan-polypyrrole nanocomposites to *Pseudomonas aeruginosa*. Microb Pathog 128: 363–373. https://doi.org/10.1016/j.micpath.2019.01.033

Khan F, Lee JW, Manivasagan P, Pham DTN, Oh J, Kim YM (2019d) Synthesis and characterization of chitosan oligosaccharide-capped gold nanoparticles as an effective antibiofilm drug against the *Pseudomonas aeruginosa* PAO1. Microb Pathog 135:103623. https://doi.org/10.1016/j.micpath.2019.103623

Kim B, Park JS, Choi HY, Yoon SS, Kim WG (2018) Terrein is an inhibitor of quorum sensing and c-di-GMP in *Pseudomonas aeruginosa*: a connection between quorum sensing and c-di-GMP. Sci Rep 8:8617. https://doi.org/10.1038/s41598-018-26974-5

Kiymaci ME, Altanlar N, Gumustas M, Ozkan SA, Akin A (2018) Quorum sensing signals and related virulence inhibition of *Pseudomonas aeruginosa* by a potential probiotic strain's organic acid. Microb Pathog 121:190–197. https://doi.org/10.1016/j.micpath.2018.05.042

Kolter R, Greenberg EP (2006) Microbial sciences: the superficial life of microbes. Nature 441:300–302. https://doi.org/10.1038/441300a

Kostakioti M, Hadjifrangiskou M, Hultgren SJ (2013) Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med 3:a010306. https://doi.org/10.1101/cshperspect.a010306

Kumar L, Chhibber S, Harjai K (2013) Zingerone inhibit biofilm formation and improve antibiofilm efficacy of ciprofloxacin against *Pseudomonas aeruginosa* PAO1. Fitoterapia 90:73–78. https://doi.org/10.1016/j.fitote.2013.06.017

Lee J, Attila C, Cirillo SL, Cirillo JD, Wood TK (2009) Indole and 7-hydroxyindole diminish *Pseudomonas aeruginosa* virulence. Microb Biotechnol 2:75–90. https://doi.org/10.1111/j.1751-7915.2008.00061.x

Lee JH, Kim YG, Cho MH, Kim JA, Lee J (2012) 7-Fluoroindole as an antivirulence compound against *Pseudomonas aeruginosa*. FEMS Microbiol Lett 329:36–44. https://doi.org/10.1111/j.1574-6968.2012.02500.x

Leighton TL, Buensuceso RN, Howell PL, Burrows LL (2015) Biogenesis of *Pseudomonas aeruginosa* type IV pili and regulation of their function. Environ Microbiol 17:4148–4163. https://doi.org/10.1111/1462-2920.12849

Li J, Metruccio MME, Evans DJ, Fleiszig SMJ (2017) Mucosal fluid glycoprotein DMBT1 suppresses twitching motility and virulence of the opportunistic pathogen *Pseudomonas aeruginosa*. PLoS Pathog 13:e1006392. https://doi.org/10.1371/journal.ppat.1006392

Li WR, Ma YK, Shi QS, Xie XB, Sun TL, Peng H, Huang XM (2018) Diallyl disulfide from garlic oil inhibits *Pseudomonas aeruginosa* virulence factors by inactivating key quorum sensing genes. Appl Microbiol Biotechnol 102:7555–7564. https://doi.org/10.1007/s00253-018-9175-2

Lidor O, Al-Quntar A, Pesci EC, Steinberg D (2015) Mechanistic analysis of a synthetic inhibitor of the *Pseudomonas aeruginosa* LasI quorum-sensing signal synthase. Sci Rep 5:16569. https://doi.org/10.1038/srep16569

Lin Chua S, Liu Y, Li Y, Jun Ting H, Kohli GS, Cai Z, Suwanchaikasem P, Kau Kit Goh K, Pin Ng S, Tolker-Nielsen T, Yang L, Givskov M (2017) Reduced intracellular c-di-GMP content increases expression of quorum sensing-regulated genes in *Pseudomonas aeruginosa*. Front Cell Infect Microbiol 7:451. https://doi.org/10.3389/fcimb.2017.00451

Liu H, Gong Q, Luo C, Liang Y, Kong X, Wu C, Feng P, Wang Q, Zhang H, Wireko MA (2019) Synthesis and biological evaluation of novel L-homoserine lactone analogs as quorum sensing inhibitors of *Pseudomonas aeruginosa*. Chem Pharm Bull (Tokyo) 67:1088–1098. https://doi.org/10.1248/cpb.c19-00359

Lou Z, Letsididi KS, Yu F, Pei Z, Wang H, Letsididi R (2019) Inhibitive effect of eugenol and its nanoemulsion on quorum sensing-mediated virulence factors and biofilm formation by *Pseudomonas aeruginosa*. J Food Prot 82:379–389. https://doi.org/10.4315/0362-028X.JFP-18-196

Luo Y, Zhao K, Baker AE, Kuchma SL, Coggan KA, Wolfgang MC, Wong GC, O'Toole GA (2015) A hierarchical cascade of second messengers regulates *Pseudomonas aeruginosa* surface behaviors. MBio:6. https://doi.org/10.1128/mBio.02456-14

Luo J, Dong B, Wang K, Cai S, Liu T, Cheng X, Lei D, Chen Y, Li Y, Kong J, Chen Y (2017) Baicalin inhibits biofilm formation, attenuates the quorum sensing-controlled virulence and enhances *Pseudomonas aeruginosa* clearance in a mouse peritoneal implant infection model. PLoS One 12:e0176883. https://doi.org/10.1371/journal.pone.0176883

Mah TF, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9:34–39

Maier B, Wong GCL (2015) How bacteria use type IV pili machinery on surfaces. Trends Microbiol 23:775–788. https://doi.org/10.1016/j.tim.2015.09.002

Majik MS, Naik D, Bhat C, Tilve S, Tilvi S, D'Souza L (2013) Synthesis of (R)-norbgugaine and its potential as quorum sensing inhibitor against *Pseudomonas aeruginosa*. Bioorg Med Chem Lett 23:2353–2356. https://doi.org/10.1016/j.bmcl.2013.02.051

Manner S, Fallarero A (2018) Screening of natural product derivatives identifies two structurally related flavonoids as potent quorum sensing inhibitors against Gram-negative bacteria. Int J Mol Sci:19. https://doi.org/10.3390/ijms19051346

Marko VA, Kilmury SLN, MacNeil LT, Burrows LL (2018) *Pseudomonas aeruginosa* type IV minor pilins and PilY1 regulate virulence by modulating FimS-AlgR activity. PLoS Pathog 14:e1007074. https://doi.org/10.1371/journal.ppat.1007074

Masak J, Cejkova A, Schreiberova O, Rezanka T (2014) *Pseudomonas* biofilms: possibilities of their control. FEMS Microbiol Ecol 89:1–14. https://doi.org/10.1111/1574-6941.12344

Maurice NM, Bedi B, Sadikot RT (2018) *Pseudomonas aeruginosa* biofilms: host response and clinical implications in lung infections. Am J Respir Cell Mol Biol 58:428–439. https://doi.org/10.1165/rcmb.2017-0321TR

Mulcahy LR, Isabella VM, Lewis K (2014) *Pseudomonas aeruginosa* biofilms in disease. Microb Ecol 68:1–12. https://doi.org/10.1007/s00248-013-0297-x

Musthafa KS, Balamurugan K, Pandian SK, Ravi AV (2012) 2,5-Piperazinedione inhibits quorum sensing-dependent factor production in *Pseudomonas aeruginosa* PAO1. J Basic Microbiol 52:679–686. https://doi.org/10.1002/jobm.201100292

Nirody JA, Sun Y-R, Lo C-J (2017) The biophysicist's guide to the bacterial flagellar motor. Advances in Physics: X 2:324–343. https://doi.org/10.1080/23746149.2017.1289120

Nolan LM, Cavaliere R, Turnbull L, Whitchurch CB (2015) Extracellular ATP inhibits twitching motility-mediated biofilm expansion by *Pseudomonas aeruginosa*. BMC Microbiol 15:55. https://doi.org/10.1186/s12866-015-0392-x

Norizan SN, Yin WF, Chan KG (2013) Caffeine as a potential quorum sensing inhibitor. Sensors (Basel) 13:5117–5129. https://doi.org/10.3390/s130405117

Oloketuyi SF, Khan F (2017) Strategies for biofilm inhibition and virulence attenuation of foodborne pathogen-*Escherichia coli* O157:H7. Curr Microbiol 74:1477–1489. https://doi.org/10.1007/s00284-017-1314-y

O'May C, Ciobanu A, Lam H, Tufenkji N (2012) Tannin derived materials can block swarming motility and enhance biofilm formation in *Pseudomonas aeruginosa*. Biofouling 28:1063–1076. https://doi.org/10.1080/08927014.2012.725130

Oura H, Tashiro Y, Toyofuku M, Ueda K, Kiyokawa T, Ito S, Takahashi Y, Lee S, Nojiri H, Nakajima-Kambe T, Uchiyama H, Futamata H, Nomura N (2015) Inhibition of *Pseudomonas aeruginosa* swarming motility by 1-naphthol and other bicyclic compounds bearing hydroxyl groups. Appl Environ Microbiol 81:2808–2818. https://doi.org/10.1128/AEM.04220-14

Packiavathy IA, Priya S, Pandian SK, Ravi AV (2014) Inhibition of biofilm development of uropathogens by curcumin - an anti-quorum sensing agent from *Curcuma longa*. Food Chem 148:453–460. https://doi.org/10.1016/j.foodchem.2012.08.002

Parai D, Banerjee M, Dey P, Chakraborty A, Islam E, Mukherjee SK (2018) Effect of reserpine on *Pseudomonas aeruginosa* quorum sensing mediated virulence factors and biofilm formation. Biofouling 34:320–334. https://doi.org/10.1080/08927014.2018.1437910

Patriquin GM, Banin E, Gilmour C, Tuchman R, Greenberg EP, Poole K (2008) Influence of quorum sensing and iron on twitching motility and biofilm formation in *Pseudomonas aeruginosa*. J Bacteriol 190:662–671. https://doi.org/10.1128/JB.01473-07

Pattnaik SS, Ranganathan S, Ampasala DR, Syed A, Ameen F, Busi S (2018) Attenuation of quorum sensing regulated virulence and biofilm development in *Pseudomonas aeruginosa* PAO1 by Diaporthe phaseolorum SSP12. Microb Pathog 118:177–189. https://doi.org/10.1016/j.micpath.2018.03.031

Pejin B, Ciric A, Markovic JD, Glamoclija J, Nikolic M, Stanimirovic B, Sokovic M (2015) Quercetin potently reduces biofilm formation of the strain *Pseudomonas aeruginosa* PAO1 in vitro. Curr Pharm Biotechnol 16:733–737

Petrov A, Lombardo S, Audette GF (2013) Fibril-mediated oligomerization of pilin-derived protein nanotubes. J Nanobiotechnology 11:24. https://doi.org/10.1186/1477-3155-11-24

Qu L, She P, Wang Y, Liu F, Zhang D, Chen L, Luo Z, Xu H, Qi Y, Wu Y (2016) Effects of norspermidine on *Pseudomonas aeruginosa* biofilm formation and eradication. Microbiologyopen 5:402–412. https://doi.org/10.1002/mbo3.338

Römling U, Galperin MY, Gomelsky M (2013) Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. Microbiol Mol Biol Rev 77:1–52. https://doi.org/10.1128/MMBR.00043-12

Roudashti S, Zeighami H, Mirshahabi H, Bahari S, Soltani A, Haghi F (2017) Synergistic activity of sub-inhibitory concentrations of curcumin with ceftazidime and ciprofloxacin against *Pseudomonas aeruginosa* quorum sensing related genes and virulence traits. World J Microbiol Biotechnol 33:50. https://doi.org/10.1007/s11274-016-2195-0

Roy R, Tiwari M, Donelli G, Tiwari V (2018) Strategies for combating bacterial biofilms: a focus on anti-biofilm agents and their mechanisms of action. Virulence 9:522–554. https://doi.org/10.1080/21505594.2017.1313372

Scoffone VC, Trespidi G, Chiarelli LR, Barbieri G, Buroni S (2019) Quorum sensing as antivirulence target in cystic fibrosis pathogens. Int J Mol Sci:20. https://doi.org/10.3390/ijms20081838

Sheng JY, Chen TT, Tan XJ, Chen T, Jia AQ (2015) The quorum-sensing inhibiting effects of stilbenoids and their potential structure-activity relationship. Bioorg Med Chem Lett 25:5217–5220. https://doi.org/10.1016/j.bmcl.2015.09.064

Shrout JD, Chopp DL, Just CL, Hentzer M, Givskov M, Parsek MR (2006) The impact of quorum sensing and swarming motility on *Pseudomonas aeruginosa* biofilm formation is nutritionally conditional. Mol Microbiol 62:1264–1277. https://doi.org/10.1111/j.1365-2958.2006.05421.x

Singh VK, Mishra A, Jha B (2019) 3-Benzyl-Hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione extracted from exiguobacterium indicum showed anti-biofilm activity against *Pseudomonas aeruginosa* by attenuating quorum sensing. Front Microbiol 10:1269. https://doi.org/10.3389/fmicb.2019.01269

Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrock-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, Lim R, Smith K, Spencer D, Wong GK, Wu Z, Paulsen IT, Reizer J, Saier MH, Hancock RE, Lory S, Olson MV (2000) Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature 406:959–964. https://doi.org/10.1038/35023079

Tapia-Rodriguez MR, Bernal-Mercado AT, Gutierrez-Pacheco MM, Vazquez-Armenta FJ, Hernandez-Mendoza A, Gonzalez-Aguilar GA, Martinez-Tellez MA, Nazzaro F, Ayala-Zavala JF (2019) Virulence of *Pseudomonas aeruginosa* exposed to carvacrol: alterations of the quorum sensing at enzymatic and gene levels. J Cell Commun Signal:1–7. https://doi.org/10.1007/s12079-019-00516-8

Topa SH, Subramoni S, Palombo EA, Kingshott P, Rice SA, Blackall LL (2018) Cinnamaldehyde disrupts biofilm formation and swarming motility of *Pseudomonas aeruginosa*. Microbiology 164:1087–1097. https://doi.org/10.1099/mic.0.000692

Tremblay J, Deziel E (2010) Gene expression in *Pseudomonas aeruginosa* swarming motility. BMC Genomics 11:587. https://doi.org/10.1186/1471-2164-11-587

Turkina MV, Vikstrom E (2019) Bacteria-host crosstalk: sensing of the quorum in the context of *Pseudomonas aeruginosa* infections. J Innate Immun 11:263–279. https://doi.org/10.1159/000494069

Turner KH, Everett J, Trivedi U, Rumbaugh KP, Whiteley M (2014) Requirements for *Pseudomonas aeruginosa* acute burn and chronic surgical wound infection. PLoS Genet 10:e1004518. https://doi.org/10.1371/journal.pgen.1004518

Tyers M, Wright GD (2019) Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nat Rev Microbiol 17:141–155. https://doi.org/10.1038/s41579-018-0141-x

Ugurlu A, Karahasan Yagci A, Ulusoy S, Aksu B, Bosgelmez-Tinaz G (2016) Phenolic compounds affect production of pyocyanin, swarming motility and biofilm formation of *Pseudomonas aeruginosa*. Asian Pacific Journal of Tropical Biomedicine 6:698–701. https://doi.org/10.1016/j.apjtb.2016.06.008

Ulrey RK, Barksdale SM, Zhou W, van Hoek ML (2014) Cranberry proanthocyanidins have anti-biofilm properties against *Pseudomonas aeruginosa*. BMC Complement Altern Med 14:499. https://doi.org/10.1186/1472-6882-14-499

Usjak D, Ivkovic B, Bozic DD, Boskovic L, Milenkovic M (2019) Antimicrobial activity of novel chalcones and modulation of virulence factors in hospital strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Microb Pathog 131:186–196. https://doi.org/10.1016/j.micpath.2019.04.015

Uusitalo P, Hagglund U, Rhoos E, Scherman Norberg H, Elofsson M, Sundin C (2017) The salicylidene acylhydrazide INP0341 attenuates *Pseudomonas aeruginosa* virulence in vitro and in vivo. J Antibiot (Tokyo) 70:937–943. https://doi.org/10.1038/ja.2017.64

Vadekeetil A, Saini H, Chhibber S, Harjai K (2016) Exploiting the antivirulence efficacy of an ajoene-ciprofloxacin combination against *Pseudomonas aeruginosa* biofilm associated murine acute pyelonephritis. Biofouling 32:371–382. https://doi.org/10.1080/08927014.2015.1137289

Valentini M, Filloux A (2016) Biofilms and cyclic di-GMP (c-di-GMP) signaling: lessons from *Pseudomonas aeruginosa* and other bacteria. J Biol Chem 291:12547–12555. https://doi.org/10.1074/jbc.R115.711507

Valentini M, Gonzalez D, Mavridou DA, Filloux A (2018) Lifestyle transitions and adaptive pathogenesis of *Pseudomonas aeruginosa*. Curr Opin Microbiol 41:15–20. https://doi.org/10.1016/j.mib.2017.11.006

Valentini M, Laventie B-J, Moscoso J, Jenal U, Filloux A (2016) The diguanylate cyclase HsbD intersects with the hptb regulatory cascade to control *Pseudomonas aeruginosa* biofilm and motility. PLoS Genet 12:e1006354–e1006354. https://doi.org/10.1371/journal.pgen.1006354

Vasavi HS, Sudeep HV, Lingaraju HB, Shyam Prasad K (2017) Bioavailability-enhanced Resveramax modulates quorum sensing and inhibits biofilm formation in *Pseudomonas aeruginosa* PAO1. Microb Pathog 104:64–71. https://doi.org/10.1016/j.micpath.2017.01.015

Wang H, Chu W, Ye C, Gaeta B, Tao H, Wang M, Qiu Z (2019a) Chlorogenic acid attenuates virulence factors and pathogenicity of *Pseudomonas aeruginosa* by regulating quorum sensing. Appl Microbiol Biotechnol 103:903–915. https://doi.org/10.1007/s00253-018-9482-7

Wang TN, Guan QT, Pain A, Kaksonen AH, Hong PY (2019b) Discovering, characterizing, and applying acyl homoserine lactone-quenching enzymes to mitigate microbe-associated problems under saline conditions. Front Microbiol 10:823. https://doi.org/10.3389/fmicb.2019.00823

Wilhelm S, Gdynia A, Tielen P, Rosenau F, Jaeger KE (2007) The autotransporter esterase EstA of *Pseudomonas aeruginosa* is required for rhamnolipid production, cell motility, and biofilm formation. J Bacteriol 189:6695–6703. https://doi.org/10.1128/JB.00023-07

Wolfe AJ, Visick KL (2008) Get the message out: cyclic-Di-GMP regulates multiple levels of flagellum-based motility. J Bacteriol 190:463–475. https://doi.org/10.1128/JB.01418-07

Wu H, Lee B, Yang L, Wang H, Givskov M, Molin S, Høiby N, Song Z (2011) Effects of ginseng on *Pseudomonas aeruginosa* motility and biofilm formation. FEMS Immunol Med Microbiol 62:49–56. https://doi.org/10.1111/j.1574-695X.2011.00787.x

Wu H, Moser C, Wang HZ, Hoiby N, Song ZJ (2015a) Strategies for combating bacterial biofilm infections. Int J Oral Sci 7:1–7. https://doi.org/10.1038/ijos.2014.65

Wu DQ, Huang WF, Duan QJ, Cheng HJ, Wang CZ (2015b) Sodium houttuyfonate inhibits virulence related motility of *Pseudomonas aeruginosa*. Zhongguo Zhong Yao Za Zhi 40:1585–1588

Yang R, Guan Y, Zhou J, Sun B, Wang Z, Chen H, He Z, Jia A (2017) Phytochemicals from camellia nitidissima chi flowers reduce the pyocyanin production and motility of *Pseudomonas aeruginosa* PAO1. Front Microbiol 8:2640. https://doi.org/10.3389/fmicb.2017.02640

Yeung AT, Torfs EC, Jamshidi F, Bains M, Wiegand I, Hancock RE, Overhage J (2009) Swarming of *Pseudomonas aeruginosa* is controlled by a broad spectrum of transcriptional regulators, including. MetR J Bacteriol 191:5592–5602. https://doi.org/10.1128/JB.00157-09

Yin H, Deng Y, Wang H, Liu W, Zhuang X, Chu W (2015) Tea polyphenols as an antivirulence compound disrupt quorum-sensing regulated pathogenicity of *Pseudomonas aeruginosa*. Sci Rep 5:16158. https://doi.org/10.1038/srep16158

Zhang M, Wang M, Zhu X, Yu W, Gong Q (2018) Equisetin as potential quorum sensing inhibitor of *Pseudomonas aeruginosa*. Biotechnol Lett 40:865–870. https://doi.org/10.1007/s10529-018-2527-2

Zhao K, Tseng BS, Beckerman B, Jin F, Gibiansky ML, Harrison JJ, Luijten E, Parsek MR, Wong GCL (2013) Psl trails guide exploration and microcolony formation in Pseudomonas aeruginosa biofilms. Nature 497:388–391. https://doi.org/10.1038/nature12155

Zhao J, Jiang H, Cheng W, Wu J, Zhao J, Wang J, Dong L (2015) The role of quorum sensing system in antimicrobial induced ampC expression in *Pseudomonas aeruginosa* biofilm. J Basic Microbiol 55: 671–678. https://doi.org/10.1002/jobm.201300987

Zhao J, Li X, Hou X, Quan C, Chen M (2019) Widespread existence of quorum sensing inhibitors in marine bacteria: potential drugs to combat pathogens with novel strategies. Mar Drugs:17. https://doi.org/10.3390/md17050275

Zhou JW, Luo HZ, Jiang H, Jian TK, Chen ZQ, Jia AQ (2018) Hordenine: a novel quorum sensing inhibitor and antibiofilm agent against *Pseudomonas aeruginosa*. J Agric Food Chem 66:1620–1628. https://doi.org/10.1021/acs.jafc.7b05035

Zhou G, Peng H, Wang YS, Huang XM, Xie XB, Shi QS (2019) Enhanced synergistic effects of xylitol and isothiazolones for inhibition of initial biofilm formation by *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538. J Oral Sci 61:255–263. https://doi.org/10.2334/josnusd.18-0102