World J Microbiol Biotechnol (2016) 32:150
DOI 10.1007/s11274-016-2105-5

ORIGINAL PAPER

Lichen secondary metabolite evernic acid as potential quorum sensing inhibitor against *Pseudomonas aeruginosa*

Barış Gökalsın[1] · **Nüzhet Cenk Sesal**[1]

Received: 19 February 2016 / Accepted: 27 June 2016 / Published online: 27 July 2016

Abstract Cystic Fibrosis is a genetic disease and it affects the respiratory and digestive systems. *Pseudomonas aeruginosa* infections in Cystic Fibrosis are presented as the main cause for high mortality and morbidity rates. *Pseudomonas aeruginosa* populations can regulate their virulence gene expressions via the bacterial communication system: quorum sensing. Inhibition of quorum sensing by employing quorum sensing inhibitors can leave the bacteria vulnerable. Therefore, determining natural sources to obtain potential quorum sensing inhibitors is essential. Lichens have ethnobotanical value for their medicinal properties and it is possible that their secondary metabolites have quorum sensing inhibitor properties. This study aims to investigate an alternative treatment approach by utilizing lichen secondary metabolite evernic acid to reduce the expressions of *Pseudomonas aeruginosa* virulence factors by inhibiting quorum sensing. For this purpose, fluorescent monitor strains were utilized for quorum sensing inhibitor screens and quantitative reverse-transcriptase PCR analyses were conducted for comparison. Results indicate that evernic acid is capable of inhibiting *Pseudomonas aeruginosa* quorum sensing systems.

Keywords Quorum sensing · *Pseudomonas aeruginosa* · Lichen secondary metabolites · Evernic acid

✉ Nüzhet Cenk Sesal
csesal@marmara.edu.tr

[1] Department of Biology, Faculty of Arts and Sciences, Marmara University, Göztepe Campus, 34722 Istanbul, Turkey

Introduction

Cystic Fibrosis (CF) is a genetic disease seen in the lungs, intestines, pancreas and sweat glands; it affects the respiratory and digestive systems through elevated viscosity of fluids. CF especially impairs the respiratory system and causes degraded lung activity (Caldas and Boisrame 2015). *Pseudomonas aeruginosa* infections in CF are presented as the main cause for high mortality and morbidity rates. Several antibiotics are utilized in treating CF for repressing chronic infections. However, medical treatments are not sufficient in some cases. Studies suggest that the failure to cure the disease might be caused by the biofilm form of *P. aeruginosa's* resistance to antibiotics (Proesmans et al. 2013). This study investigates the possibility of an alternative treatment approach by utilizing lichen secondary metabolites to reduce the expressions of *P. aeruginosa* virulence factors.

P. aeruginosa is an opportunistic pathogen. It is resistant to multiple antibiotics and causes pneumonia, sepsis, acute infections and chronic lung infections (Jones et al. 2009; Lambert et al. 2011; Zhanel et al. 2004). *P. aeruginosa* can adapt and grow in diverse environmental conditions. They can receive signals from their surroundings and react to these signals by organizing their gene expressions. However, bacteria can also receive signals from other bacteria and regulate gene expressions as a population. This mechanism is called quorum sensing (QS) (Bassler 2015).

Gram-negative bacteria utilize the QS system to express virulence factors by constantly releasing signal molecules called N-acyl-homoserine lactones (AHLs) (Jakobsen et al. 2013). As the bacteria population grows, the concentration of AHLs increase and they reach a threshold. At this point the expression of the genes controlled by QS are induced. The QS system is known to regulate many physiological

characteristics such as virulence, motility, conjugation and bioluminescence (Girard and Bloemberg 2008).

Pseudomonas aeruginosa QS consist of two important systems: LasIR and RhlIR. LasI synthesizes *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) and RhlI synthesizes *N*-butanoyl homoserine lactone (C4-HSL). At high concentrations, these signal molecules called autoinducers (AIs) bind to their receptors and activate transcriptional regulators LasR and RhlR. The AI-Regulator complex then binds to the QS related genes' promoter sites and start the expression of the genes (Zhang et al. 2002). *P. aeruginosa* has a third QS system that utilizes *Pseudomonas* quinolone signal (PQS). This system is believed to act as a mediatory between the other two systems (Diggle et al. 2003; McGrath et al. 2004).

Utilizing QS, *P. aeruginosa* form biofilm to protect them against harsh conditions such as pH, ultraviolet light, heat etc. (Boor 2006; Shannon and French 2004). The biofilm form gives them a substantial advantage over planktonic form, one of which is antibiotic resistance. (Brackman and Coenye 2015). Antibiotic resistance of microorganisms has become a significant problem in recent years. The novel approach to fight against bacteria without risking antibiotic resistance is to target their QS systems (Hentzer et al. 2003a). Inhibition of QS by employing quorum sensing inhibitors (QSIs) can leave the bacteria vulnerable and easier to fight against (Zeng et al. 2008).

At this stage, the need for determining natural sources that can inhibit quorum sensing with secondary metabolites arises (Pompilio et al. 2013). Plants or natural or semi-synthetic products obtained from them are preferred for the development of pharmaceutical raw materials. Like plants, lichens also have been used in the treatment of many diseases for their medicinal properties since ancient times. Therefore they can also be used for developing new pharmaceutical products.

Lichens are complex organisms composed of algae or cyanobacteria (photobiont) living together with fungi (mycobiont) in a symbiotic relationship (Nash 2008). Studies demonstrate that the healing properties of lichens used in the prevention of diseases results from their secondary metabolite components with acidic properties (Acikgoz et al. 2013; Nguyen et al. 2014). Lichens are exposed to a number of biotic and abiotic stresses and the production of secondary metabolites is one way of coping with this (Deduke et al. 2012; Fernandez-Moriano et al. 2016). Lichens have approximately 1150 secondary metabolites as a result of their metabolism (Shukla et al. 2010). These metabolites have antibacterial, antiviral, antimicrobial, antifungal, antioxidant, antitumor and enzyme inhibiting properties (Acikgoz et al. 2013; Basile et al. 2015; Bhattarai et al. 2008; Nguyen et al. 2014). Therefore, it is essential to determine QSI potentials and healing properties of lichens, and have specific knowledge of the active compounds. Such a compound is evernic acid: a secondary metabolite that can be isolated from *Evernia* species, and is known to have antifungal properties (Marijana and Branislav 2011).

In this study, a lichen secondary metabolite evernic acid was tested for its QSI activity against *P. aeruginosa*. Fluorescent monitor strains were utilized for QSI screens and quantitative reverse-transcriptase PCR (qRT-PCR) analyses were conducted for comparison.

Materials and methods

Bacterial strains

Pseudomonas aeruginosa strains used in this study are listed in Table 1. Bacteria strains were grown in M9 minimal media supplemented with 2.5 mg of thiamine per liter, 0.5 % (wt/vol) glucose, and 0.5 % (wt/vol) Casamino Acids. *lasB-gfp* and *rhlA-gfp* strains were used for QSI screens and wild type *P. aeruginosa* strain PAO1 was used for qRT-PCR analyses. The monitor strains *lasB-gfp* and *rhlA-gfp* are developed by Hentzer et al. (2002) and Yang et al. (2009). They contain a mini-Tn*5* insert containing well characterized *lasB* and *rhlA* promoters that are regulated by *lasR* and *rhlR*, and genes for an unstable green fluorescent protein (GFP). Strains are highly sensitive even with a single chromosomal copy and very low AHL concentrations. All strains were obtained from Tim Holm Jakobsen (University of Copenhagen, Copenhagen, Denmark).

P. aeruginosa QSI screens

QSI screenings were performed according to a modified method of that described by Bjarnsholt et al. (2010). Growth medium (100 µl M9 medium with 0.5 % [w/v] Casamino Acids, 2.5 mg of thiamine liter^{-1} and 0.5 % [w/v] glucose) were added to each well of 96-well black microplates (Nunc, Thermo Scientific) and two-fold serial dilutions of evernic acid were made. An overnight culture of the *lasB-gfp* or *rhlA-gfp* monitor strain were added to

Table 1 Strains used in this study. PAO1 strain was used as wild type *rhlA-gfp* and *lasB-gfp* strains were used as fluorescent monitors for QS inhibition

Species	Name/genotype	Reference
P. aeruginosa	PAO1 (wild type)	(Holloway and Morgan 1986)
	rhlA-gfp	(Yang et al. 2009)
	lasB-gfp	(Hentzer et al. 2002)

obtain a total volume of 200 µl and give an OD 450 nm of 0.1. As a result, 116 µM, 58 µM, 29 µM, 14.5 µM and 7.25 µM final concentrations of evernic acid were achieved. Growth and green fluorescent protein (GFP) expressions were monitored using Cytation 3 multimode microplate reader (Biotek) at 34 °C for 15 h, measuring every 15 min. Fluorescence of GFP expression were measured at 485 nm excitation and 535 nm emission wavelengths and growth was measured at OD 450 nm.

qRT-PCR

Overnight cultures of *P. aeruginosa* PAO1 strain were treated with same concentrations of evernic acid (Extrasynthese) as in the QSI screens. Cultures were incubated at 34 °C for 15 h. After incubation, samples were retrieved and RNA isolation was performed with Roche High Pure RNA Isolation Kit (Roche). RNA purities were measured and concentrations were calculated using Cytation 3 with Take3 plate (Biotek). cDNA synthesis was performed with Transcriptor First Strand cDNA Synthesis Kit (Roche). Gene expression analyses were performed using *FastStart Essential DNA Probes Master* Kit (Roche) and TaqMan probes. 45 cycles were run and the *rpoD* gene was used as control for normalization. The primer sequences are listed in Table 2.

Biofilm inhibition assay

Overnight cultures of *P. aeruginosa* PAO1 strain were diluted 1:100 into growth medium (M9 medium with 0.5 % [w/v] Casamino Acids, 2.5 mg of thiamine liter^{-1} and 0.5 % [w/v] glucose) for biofilm assays. Serial dilutions were made and 116 µM, 58 µM, 29 µM, 14.5 µM and 7.25 µM final concentrations of evernic acid were achieved on microplate. After a day of incubation at 37 °C, plate was rinsed and stained with 0.1 % crystal violet solution for 5 min. Plate was rinsed again and then allowed to dry. Finally, stains in the plate was dissolved using ethanol and absorbance measurements were made at 590 nm.

Table 2 Primer sequences used in this study

Primer	Position	Sequence
lasB F	418–436	cgcaagaccgagaatgaca
lasB R	469–487	agaccagttgggcgatgtt
rhlA F	512–529	aagccagcaaccatcagc
rhlA R	571–589	gcacctggtcgatgtgaaa
rpoD F	435–454	tccaccgacaacagctacg
rpoD R	478–495	gagctggaaaccgtggact

Results

QSI activity of evernic acid

Evernic acid concentrations have presented QS inhibition for both *las* and *rhl* systems. The results of the GFP measurements are means of three experiments and shown in Fig. 1. The results are presented as GFP expression/bacterial cell growth to make sure the reduced fluorescence is due to QS inhibition. It was observed that evernic acid is capable of inhibiting approximately 54 % of *gfp* expression of *lasB-gfp* and 50 % of *rhlA-gfp* at 116 µM when compared to the untreated control group.

qRT-PCR

The regulations of *rhlA* and *lasB* genes were analyzed and relatively quantified for treated and untreated PAO1 strain samples. The results are shown in Fig. 2. It is indicated that there is a downregulation of the genes depending on QSI concentration. Compared to *lasB*, expression of *rhlA* gene seem to be inhibited slightly more. According to results, the expression of *lasB* was downregulated by 74 % at 116 µM, 55 % at 58 µM, 46 % at 29 µM, 32 % at 14,5 µM and 18 % at 7,25 µM doses. The expression of *rhlA* was downregulated by 78 % at 116 µM, 63 % at 58 µM, 62 % at 29 µM, 44 % at 14,5 µM and 21 % at 7,25 µM doses.

Biofilm assays

Results indicate that in samples treated with evernic acid, biofilm formation was significantly less than the untreated control group. Measured absorbance (590 nm) levels are shown in Fig. 3. Results are means of 5 experiments.

Discussion

The antibiotic resistance of bacteria and resulting ineffective treatments are a growing concern worldwide. Numerous studies focus on this problem and seek alternative treatment solutions. QS studies carry weight in current studies to find an alternative treatment to antibiotics. Trying to exterminate the bacteria induces stress and causes them to protect themselves via biofilm. The biofilm forms enable bacteria to grow in more secure conditions and gain antibiotic resistance (Brackman and Coenye 2015; Fux et al. 2005). Instead of attacking bacteria, the safer approach appears to be to block their communication and thus reduce virulence factors by inhibiting QS (Hentzer et al. 2003a). In this regard, it is important to discover potential QSIs from natural resources.

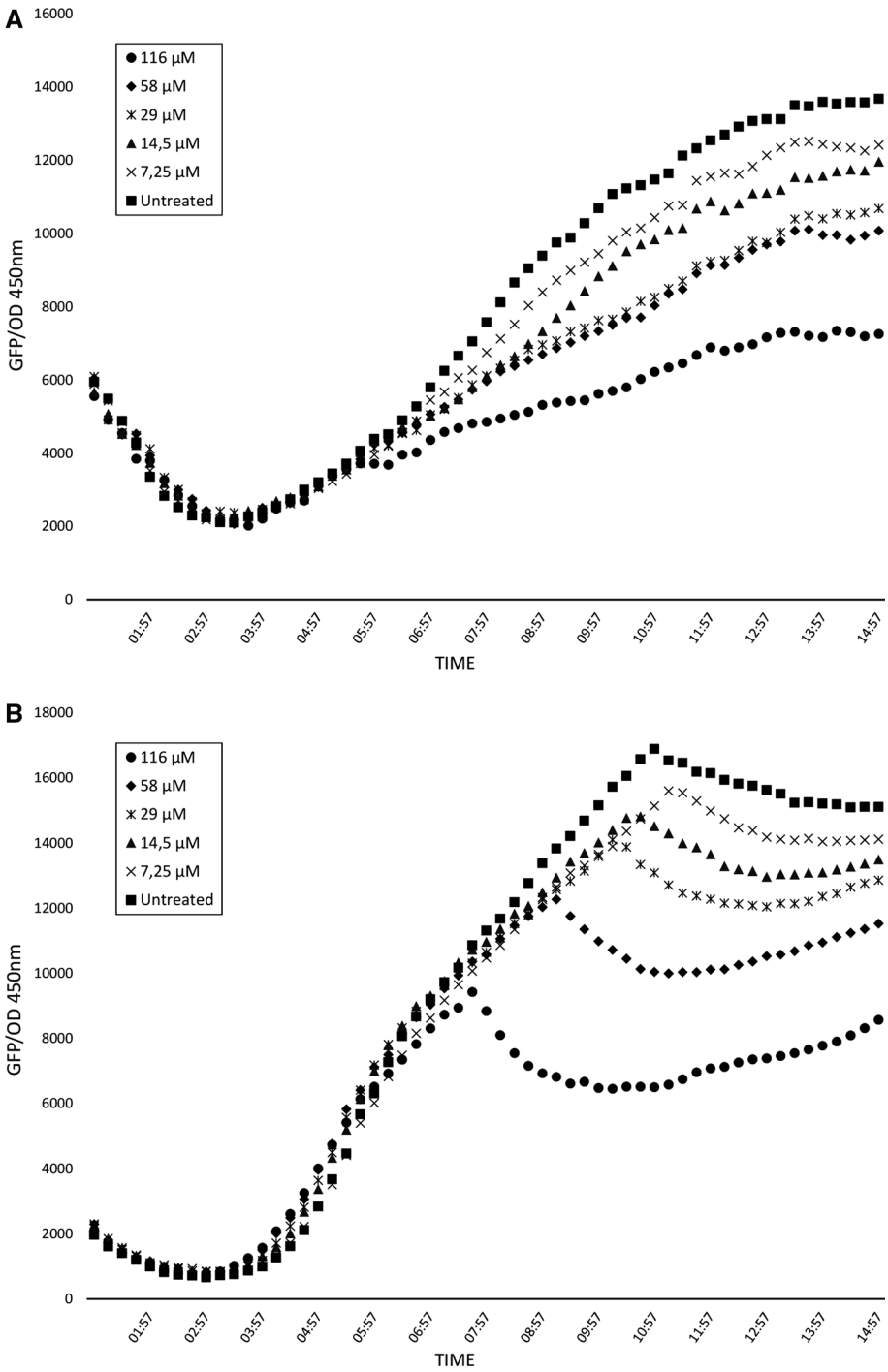

Fig. 1 Dose response curves of *lasB-gfp* **a** and *rhlA-gfp* **b** monitor strains treated with evernic acid concentrations between 7.25 and 116 µM, compared to untreated samples

Plants and fungi are notable resources with their natural products for drug research and QS inhibition studies. Several strong QSIs have been identified to date (Al-Ani et al. 2015; Azimi et al. 2012; Savo et al. 2015; Truchado et al. 2012). Lichens, a symbiotic relationship between fungi and algae or cyanobacteria, are also known to have ethnobotanical value and produce unique secondary metabolites. Therefore, it is necessary to explore the QSI properties of lichen secondary metabolites such as evernic acid.

Pseudomonas aeruginosa has become a model organism for QS studies (Hentzer et al. 2002). As in many bacteria, *P. aeruginosa* can also determine its population density by excreting and translating communication signals with the QS mechanism (De Kievit 2009). Moreover, this cell density centered system regulates various genes most of

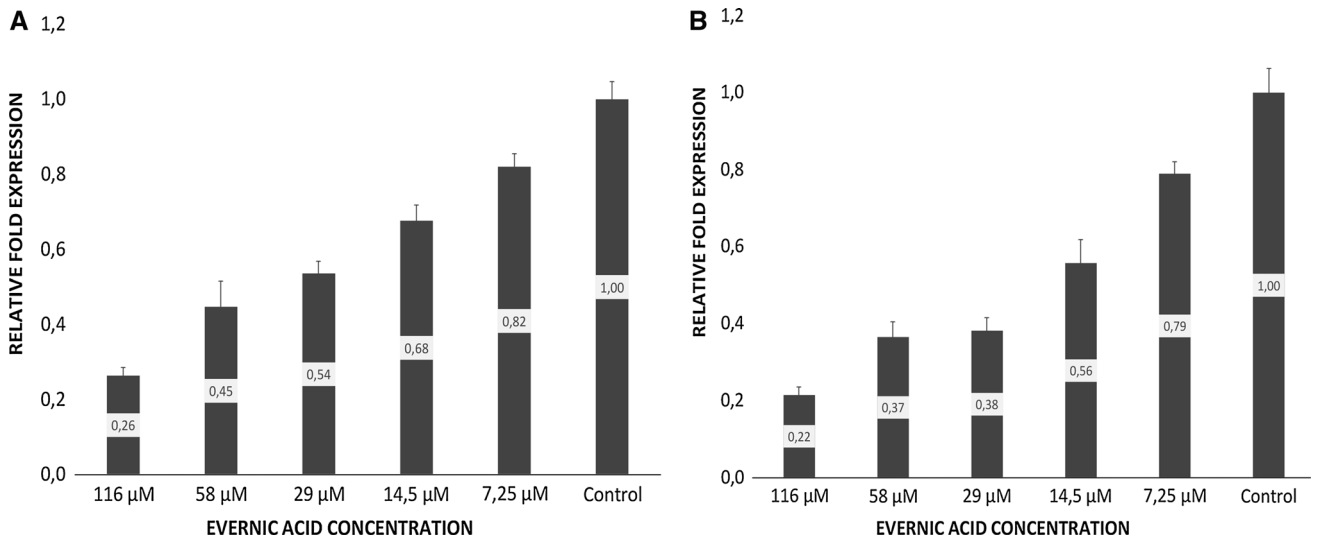

Fig. 2 Gene expression levels of *lasB* **a** and *rhlA* **b** compared to the untreated control group. Data show representative experiment from three individual qRT-PCR determinations. Gene expressions were normalized to expression of *rpoD*

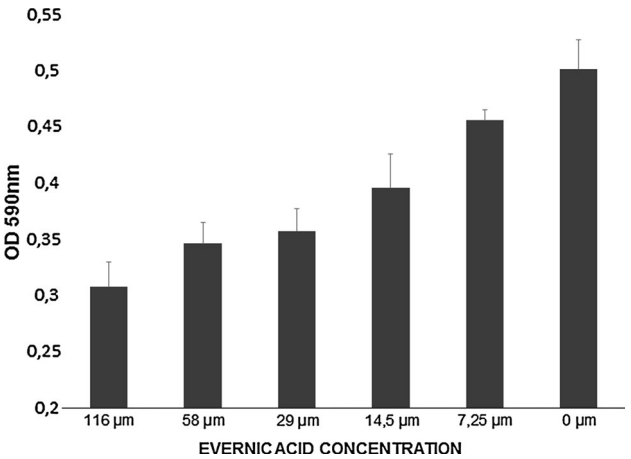

Fig. 3 Biofilm formation of PAO1 strain treated with evernic acid concentrations, compared to untreated control sample. Results are means of 5 individual experiments

which are virulence factors of *P. aeruginosa*. These genes include lasB (elastase), the rhlAB operon (rhamnolipid production), the hcnABC operon (hydrogen cyanide production) and chiC (chitinase) (Hentzer et al. 2003b).

In addition to two important QS systems of *P. aeruginosa*, there are two other systems that are activated by their unique signals: Pseudomonas Quinolone Signal (PQS) and Integrated Quorum Sensing Signal (IQS) (Pesci et al. 1999; Lee et al. 2013). PQS system has a role in regulation of virulence factors by mediating between *las* and *rhl* systems, but its importance in virulence remains debatable (Lee and Zhang 2015). Since both *rhl* and PQS systems are governed by the *las* system, its inhibition takes priority regarding virulence. Recently discovered IQS on the other hand, is reported to take over *las* system under phosphate depletion stress conditions (Lee et al. 2013).

In this study, two monitor strains *lasB-gfp* and *rhlA-gfp* were treated with evernic acid concentrations between 7.25 and 116 µM, and monitored for QSI screenings. PAO1 strain was used in biofilm assays. In addition, the expression levels of *lasB* and *rhlA* genes were analyzed using qRT-PCR method for confirmation. These genes are pivitol due to the virulence factors elastase and rhamnolipids encoded by them.

Measurements with fluorescent monitor strains indicate that both QS systems (LasIR and RhlIR) of *P. aeruginosa* were inhibited to approximately 50 % at 116 µM evernic acid concentration. In 2012, Jakobsen et al. have tested "iberin" from horseradish on *P. aeruginosa* and present that iberin can inhibit the expression of *lasB-gfp* fusion completely at 100 µM without affecting growth (Jakobsen et al. 2012). In this regard, we couldn't achieve total inhibition with evernic acid, but it seems possible if it is applied in higher concentrations. According to the results, qRT-PCR method also confirms the down-regulation of *lasB* and *rhlA* genes and thus the inhibition of QS systems by evernic acid. This suggests that PQS system is also presumably inhibited since its regulation is also governed by the *las* system. Therefore, the outcomes of this study presents unique opportunities concerning the utilization of lichen secondary metabolites for QS inhibition studies.

Inhibition of QS is an effective strategy against infections caused by pathogenic bacteria. Determining potential QSIs and effective screenings are essential for this strategy. This study shows that a lichen secondary metabolite evernic acid is capable of inhibiting QS systems of the

opportunistic pathogen *P. aeruginosa*. Findings of this study indicate that lichens are probably a wide resource for secondary metabolites capable of inhibiting QS. Their unique secondary metabolites present an opportunity for discovering new therapeutic agents and raw materials for future anti-QS drugs. Therefore, with additional research it is safe to assume that an alternative treatment can be developed for of CF patients with *P. aeruginosa* infections.

Acknowledgments We thank Tim Holm Jakobsen and Michael Givskov for providing us the monitor strains. This study was funded by the projects: TUBITAK 113S306 (COST FA1202) and BAPKO FEN-A-130515-0177.

References

Acikgoz B, Karalti I, Ersoz M, Coskun ZM, Cobanoglu G, Sesal C (2013) Screening of antimicrobial activity and cytotoxic effects of two Cladonia species. Z Naturforsch C 68:191–197

Al-Ani I, Zimmermann S, Reichling J, Wink M (2015) Pharmacological synergism of bee venom and melittin with antibiotics and plant secondary metabolites against multi-drug resistant microbial pathogens. Phytomedicine 22:245–255. doi:10.1016/j.phymed.2014.11.019

Azimi H, Khakshur AA, Aghdasi I, Fallah-Tafti M, Abdollahi M (2012) A review of animal and human studies for management of benign prostatic hyperplasia with natural products: perspective of new pharmacological agents. Inflamm Allergy Drug Targets 11:207–221

Basile A et al (2015) Antiproliferative, antibacterial and antifungal activity of the lichen *Xanthoria parietina* and its secondary metabolite parietin. Int J Mol Sci 16:7861–7875

Bassler B (2015) Manipulating quorum sensing to control bacterial pathogenicity. FASEB J 29(Suppl 1):88-1

Bhattarai HD, Paudel B, Hong SG, Lee HK, Yim JH (2008) Thin layer chromatography analysis of antioxidant constituents of lichens from Antarctica. J Nat Med 62:481–484

Bjarnsholt T, van Gennip M, Jakobsen TH, Christensen LD, Jensen PO, Givskov M (2010) In vitro screens for quorum sensing inhibitors and in vivo confirmation of their effect. Nat Protoc 5:282–293. doi:10.1038/nprot.2009.205

Boor KJ (2006) Bacterial stress responses: what doesn't kill them can make them stronger. PLoS Biol 4:18–20. doi:10.1371/journal.pbio.0040023

Brackman G, Coenye T (2015) Quorum sensing ınhibitors as anti-biofilm agents. Curr Pharm Des 21:5–11

Caldas RR, Boisrame S (2015) Upper aero-digestive contamination by *Pseudomonas aeruginosa* and implications in Cystic Fibrosis. J Cystic Fibros 14:6–15. doi:10.1016/j.jcf.2014.04.008

De Kievit T (2009) Quorum sensing in *Pseudomonas aeruginosa* biofilms. Environ Microbiol 11:279–288

Deduke C, Timsina B, Piercey-Normore MD (2012) Effect of environmental change on secondary metabolite production in lichen-forming fungi. In: Young S (ed) International perspectives on global environmental change. InTech, Rejika, Croatia

Diggle SP, Winzer K, Chhabra SR, Chhabra SR, Worrall KE, Camara M, Williams P (2003) The *Pseudomonas aeruginosa* quinolone signal molecule overcomes the cell density-dependency of the quorum sensing hierarchy, regulates rhl-dependent genes at the onset of stationary phase and can be produced in the absence of LasR. Mol Microbiol 50:29–43. doi:10.1046/j.1365-2958.2003.03672.x

Fernandez-Moriano C, Gomez-Serranillos MP, Crespo A (2016) Antioxidant potential of lichen species and their secondary metabolites. A systematic review. Pharm Biol 54:1–17. doi:10.3109/13880209.2014.1003354

Fux CA, Costerton JW, Stewart PS, Stoodley P (2005) Survival strategies of infectious biofilms. Trends Microbiol 13:34–40. doi:10.1016/j.tim.2004.11.010

Girard G, Bloemberg GV (2008) Central role of quorum sensing in regulating the production of pathogenicity factors in *Pseudomonas aeruginosa*. Future Microbiol 3:97–106. doi:10.2217/17460913.3.1.97

Hentzer M et al (2002) Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. Microbiology-Sgm 148:87–102

Hentzer M, Eberl L, Nielsen J, Givskov M (2003a) Quorum sensing—a novel target for the treatment of biofilm infections. Biodrugs 17:241–250. doi:10.2165/00063030-200317040-00003

Hentzer M et al (2003b) Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J 22:3803–3815. doi:10.1093/emboj/cdg366

Holloway BW, Morgan AF (1986) Genome organization in *Pseudomonas*. Annu Rev Microbiol 40:79–105. doi:10.1146/annurev.micro.40.1.79

Jakobsen TH et al (2012) Food as a source for quorum sensing inhibitors: iberin from horseradish revealed as a quorum sensing inhibitor of *Pseudomonas aeruginosa*. Appl Environ Microbiol 78:2410–2421. doi:10.1128/Aem.05992-11

Jakobsen TH, Bjarnsholt T, Jensen PO, Givskov M, Hoiby N (2013) Targeting quorum sensing in *Pseudomonas aeruginosa* biofilms: current and emerging inhibitors. Future Microbiol 8:901–921. doi:10.2217/fmb.13.57

Jones RN, Stilwell MG, Rhomberg PR, Sader HS (2009) Antipseudomonal activity of piperacillin/tazobactam: more than a decade of experience from the SENTRY Antimicrobial Surveillance Program (1997–2007). Diagn Microbiol Infect Dis 65:331–334. doi:10.1016/j.diagmicrobio.2009.06.022

Lambert ML et al (2011) Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. Lancet Infect Dis 11:30–38. doi:10.1016/S1473-3099(10)70258-9

Lee J, Zhang L (2015) The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. Protein Cell 6:26–41

Lee J et al (2013) A cell-cell communication signal integrates quorum sensing and stress response. Nat Chem Biol 9:339–343

Marijana K, Branislav R (2011) Antibacterial and antifungal activity of different lichens extracts and lichen acid. Res J Biotechnol 6:23–26

McGrath S, Wade DS, Pesci EC (2004) Dueling quorum sensing systems in *Pseudomonas aeruginosa* control the production of the *Pseudomonas* quinolone signal (PQS). FEMS Microbiol Lett 230:27–34. doi:10.1016/S0378-1097(03)00849-8

Nash TH (ed) (2008) Lichen biology. Cambridge University Press, Cambridge

Nguyen TT et al (2014) Lichen secondary metabolites in *Flavocetraria cucullata* exhibit anti-cancer effects on human cancer cells through the induction of apoptosis and suppression of tumorigenic potentials. PLoS ONE 9:e111575. doi:10.1371/journal.pone.0111575

Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP, Iglewski BH (1999) Quinolone signaling in the cell-to-cell communication system of Pseudomonas aeruginosa. Proc Natl Acad Sci 96:11229–11234

Pompilio A et al (2013) Antimicrobial and antibiofilm activity of secondary metabolites of lichens against methicillin-resistant *Staphylococcus aureus* strains from cystic fibrosis patients. Future Microbiol 8:281–292. doi:10.2217/Fmb.12.142

Proesmans M, Vermeulen F, Boulanger L, Verhaegen J, De Boeck K (2013) Comparison of two treatment regimens for eradication of *Pseudomonas aeruginosa* infection in children with cystic fibrosis. J Cystic Fibros 12:29–34. doi:10.1016/j.jcf.2012.06.001

Savo V, Joy R, Caneva G, McClatchey WC (2015) Plant selection for ethnobotanical uses on the Amalfi Coast (Southern Italy). J Ethnobiol Ethnomed 11:58. doi:10.1186/s13002-015-0038-y

Shannon KP, French GL (2004) Increasing resistance to antimicrobial agents of Gram-negative organisms isolated at a London teaching hospital, 1995–2000. J Antimicrob Chemother 53:818–825. doi:10.1093/jac/dkh135

Shukla V, Joshi GP, Rawat M (2010) Lichens as a potential natural source of bioactive compounds: a review. Phytochem Rev 9:303–314

Truchado P, Tomas-Barberan FA, Larrosa M, Allende A (2012) Food phytochemicals act as quorum sensing inhibitors reducing production and/or degrading autoinducers of *Yersinia enterocolitica* and *Erwinia carotovora*. Food Control 24:78–85. doi:10.1016/j.foodcont.2011.09.006

Yang L, Rybtke MT, Jakobsen TH, Hentzer M, Bjarnsholt T, Givskov M, Tolker-Nielsen T (2009) Computer-aided identification of recognized drugs as *Pseudomonas aeruginosa* quorum-sensing inhibitors. Antimicrob Agents Chemother 53:2432–2443. doi:10.1128/Aac.01283-08

Zeng Z et al (2008) Virtual screening for novel quorum sensing inhibitors to eradicate biofilm formation of *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 79:119–126. doi:10.1007/s00253-008-1406-5

Zhanel GG, Hoban DJ, Schurek K, Karlowsky JA (2004) Role of efflux mechanisms on fluoroquinolone resistance in *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. Int J Antimicrob Agents 24:529–535. doi:10.1016/j.ijantimicag.2004.08.003

Zhang RG et al (2002) Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. Nature 417:971–974. doi:10.1038/nature00833