Cranberry-derived proanthocyanidins impair virulence and inhibit quorum sensing of *Pseudomonas aeruginosa*

Vimal B. Maisuria¹, Yossef Lopez-de Los Santos², Nathalie Tufenkji¹ & Eric Déziel²

Bacteria have evolved multiple strategies for causing infections that include producing virulence factors, undertaking motility, developing biofilms, and invading host cells. *N*-acylhomoserine lactone (AHL)-mediated quorum sensing (QS) tightly regulates the expression of multiple virulence factors in the opportunistic pathogenic bacterium *Pseudomonas aeruginosa*. Thus, inhibiting QS could lead to health benefits. In this study, we demonstrate an anti-virulence activity of a cranberry extract rich in proanthocyanidins (cerPAC) against *P. aeruginosa* in the model host *Drosophila melanogaster* and show this is mediated by QS interference. cerPAC reduced the production of QS-regulated virulence determinants and protected *D. melanogaster* from fatal infection by *P. aeruginosa* PA14. Quantification of AHL production using liquid chromatography-mass spectrometry confirmed that cerPAC effectively reduced the level of AHLs produced by the bacteria. Furthermore, monitoring QS signaling gene expression revealed that AHL synthases LasI/RhlI and QS transcriptional regulators LasR/RhlR genes were inhibited and antagonized, respectively, by cerPAC. Molecular docking studies suggest that cranberry-derived proanthocyanidin binds to QS transcriptional regulators, mainly interacting with their ligand binding sites. These findings provide insights into the underlying mechanisms of action of a cerPAC to restrict the virulence of *P. aeruginosa* and can have implications in the development of alternative approaches to control infections.

As antibiotic resistance in microbial pathogens embodies a global threat to public health, it demands the development of novel strategies for managing microbial infections. The long-term effectiveness of most antibiotic treatments is restricted by both pathogen drug resistance and non-target effects on the host’s commensal microbial community. Over the last decade, research on antimicrobials has shifted towards an alternative approach to combat pathogens using anti-infective drugs that selectively interrupt virulence pathways to help prevent or cure bacterial infections. Anti-infective drugs that do not perturb survival or viability of bacterial pathogens should be less likely to promote resistance than conventional antibiotics. Until now, the development of anti-infective synthetic drugs has been limited to the laboratory and preclinical studies. Natural bioactive compounds derived from plant species show promising therapeutic properties to combat the emerging resistance in microbial pathogens, which can be exploited as next generation anti-infective drugs.

The fruit of the American cranberry (*Vaccinium macrocarpon* L.) has been anecdotally reported as a natural remedy for urinary tract infections for centuries. Accordingly, a growing number of studies have examined the potential anti-oxidant, anti-adhesion, anti-motility and anti-cancer properties of cranberry-derived compounds. A number of these studies focused on the bioactivity of a specific fraction of cranberry phytochemicals known as proanthocyanidins (cPACs). Research shows that these condensed tannins hinder bacterial attachment to cellular or biomaterial surfaces, impair motility of the pathogens *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*, and can induce a state of iron limitation in uropathogenic *E. coli*. O’May et al. also reported that a different cranberry derived material, the cranberry powder derived from whole, crushed cranberries, impairs the surface-associated swarming motility of *P. aeruginosa* strain PA01 while enhancing biofilm formation. Also, cPACs block invasion of intestinal pathogens as a result of rearrangement of...
host cell cytoskeleton \textit{in vivo} \textsuperscript{20}. While many studies have suggested that consumption of cranberry-derived materials, namely cranberry capsules and cranberry juice, is effective in preventing bacterial infections\textsuperscript{22–26}, few have looked at the effects of these cranberry-derived materials \textit{in vitro} and \textit{in vivo} after consumption\textsuperscript{24,27,28}. Indeed, the effect of bioactive cPACs on bacterial virulence \textit{in vivo} and mechanisms by which they do so are poorly understood. To date, not much attention has been given to the anti-virulence properties of cPACs.

\textit{P. aeruginosa} is an opportunistic and versatile \textgreek{gamma}-proteobacterium that readily develops antibiotic resistance and is responsible for various infections affecting immunocompromised individuals, such as those suffering from cystic fibrosis\textsuperscript{29–31}. \textit{P. aeruginosa} regulates most of its virulence factors in a cell density-dependent manner via cell-to-cell communication, commonly known as quorum sensing (QS)\textsuperscript{32}. \textit{P. aeruginosa} has two major \textgreek{N}-acylhomoserine lactone (AHL)-based QS pathways, the Las and Rhl QS systems, and one 2-alkyl-4-(1H)-quinolone (AQ)-based QS system, which function in a cascade manner\textsuperscript{33,34}. The Las system is positioned at the top of the QS hierarchy and uses \textgreek{N}-3-(oxo-dodecanoyl)-L-homoserine lactone (3-oxo-C\textsubscript{12}-HSL) as its signal molecule, and involves LasI and LasR as the synthase and regulator, respectively\textsuperscript{35,36}. The Rhl system uses \textgreek{N}-butanoyl-L-homoserine lactone (C\textsubscript{4}-HSL) as its signal, and involves RhlI and RhlR as the synthase and regulator, respectively\textsuperscript{35,36}. The LasR initiates the QS regulatory systems, partially activates the transcription of RhlR and other regulators of \textit{Pseudomonas} quinolone signal (PQS) and integrated quorum sensing (IQS) systems\textsuperscript{33,37}. The complex QS regulation network influences, both positively and negatively, the transcription of possibly 5–10\% genes of \textit{P. aeruginosa}\textsuperscript{38,39}. The QS system is an essential part of the organism’s virulence and is required to establish infection in the mammalian host\textsuperscript{40}.

Bacterial QS is considered an attractive therapeutic target in efforts to diminish bacterial virulence by reducing the level of QS signals, or by interfering with other QS system targets\textsuperscript{41,42}. Several attempts were made to find suitable candidate molecules with anti-QS and anti-biofilm activity against \textit{P. aeruginosa}\textsuperscript{43}. Experimental and modeling studies suggest that evolution and spread of resistance in bacteria against QS inhibiting compounds may be occurring\textsuperscript{44,45}. Thus, the identification of new compounds aimed at inhibiting bacterial QS will mitigate the emergence of antimicrobial resistance in bacterial pathogens.

The goal of this study was to examine the anti-virulence potential of a cranberry extract rich in proanthocyanidins (cPAC) in combating the opportunistic human pathogen \textit{P. aeruginosa}. Our results clearly show the anti-infectious properties of a cerPAC against \textit{P. aeruginosa} strain PA14 and reveal the multi-modal action of cerPAC in impairing QS function.

\textbf{Results}

\textbf{A cranberry extract rich in proanthocyanidins inhibits \textit{P. aeruginosa} virulence towards \textit{D. melanogaster}.} Treatment with cerPAC significantly inhibited the staphylolytic (LasA, \(F_{3,8} = 21.41, p < 0.001\)), elastolytic (LasB, \(F_{3,8} = 84.29, p < 0.001\)) and alkaline proteolytic (AprA, \(F_{3,8} = 34.41, p < 0.001\)) activities of \textit{P. aeruginosa} PA14 (Fig. 1a). Importantly, this inhibition was achieved without affecting bacterial growth (Fig. 1b).

Next, to verify whether cerPAC can limit infection \textit{in vivo}, we used a fruit fly killing assay in which we administered cerPAC to \textit{Drosophila melanogaster} infected with WT \textit{P. aeruginosa} PA14. As shown in Fig. 2, the median survival of \textit{D. melanogaster} after exposure to \textit{P. aeruginosa} was 168 h without cerPAC, but 240 h with cerPAC treatment, which is significantly \((\chi^2 = 4.14, df = 1, p < 0.05)\) less virulence based on the comparison of survival curves. The survival of uninfected \textit{D. melanogaster} was identical to the treatment with only cerPAC.
The difference in the treated or untreated PA14 strains’ ability to kill *D. melanogaster* in this feeding assay may have been due to modified survival of the bacteria on the filter papers used for exposure during incubation. To address this possibility, we analyzed the survival of PA14 on the paper discs without and with 200 μg mL⁻¹ cerPAC under the same conditions as the fly feeding assay. There was no significant difference (F₁₀₀ = 0.54, p = 0.74) in culturability of the bacterium on the filter paper discs in the absence and presence of cerPAC during incubation (see Supplementary Fig. S1), indicating that an alteration in survival ability of bacteria could not account for the observed differences in fly killing. Overall, these results indicate that cerPAC protect *D. melanogaster* from *P. aeruginosa* infection.

**Cranberry extract rich in proanthocyanidins modulates the AHL-mediated quorum sensing system in *P. aeruginosa* PA14.** Since QS regulates multiple virulence determinants in *P. aeruginosa*, we hypothesized that cerPAC contain molecule(s) that might interfere with QS in *P. aeruginosa* PA14. Therefore, to determine the ability of cerPAC to modulate the production of the two principal AHL molecules by *P. aeruginosa* PA14, we determined AHL production kinetics in absence or presence of 200 μg mL⁻¹ cerPAC. As shown in Fig. 3A,B, cerPAC significantly impairs the production of 3-oxo-C₁₂-HSL (t = 7.45, df = 4, p < 0.001) and C₄-HSL (t = 3.54, df = 4, p < 0.05), in *P. aeruginosa* PA14 at exponential and late stationary phase, respectively. This reduction in the production of the QS signals was observed without affecting bacterial growth (Fig. 3C).

To understand the mechanism for the reduction in AHL levels, β-galactosidase transcriptional fusion reporters of *lasI* (3-oxo-C₁₂-HSL synthase) and *rhlI* (C₄-HSL synthase) were assayed in *P. aeruginosa* PA14 bioreporter strain with the same 200 μg mL⁻¹ cerPAC exposure. These bioassays revealed that expression of both AHL synthase genes (*lasI* and *rhlI*) is repressed by cerPAC (Fig. 4A,B). Similarly, we also investigated whether presence of cerPAC affects the expression of the two cognate transcriptional regulator genes *lasR* and *rhlR* using *lacZ* transcriptional fusion reporters. Expression of both regulator gene fusions was partially repressed in the presence of cerPAC (Fig. 4C,D). Thus, cerPAC inhibits both AHL synthases and partially represses the LuxR-type regulator genes associated with the production of the two AHL signals in *P. aeruginosa* PA14.

**Cranberry extract rich in proanthocyanidins act as an antagonist of AHL-mediated quorum sensing in *P. aeruginosa* PA14.** Considering that AHLs act as autoinducing ligands of LasR and RhlR, we hypothesized that cerPAC component(s) interfere with LasR/RhlR activation by AHLS. We thus investigated whether cerPAC affects LasR and/or RhlR induction by exogenous AHLS using bioreporter AHL-negative PA14 mutants with *lacZ* fusions. As expected, when 3-oxo-C₁₂-AHL or C₄-HSL were supplied to their respective bioreporters, they activated the expression of *lasI* and *rhlI*, respectively (Fig. 5A,B). While cerPAC had no effect on the activity of the reporters, there was a significant (p < 0.05) reduced activation by either AHLS in presence of cerPAC (Fig. 5A,B). This indicates that cerPAC partially inhibits the activation of both LasR- and RhlR-directed transcription of *lasI* and *rhlI*, respectively, the primary targets of these LuxR-type regulators. Additionally, LasR and RhlR activation titration was performed in absence and presence of three different concentrations of cerPAC, which resulted in lower activation of LasR and RhlR (Fig. 5C,D). This indicates that cerPAC can reduce the activation of both regulators by their native AHLS, likely as a potential antagonist.

**Cranberry extract rich in proanthocyanidins inhibits LasR activity without binding to AHL molecules and also interact with LasI.** To assess a possible physical interaction between cerPAC components and either AHL molecule, we quantified C₄-HSL and 3-oxo-C₁₂-HSL in cell-free growth medium using an ethyl acetate extraction procedure followed by LC-MS analysis. If the cerPAC binds to the AHLS, we would expect to...
Figure 3. cerPAC (200μg mL⁻¹) impairs the production of AHL-type QS molecules in P. aeruginosa PA14. Concentrations of (A) 3-oxo-dodecanoyl-homoserine lactone (3-oxo-C₁₂-HSL), and (B) butanoyl-homoserine lactone (C₄-HSL) are shown as a function of cell growth (OD₆₀₀). (C) Total cell dry weight of 3 mL culture is shown as a function of cell growth (OD₆₀₀). Data points represent the average of triplicate experiments and the error bars show the standard deviation.

Figure 4. Effect of cerPAC on the expression of quorum sensing genes. P. aeruginosa PA14 carrying reporter fusion plasmids (A) lasI'-lacZ (B) rhlI'-lacZ, (C) lasR'-lacZ and (D) rhlR'-lacZ were grown in TSB medium without or with 200μg mL⁻¹ cerPAC, and expression was quantified by measuring β-galactosidase activity. Data points represent the average of triplicate experiments. The error bars show the standard deviation.
observe a reduction in AHL concentration due to compromised extraction. As shown in Supplementary Fig. S2, there was no difference in the concentration of AHLs with or without cerPAC, demonstrating that cerPAC components do not bind to the AHLs and therefore do not inhibit QS by physical interaction.

Inhibition of Las-type QS regulators’ activities by cerPAC may be due to structural interactions, important for the functional activity of transcriptional regulatory proteins. To address this possibility, in silico docking analysis was performed using protein structures of LasR (2UV046), LasI (1RO547), the monomer and dimer of epicatechin molecules (important components of cPACs48). To test our docking method, we compared the interaction energy scores (obtained using Moldock tools) of the predicted docking complex and the known crystallographic complex structures of the LasR with ligand 3-oxo-C12-HSL. The Moldock interaction energy score of $-144.1 \text{ kcal mol}^{-1}$ for the predicted complex of LasR with 3-oxo-C12-HSL was marginally lower than the Moldock interaction energy score of $-157.5 \text{ kcal mol}^{-1}$ obtained for the crystallographic complex of LasR with ligand 3-oxo-C12-HSL (Fig. 6a and see Supplementary Table S1). The epicatecin and its dimer (proanthocyanidin) molecules were docked separately in the internal cavity of LasR (Fig. 6b,c). Ligand binding domain (LBD) of LasR with a volume of 653 Å$^3$, exhibits sufficient space to accommodate the monomer or dimer of epicatechin with a volume of 225 Å$^3$ or 466 Å$^3$, respectively. The in silico docking analysis suggests that the complex formation between the epicatechin and LasR, with a Moldock interaction energy score of $-127.1 \text{ kcal mol}^{-1}$, is more favorable than LasR-proanthocyanidin complex with Moldock score of $-68 \text{ kcal mol}^{-1}$ (see Supplementary Table S1). The proanthocyanidin formed six hydrogen bonds at the internal binding cavity of LasR compared to four hydrogen bonds of the LasR-3-oxo-C12-HSL or LasR-epicatechin complex (Fig. 6). The increase in the Moldock score for the docking complex of LasR with proanthocyanidin compared to LasR-epicatechin complex was observed due to the steric constraints of the proanthocyanidin structure in the internal cavity space of LasR identified by the comparison of their internal energies (see Supplementary Table S1).
Due to the lack of crystallographic structure of LasI protein bound with its natural substrates or functional analogues, we performed in silico docking analysis to predict a complex of LasI with its natural substrate S-adenosyl L methionine (SAM) (Fig. 7a). This putative complex with LasI was used as a reference for both docking analyses of epicatechin and proanthocyanidin. The best five structural positions of SAM with higher interaction energies occupied the same binding cavity on the LasI protein. The docking analysis showed the formation of hydrogen bonds of SAM with residues that surround the putative binding cavity with Moldock interaction energy score of $-126.2 \text{ kcal mol}^{-1}$ (Fig. 7a and see Supplementary Table S1). The binding cavity known for the second substrate of LasI, the acyl-acyl carrier protein (acyl-ACP) was not identified as a potential binding site for either of the tested cerPAC components (epicatechin or proanthocyanidin). The LasI-epicatechin complex showed single hydrogen bond with Moldock interaction energy score of $-106.8 \text{ kcal mol}^{-1}$ (Fig. 7b and see Supplementary Table S1). The docking complex of the LasI protein with the proanthocyanidin molecule suggests the more favorable complex formation with five hydrogen bonds and Moldock interaction energy score of $-153.6 \text{ kcal mol}^{-1}$ compared to the LasI-SAM complex (Fig. 7c). This in silico docking analysis suggests that both main components of cerPAC have the potential to form complexes with LasR and LasI proteins to compete with their native ligands 3-oxo-C12-HSL and SAM, respectively.

Cranberry extract rich in proanthocyanidins impairs AHL production in other pathogenic strains. According to our data, cerPAC can act as a general QS inhibitor by interfering with the binding of the AHL ligand to LuxR-type transcriptional regulators. To verify that cerPAC is able to impede QS in other bacterial species, we performed an AHL production kinetics assay to examine the effect of administering cerPAC to wild type strains of Burkholderia ambifaria and Chromobacterium violaceum. The addition of cerPAC to growth medium significantly impairs the production of the two main AHLs (C8-HSL and C6-HSL) in B. ambifaria (Fig. 8A) and C6-HSL in C. violaceum (Fig. 8B). Since the primary target of LuxR regulators are luxI homologues, these observations validate the capacity of cerPAC to interfere with AHL-mediated QS in different bacterial species.

Figure 6. Molecular docking analysis of the LasR protein with AHL molecule and two main components of the cerPAC. (a) Left panel represents full view of the ribbon structure of LasR protein with the ligand binding cavity (highlighted in golden color) between four $\beta$-sheets ($\beta_1$, $\beta_2$, $\beta_4$ and $\beta_5$) and two $\alpha$-helices ($\alpha_3$ and $\alpha_4$). Upper right panel represents the inset view of docked complex with known binding position (reported crystallographic structure) of ligand 3-oxo-C12-HSL (shown in magenta color) and the predicted binding position of 3-oxo-C12-HSL (shown in black color) during in silico docking. The docking complexes of LasR with (b) the monomer of epicatechin (shown in blue color) and (c) the dimeric form of the epicatechin (proanthocyanidin, shown in green aqua color) are shown in the presence of 3-oxo-C12-HSL (shown in magenta color) for the comparison of binding positions. All possible hydrogen bonds are shown using black lines and binding residues shown in bright green color.
**Figure 7.** Molecular docking analysis of the LasI protein with substrate S-adenosyl L methionine (SAM) and two main components of the cerPAC. (a) Left panel represents full view of the ribbon structure of LasI protein with its substrates binding cavities and right panel represents the inset view of docked complex with substrate SAM (shown in magenta color). The docking complexes of LasI with (b) the monomer of epicatechin (shown in blue color) and (c) the dimeric form of the epicatechin (proanthocyanidin, shown in green aqua color) are shown with predicted binding residues (shown in bright green color). The surface structures are shown in red and blue for hydrophobic and hydrophilic attributes, respectively, and possible hydrogen bonds are depicted using black lines.

**Figure 8.** cerPAC (200 μg mL⁻¹) impairs production of AHL-type QS molecules in wild type strains *B. ambifaria* HSJ1 and *C. violaceum*. Concentrations of (A) N-octanoyl-homoserine lactone (C8-HSL) and N-hexanoyl-homoserine lactone (C6-HSL) in *B. ambifaria* HSJ1, and (B) N-hexanoyl-homoserine lactone (C6-HSL) in *C. violaceum* are shown as a function of cell growth (OD600). Results are expressed as average and standard deviations (SD) from values obtained from three replications.
Discussion

The identification of selective anti-virulence therapies that abolish the production of virulence determinants without affecting the viability of pathogenic bacteria would be extremely useful in combating bacterial infections caused by broad-spectrum antibiotic-resistant pathogens. The search for QS inhibitors or quenchers is a promising strategy aimed at development of innovative anti-microbial strategy to attenuate the virulence of infectious bacteria. Here, we showed that a cranberry extract enriched in PACs restricts virulence of *P. aeruginosa* in a fruit fly animal model and inhibits QS mechanisms. In addition, the cerPAC does not perturb cell viability of *P. aeruginosa*, indicating that use of these molecules may provide less selective pressure towards the development of resistance than conventional antibiotics (bactericidal and bacteriostatic, which pose strong selective pressure in any environment45). However, it would be naive to presume that QS inhibition is unlikely to put any selective pressures on pathogenic bacteria that may lead to resistance against QS inhibition, quite a few studies suggest the possibility of resistance development against QS inhibition44,45. Nonetheless, it is important to continue to expand our strategies for combating pathogen resistance by identifying novel anti-microbial and anti-virulence agents49. Our findings have elucidated the likely mechanisms of action behind the anti-virulence efficacy of cerPAC: 1) it reduces the production of AHL signaling molecules; 2) it represses the expression of the QS regulators LasR and RhlR and autoinducer synthases LasI and RhlI; 3) it antagonizes the activation of LasR and RhlR by their cognate autoinducers; and 4) epicatechin and proanthocyanidin, the main components of cerPAC, are modeled in *silsico* to interact with the LBD of LasR and LasI. In addition, cerPAC also inhibits AHL production in strains of the Gram-negative species *B. ambifaria* and *C. violaceum*. Thus, cerPAC could have anti-virulence activity against various pathogens with clinical importance.

For *in vivo* study, we used a fly feeding assay which represents a long-term infection model and involves feeding starved flies with bacterial cultures. This method is better adapted to chronic infections compared to the fly nicking model50. A dose of cerPAC was supplied at the start of infection and virulence was subsequently reduced, indicating that a cranberry extract enriched in PACs could function as a prophylactic. These results, when considered with other literature, indicate that the use of effective prophylactic molecules with anti-virulence activity, specifically for *P. aeruginosa*, could be a best practice in the clinical setting51. It is noteworthy that the extract used herein contains approximately 95% proanthocyanidins, and thus, it is presumed that the bioactivity observed can be mostly attributed to these molecules. Nonetheless, there is also a possibility that other, as yet unidentified molecules present in the cranberry extract, also act as potent quorum sensing inhibitors. Additional studies conducted with fractionated cranberry extracts are needed to achieve more insights into the bioactivity of specific fractions of cranberry derivatives.

That a cerPAC alone inhibits QS has not been previously reported in the peer-reviewed scientific literature. An earlier study reported the impairment of QS in *P. aeruginosa* in the presence of a commercial cranberry juice cocktail52; however, the mechanisms of action of this complex mixture of molecules was not identified. We targeted Las and Rhl QS systems because they are at the top of the *P. aeruginosa* quorum sensing hierarchy53. Both AHL molecules induce their own production and activate the corresponding LuxR-type transcriptional regulators LasR and RhlR54,55. In the presence of the cranberry extract, we observed impairment in AHL production, along with reduced gene expression of AHL synthase (LasI and RhlI) and partial repression of their regulators (LasR and RhlR). Similar observations have been made using other QS inhibitor molecules51,54,56. Interestingly, we found that cerPAC, a potent *in vivo* inhibitor, is an effective antagonist of both LasR and RhlR, two regulators that act reciprocally on key virulence determinants53,55,57. We propose that anti-virulence efficacy of the cerPAC is essentially due to its interference with the LasIR and RhlIR-dependent QS regulatory circuitry, though we cannot exclude a multifactorial effect.

Previous studies have identified anti-QS and anti-biofilm candidate molecules effective against *P. aeruginosa*17,44,58. Among them, plant-derived natural flavonoid and phenolic compounds are commonly studied for the inhibition of *P. aeruginosa* biofilm17,43 and QS43. The structure of a typical PAC is tetrameric in nature, composed of epicatechin units with one A-type linkage48. Catechin, an epimer of epicatechin, inhibits biofilm formation and QS-controlled virulence factors59. Consequently, other structurally-similar flavonoids, such as baicalein60, naringenin61 and quercetin55, are also anti-QS and anti-biofilm compounds. Thus, we docked monomer and dimer (proanthocyanidin) of epicatechin molecule onto LasR and LasI proteins to assess the specific interaction. Our hypothesis was that these molecules would bind to LasR and LasI, occupying the crucial LBD, which would result in inhibition of *P. aeruginosa* QS systems. The successful molecular docking of epicatechin or proanthocyanidin with LasR suggested that the inactivation of transcriptional regulators may be the primary mechanism of action for the cerPAC as anti-virulence factors *in vivo*.

Our results show that a cerPAC protects *D. melanogaster* from *P. aeruginosa* likely through an inhibition of QS without negative effect on bacterial growth. Antagonist activity and *in silico* analysis projected the potential mechanism of action to the inhibition of AHL regulators. As hopeful as these anti-virulence strategies are, they emerge with a new set of preclinical and regulatory development challenges. It remains to be verified whether plant-derived compounds cause significant side-effects in animals or humans. However, one can also conceive of important clinical application for treatment of acute and chronic infections caused by *P. aeruginosa* and potentially other bacterial pathogens, as our preliminary data suggest cerPAC is a general inhibitor of AHL-mediated QS. Taken together, our results present a promising case for cerPACs as efficient QS inhibitors for the regulation of bacterial pathogenicity.

Methods

**Cranberry-derived materials and bacterial strains.** The cranberry extract rich in proanthocyanidins (cerPAC) was obtained from Ocean Spray Cranberries Inc. The supplier prepared the sample according to well-established methods62 by enriching from cranberry fruit juice extract. While the exact composition is proprietary information undisclosed by the supplier, it contains approximately 95% proanthocyanidins. A dry powder of...
cerPAC was solubilized in deionized water and sterilized by filtration (0.22 μm PVDF membrane filter). Bacteria used in this study were *P. aeruginosa* strain PA14 (wild type) and isogenic QS mutant strains in *lasI* 
*lasR* 
*lasI* and *rhlR* as well as wild type strains *Burkholderia ambifaria* HS1 
*Chromobacterium violaceum* ATCC 31532 and *Staphylococcus aureus* ATCC 25923. Plasmids carrying lacZ fusion with genes *lasI* (pSC11 
transcriptional fusion; pME3853 
translational fusion), *lasR* (pPCS1001 
transcriptional fusion), *rhlR* (pMW3058 
transcriptional fusion; pME3846 
translational fusion) and *rhlR* (pPCS1002 
transcriptional fusion) were introduced into appropriate *P. aeruginosa* PA14 QS mutant strains by electroporation, as described previously. All bacterial strains were preserved in glycerol stock (15% v/v) culture at −80 °C and cultured in Tryptone Soy Broth (TSB) medium, with antibiotics if required for plasmid maintenance: tetracycline (75 mg L−1), carbenicillin (300 mg L−1), gentamicin (100 mg L−1), streptomycin (250 mg L−1) and spectinomycin (250 mg L−1).

**Phenotypic assay.** To assess LasB elastolytic activity, filter-sterilized culture supernatant samples (100 μL) from late stationary phase cultures of strain PA14 were mixed with 5 μg of lecinin (Sigma–Aldrich) and 300 μL of 0.1 M Tris-HCl (pH 7.2). Release of lecinin from degraded elastin was measured as A495 after 2 h of incubation at 37 °C with shaking at 100 rpm, followed by centrifugation. For assessment of LasA staphylocytic activity, 5 μL of *S. aureus* ATCC 25923 overnight cultures were boiled for 15 min, and 100 μL were mixed with 300 μL of filtered culture supernatants of PA14. The OD600 was measured after 2 h of incubation at 37 °C and 100 rpm. To analyze alkaline protease (AprA) activity, filter-sterilized culture supernatant samples (200 μL) from late stationary phase cultures of PA14 were vortexed with 25 mg of Hide–Remazol Brilliant Blue R powder (Sigma–Aldrich) in 800 μL of 20 mM Tris–HCl buffer (pH 8.0) containing 1 mM CaCl2. The tube was then incubated at 37 °C at 150 rpm for 1 h. The insoluble hide azure blue was removed by centrifugation at 10,000 × g for 4 min at 4 °C and the absorption of the supernatant was measured at 595 nm. All experiments were carried out in triplicate.

**Infection of Drosophila melanogaster.** Fruit flies (*D. melanogaster*) were infected orally in fly feeding assay as before, with some modifications. Briefly, flies were anesthetized under a gentle stream of carbon dioxide. Male flies (3- to 5-days-old) were starved of food and water for 5–6 h and separated into vials (10 per vial) containing 5 mL of 5% sucrose agar (sterile) without and with 200 μg mL−1 cerPAC and 2.3-cm filter paper disks (sterile) containing freshly grown bacterial culture suspension. To achieve this freshly grown cultures, an overnight PA14 culture was inoculated in 6 mL TSB culture and incubated at 37 °C and 100 rpm until OD600 = 3.0. This culture was centrifuged at 12,000 × g for 1 min and the resulting pellet resuspended in 150 μL of sterile 5% sucrose, without and with 200 μg mL−1 cerPAC. All filters were soaked appropriately with this culture suspension, along with sucrose agar, in feeding vials prior to transferring flies into the vial. Separate feeding vials soaked with 150 μL of 5% sucrose without and with 200 μg mL−1 cerPAC were used as negative controls for each experiment. Post-infection mortality of flies was monitored daily for 14 days, with each treatment tested twice in triplicate.

**LC-MS analyses.** Specific estimation of AHL molecules was achieved by LC-MS in the positive electro-spray ionization (ESI+) mode, combined with the MRMs, as described previously. Samples of PA14 culture exposed to cerPAC were retrieved at different time points and OD600 was measured. An aliquot of methanolic internal standard was mixed with each sample to adjust final concentration 3 mg L−1 of 5,6,7,8-tetradeutero-4-hydroxy-2-heptylquinoline (HHQ-d4) and 6 mg L−1 of 5,6,7,8-tetradeutero-3,4-dihyd roxy-2-heptylquinoline (PQS-d4). All culture samples were vortex-mixed and extracted twice with ethyl acetate (300 μL) from late stationary phase cultures of PA14, and then centrifuged at 14,000 × g for 1 min. Equal parts of organic and aqueous layers were analyzed by LC-MS/MS. Specific AHL molecules were identified and quantified by LC-MS/MS with a Waters Acquity UPLC (Waters, MA, USA) connected to a triple quadrupole mass spectrometer (Quadrupole time-of-flight mass spectrometer, TSQ Quantum Discovery Max, Thermo Fisher Scientific, Waltham, MA, USA). Samples were analyzed in positive ionization mode using the MRM mode, as described previously.

**β-galactosidase assay for LacZ expression.** β-galactosidase activity was measured by Miller, with slight modifications as reported previously. Briefly, cells were grown in TSB without and with cerPAC to various cell densities. Samples of cell culture were collected at different time points and diluted in Z-buffer (Na2HPO4 0.06 M; NaH2PO4 0.04 M; KCl 0.01 M; MgSO4·7H2O 0.001 M; β-mercaptop ethanol 0.05 M; pH 7.0). Cells in Z-buffer were permeabilized by the addition of one drop of 0.1% SDS and two drops of chloroform. Then, 200 μL of 4 mg mL−1 ONPG was added to each reaction mixture, and enzyme reaction was stopped using 100 μL of 1 M Na2CO3. Cell debris were separated by centrifugation at 14,000 × g for 30 sec and color development was monitored at 420 nm. β-galactosidase activity was expressed in Miller units (MU), calculated as follows: 1,000 × OD595/FT (min) × V (mL) × OD600.

**Antagonists/agonists assay.** To evaluate the activity of cerPAC as antagonists/agonists against the natural AHL ligand of LasR or RhlR, 3-oxo-C12-HSL (Sigma–Aldrich) and C4-HSL (Cayman Chemical) were used as inducers in this study. The AHL-deicient strain that has been engineered to produce β-galactosidase upon activa tion of LasR by 3-oxo-C12-HSL [LasI (lasI-lacZ, pME3853)] and RhlR by C4-HSL [RhlI (rhlI-lacZ, pME3846)], were grown overnight in TSB medium. The overnight culture was diluted in fresh TSB and was grown to achieve an OD600 = 0.3. An appropriate amount of sterilized cerPAC stock solution prepared in MilliQ water was added to sterile culture tube containing TSB. For control condition, either 3-oxo-C12-HSL or C4-HSL (stock solution in DMSO as a control) was added to sterile culture tube containing TSB, final DMSO concentration (after addition of cells) did not exceed 0.5% v/v. Bacterial cells were added to TSB (final OD600 = 0.05) without and with cerPAC approximately 30 min prior to the addition of the AHL at a final concentration of 3.13–50 nm (for 3-oxo-C12-HSL) or 62.5–1000 nM (for C4-HSL), to achieve final volume of 2 mL. Culture tubes were incubated at 37 °C for 3 h under shaking at 200 rpm, measurement of cell OD600 and β-galactosidase assay were performed.
at the regular time intervals after 3 h of incubation. The concentration of cerPAC that reduced or increased the β-galactosidase activity compared to controls containing 3-oxo-C12-HSL or C6-HSL (without cerPAC) was considered to determine antagonist or agonist activity.

**In silico docking analysis.** To understand the interaction between components of the cerPAC with LasR and LasI protein structures, we performed virtual docking using the Piecewise Linear Potential and Lennard-Jones algorithms that can identify steric and hydrogen bonding interactions, and the Coulomb potential for electrostatic forces. In *in silico* docking analysis was performed using the Molegro Virtual Docker 6.0 suite without the incorporation of water molecules. To maintain the search robustness, twenty rounds of iteration were used for each docking process. The S-adenosyl L methionine (NCBI Pubchem CID 34756) and the 3-oxo-C12-HSL (NCBI Pubchem CID 127864) molecular structures were used as native ligand molecules, for the LasI (RCSB Protein data base ID 1RO547) and LasR (RCSB Protein data base ID 2UV0) proteins, respectively. The components of cerPAC, epicatechin (NCBI PubChem CID 182232) and proanthocyanidin (NCBI PubChem CID 108065) molecular structures were used as ligands in virtual docking for both proteins. The MolDock search tool[22], which combines guided differential evolution and a cavity prediction algorithm was used for docking scores (in kcal mol−1) based on the interaction energies of each complex. The best five positions with high Moddock interaction energies were sampled and compared in every complex computed. The Computed Atlas of Surface Topography[22] of proteins was used to explore the volumes of the cavities in the target proteins (http://sts.bioe.uic.edu/castp/index.php). The molecular graphics and analyses were performed using the UCSF Chimera version 1.17.[17]

**Statistical analysis.** All experiments were repeated in quadruplicate, unless otherwise specified. Fruit fly survival curves were prepared using GraphPad Prism 6 (GraphPad Software, Inc, San Diego, CA) to perform a statistical Log-rank (Mantel–Cox) test. Data represented are the means of replicates and the differences among the control, and results were analyzed by using one way or two way ANOVA as required.

### References

1. Lesic, B. et al. Inhibitors of pathogen intercellular signals as selective anti-infective compounds. *PloS Pathog.*, 3, 1229–1239 (2007).
2. Shryock, T. R. The future of anti-infective products in animal health. *Nat Rev Micro.* 2, 425–430 (2004).
3. Berdy, J. Thoughts and facts about antibiotics: where we are now and where we are heading. *J Antimicrob Chemother.* 65, 385–395 (2012).
4. Jakobsen, T. H., Bjarnsholt, T., Jensen, P. O., Givskov, M. & Hoiby, N. Targeting quorum sensing in *Pseudomonas aeruginosa* biofilms: current and emerging inhibitors. *Future Microbiol.* 8, 901–921 (2013).
5. Blatherwick, N. R. The specific role of foods in relation to the composition of the urine. *Arch Intern Med.* 14, 409–450 (1914).
6. Greffin, J. K., Philpott, J. & Bass, A. L. T. *A Reference Guide to Medicinal Plants* (Duke University Press, 1990).
7. Yan, X., Murphy, T. C., Hammond, G. B., Vinson, J. A. & Neto, C. C. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *J Agric Food Chem.* 50, 5844–5849 (2002).
8. Eydelnant, I. A. & Tufenkji, N. Cranberry derived proanthocyanidins reduce bacterial adhesion to selected biomaterials. *Langmuir* 24, 10273–10281 (2008).
9. Feldman, M., Tanabe, S., Howell, A. & Grenier, D. Cranberry proanthocyanidins inhibit the adherence properties of *Candida albicans* and cytotoxicity secretion by oral epithelial cells. *BMCL Complement Altern Med.* 12, 6 (2012).
10. Howell, A. B., Vorsa, N., Der Marderosian, A. & Foo, L. Y. Inhibition of the adherence of P-fimbriated *Escherichia coli* to uropathelial-cell surfaces by proanthocyanidin extracts from cranberries. *N Engl J Med.* 339, 1085–1086 (1998).
11. Tufenkji, N., Rafaï, O. J., Harmidy, K. & Eydelnant, I. A. Cranberry derived proanthocyanidins can prevent pathogen invasion of kidney epithelial cells. *Food Res Int.* 43, 922–924 (2010).
12. Chan, M. et al. Inhibition of bacterial motility and spreading via release of cranberry derived materials from silicone substrates. *Collide Surf B Biointerface.* 110, 273–280 (2013).
13. Hidalgo, G., Chan, M. & Tufenkji, N. Inhibition of *Escherichia coli* CFT073 flic expression and motility by cranberry materials. *Appl Environ Microbiol.* 77, 6852–6857 (2011).
14. McCall, J., Hidalgo, G., Asadishad, B. & Tufenkji, N. Cranberry impairs selected behaviors essential for virulence in *Pseudomonas mirabilis* HI4320. *Can J Microbiol.* 59, 430–436 (2013).
15. O’May, C., Ciobanu, A., Linn, H. & Tufenkji, N. Tannin derived materials can block swarming motility and enhance biofilm formation in *Pseudomonas aeruginosa*. *Biofouling.* 28, 1063–1076 (2012).
16. O’May, C. & Tufenkji, N. The swarming motility of *Pseudomonas aeruginosa* is blocked by cranberry proanthocyanidins and other tannin-containing materials. *Appl Environ Microbiol.* 77, 3061–3067 (2011).
17. Ulrey, R. K., Barkdole, S. M., Zhou, W. & van Hoek, M. L. Cranberry proanthocyanidins have anti-biofilm properties against *Pseudomonas aeruginosa*. *BMC Complement Altern Med.* 14, 499 (2014).
18. Neto, C. C., Amoroso, J. W. & Liberty, A. M. Anticancer activities of cranberry phytochemicals: an update. *Mol Nutr Food Res.* 52, S18–S27 (2008).
19. Singh, A. P. et al. Cranberry proanthocyanidins are cytotoxic to human cancer cells and sensitize platinum-resistant ovarian cancer cells to paraplatin. *Phytother Res.* 23, 1066–1074 (2009).
20. Harmidy, K., Tufenkji, N. & Grunfeld, S. Perturbation of host cell cytoskeleton by cranberry proanthocyanidins and their effect on enteric infections. *PLoS One.* 6, e27267 (2011).
21. Hidalgo, G. et al. Induction of a state of iron limitation in uropathogenic *Escherichia coli* CFT073 by cranberry-derived proanthocyanidins as revealed by microarray analysis. *Appl Environ Microbiol.* 77, 1532–1535 (2011).
22. Di Martino, P. et al. Reduction of *Escherichia coli* adherence to uropathelial bladder cells after consumption of cranberry juice: a double-blind randomized placebo-controlled cross-over trial. *World J Urol.* 24, 21–27 (2006).
23. Jepson, R. G., Williams, G. & Craig, J. C. Cranberries for preventing urinary tract infections. *Cochrane Database Syst Rev.* 10, CD001321 (2012).
24. Lavigne, J. P., Bourg, G., Combesure, C., Botto, H. & Sott, A. *In-vitro* and *in-vivo* evidence of dose-dependent decrease of uropathogenic *Escherichia coli* virulence after consumption of commercial *Vaccinium macrocarpon* (cranberry) capsules. *Clinical Microbiology and Infection.* 14, 350–355 (2008).
25. Liu, Y., Black, M. A., Caron, L. & Camesano, T. A. Evaluation of the *in vitro*/*in vivo* potential of five berries (bilberry, blueberry, cranberry, elderberry, and raspberry ketones) commonly used as herbal supplements to inhibit uridine diphospho-glucuronosyltransferase. *Food Chem Toxicol.* 72, 13–19 (2014).
28. Diarra, M. S. et al. In vitro and in vivo antibacterial activities of cranberry press cake extract alone or in combination with beta-lactams against Staphylococcus aureus. BMC Complement Altern Med. 13, 90 (2013).
29. Wu, D. C., Chan, W. W., Metelitsa, A. I., Fiorillo, L. & Lin, A. N. Pseudomonas skin infection: clinical features, epidemiology, and management. Am J Clin Dermatol. 12, 157–169 (2011).
30. de Bentzmann, S. & Peslati, P. The Pseudomonas aeruginosa opportunistic pathogen and human infections. Environ Microbiol. 13, 1655–1665 (2011).
31. Navon-Venezia, S., Ben-Ami, R. & Carmeli, Y. Update on Pseudomonas aeruginosa and Acinetobacter baumannii infections in the healthcare setting. Curr Opin Infect Dis. 18, 306–313 (2005).
32. Bjarnsholt, T. & Givskov, M. The role of quorum sensing in the pathogenicity of the cunning aggressor Pseudomonas aeruginosa. Anf Bnaal Chem. 387, 409–414 (2007).
33. Lee, J. & Zhang, L. The hierarchy quorum sensing network in Pseudomonas aeruginosa. Protein Cell 6, 26–41 (2015).
34. Williams, P. & Camara, M. Quorum sensing and environmental adaptation in Pseudomonas aeruginosa: a tale of regulatory networks and multifunctional signal molecules. Curr Opin Microbiol. 12, 182–191 (2009).
35. Latifi, A., Foglino, M., Tanaka, K., Williams, P. & Lazdunski, A. A hierarchical quorum-sensing cascade in Pseudomonas aeruginosa links the transcriptional activators LasR and RhlR (VsmR) to expression of the stationary-phase sigma factor RpoS. Mol Microbiol. 21, 1137–1146 (1996).
36. Pesci, E. C., Pearson, J. P., Seed, P. C. & Igleswki, B. H. Regulation of las and rhl quorum sensing in Pseudomonas aeruginosa. J Bacteriol. 179, 3127–3132 (1997).
37. Lee, J. et al. A cell­cell communication signal integrates quorum sensing and stress response. Nat Chem Biol. 9, 339–343 (2013).
38. Hentzer, M. et al. Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. Microbiology. 148, 87–102 (2002).
39. Schuster, M., Bestroh, C. P., Ogí, T. & Greenberg, E. P. Identification, timing, and signal specificity of Pseudomonas aeruginosa quorum-controlled genes: a transcriptome analysis. J Bacteriol. 185, 2066–2079 (2003).
40. Lang, B. E. et al. Contribution of specific Pseudomonas aeruginosa virulence factors to pathogenesis of pneumonia in a neonatal mouse model of infection. Infect Immun. 64, 37–43 (1996).
41. Gonzalez, J. E. & Keshavan, N. D. Messing with bacterial quorum sensing. Microbiol Mol Biol Rev. 70, 859–875 (2006).
42. Scutera, S., Zucca, M. & Savio, D. Novel approaches for the design and discovery of quorum-sensing inhibitors. Expert Opin Drug Discov. 3, 353–366 (2014).
43. Rasamiravaka, T., Labanti, Q., Duez, P. & El Jaziri, M. The formation of biofilms by Pseudomonas aeruginosa: a review of the natural and synthetic compounds interfering with control mechanisms. Biomed Res Int. 2015, 759348 (2015).
44. Garcia-Contreras, R., Maeda, T. & Wood, T. K. Resistance to quorum-quenching compounds. Appi Environ Microbiol. 79, 6840–6846 (2013).
45. Defordi, T., Boon, N. & Bossier, P. Can bacteria evolve resistance to quorum sensing disruption? PLoS Pathog. 6, e1000989 (2010).
46. Bottomley, M. J., Muraglia, E., Rizzo, R. & Carfi, A. Molecular insights into quorum sensing in the human pathogen Pseudomonas aeruginosa from the structure of the virulence regulator LasR bound to its autoinducer. J Bacteriol. 282, 13592–13600 (2007).
47. Gould, T. A., Schweizer, H. P. & Churchill, M. E. Structure of the Pseudomonas aeruginosa acyl-homoserine lactone synthase LasL. Mol Microbiol. 53, 1135–1146 (2004).
48. Neto, C. C. Cranberry and its phytochemicals: a review of in vitro anticancer studies. J Nutr. 137, 1865–1935 (2007).
49. Njoroje, J. & Sperandio, V. Jamming bacterial communication: new approaches for the treatment of infectious diseases. EMBO Mol Med. 1, 201–210 (2009).
50. Lutter, E. I., Farra, M. M., Rabin, H. R. & Storey, D. G. Pseudomonas aeruginosa cystic fibrosis isolates from individual patients demonstrate a range of levels of lethality in two Drosophila melanogaster infection models. Infect Immun. 76, 1877–1888 (2008).
51. O’Loughlin, C. T. et al. A quorum-sensing inhibitor blocks Pseudomonas aeruginosa virulence and biofilm formation. Proc Natl Acad Sci USA 110, 17981–17986 (2013).
52. Harjai, K., Gupta, K. R. & Sehgal, H. Attenuation of quorum sensing controlled virulence of Pseudomonas aeruginosa by cranberry. Indian J Med Res. 139, 446–453 (2014).
53. Dekimpe, V. & Deziel, E. Revisiting the quorum-sensing hierarchy in Pseudomonas aeruginosa: the transcriptional regulator RhlR regulates LasR-specific regulators. Microbiology. 155, 712–723 (2009).
54. Borlee, B. R., Geske, G. D., Blackwell, H. E. & Handelsman, J. Identification of synthetic inducers and inhibitors of the quorum-sensing regulator LasR in Pseudomonas aeruginosa by high-throughput screening. Appl Environ Microbiol. 76, 8255–8258 (2010).
55. Goup, V., Meena, C. K. & Shetty, P. H. Quercetin Influences Quorum Sensing in Food Borne Bacteria: In-Vitro and In-Silico Evidence. PLoS One. 10, e0134684 (2015).
56. Rasamiravaka, T. et al. Pseudomonas aeruginosa Biofilm Formation and Persistence, along with the Production of Quorum-Sensing Virulence Factors, Are Disrupted by a Triterpenoid Coumarate Ester Isolated from Dalbergia trichocarpa, a Tropical Legume. PLoS One. 10, e0132791 (2015).
57. Parsek, M. R. & Greenberg, E. P. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. Proc Natl Acad Sci USA 97, 8789–8793 (2000).
58. Rasmussen, T. B. & Givskov, M. Quorum-sensing inhibitors as anti-pathogenic drugs. Int Med Microbiol. 296, 149–161 (2006).
59. Vandeputte, O. M. et al. Identification of catechin as one of the flavonoids from Combretum altiflorum bark extract that reduces the production of quorum-sensing-controlled virulence factors in Pseudomonas aeruginosa PA01. Appl Environ Microbiol. 76, 243–253 (2010).
60. Zeng, Z. et al. Virtual screening for novel quorum sensing inhibitors to eradicate biofilm formation of Pseudomonas aeruginosa. Appl Microbiol Biotechnol. 79, 119–126 (2008).
61. Vandeputte, O. M. et al. The flavonone naringenin reduces the production of quorum-sensing-controlled virulence factors in Pseudomonas aeruginosa PA01. Microbiology. 157, 2120–2132 (2011).
62. Howell, A. B. et al. A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. Phytochemistry. 66, 2281–2291 (2005).
63. Rahme, L. G. et al. Common virulence factors for bacterial pathogenicity in plants and animals. Science. 268, 1899–1902 (1995).
64. Deziel, E. et al. The contribution of Mvr to Pseudomonas aeruginosa pathogenesis and quorum sensing circuitry regulation: multiple quorum sensing-regulated genes are modulated without affecting lasR, rhlR, or the production of N-acyl-L-homoserine lactones. Mol Microbiol. 55, 998–1014 (2005).
65. Deziel, E. et al. Analysis of Pseudomonas aeruginosa 4-hydroxy-2-alkylquinolines (HAs) reveals a role for 4-hydroxy-2-heptylquinoline in cell-to-cell communication. Proc Natl Acad Sci USA 101, 1339–1344 (2004).
66. Vial, L. et al. Burkholderia pseudomallei, B. thailandensis, and B. ambifaria produce 4-hydroxy-2-alkylquinolines analogues with a methyl group at the 3 position that is required for quorum-sensing regulation. J Bacteriol. 190, 5339–5352 (2008).
67. Chugani, S. A. et al. QscR, a modulator of quorum-sensing signal synthesis and virulence in Pseudomonas aeruginosa. Proc Natl Acad Sci USA 98, 2752–2757 (2001).
68. Pesi, D. et al. The global posttranscriptional regulator RsmA modulates production of virulence determinants and N-acylhomoserine lactones in Pseudomonas aeruginosa. J Bacteriol. 183, 6676–6683 (2001).
69. Whiteley, M., Parsek, M. R. & Greenberg, E. P. Regulation of quorum sensing by RpoS in Pseudomonas aeruginosa. J Bacteriol. 182, 4356–4360 (2000).
70. Bjorn, M. J., Sokol, P. A. & Iglewski, B. H. Influence of iron on yields of extracellular products in Pseudomonas aeruginosa cultures. *J Bacteriol.* 138, 193–200 (1979).

71. Howe, T. R. & Iglewski, B. H. Isolation and characterization of alkaline protease-deficient mutants of Pseudomonas aeruginosa in vitro and in a mouse eye model. *Infect Immun.* 43, 1058–1063 (1984).

72. Apidianakis, Y. & Rahme, L. G. Drosophila melanogaster as a model host for studying Pseudomonas aeruginosa infection. *Nat Protoc.* 4, 1285–1294 (2009).

73. Lepine, F. & Deziel, E. Liquid chromatography/mass spectrometry for the detection and quantification of N-acyl-L-homoserine lactones and 4-hydroxy-2-alkylquinolines. *Methods Mol Biol.* 692, 61–69 (2011).

74. Miller, F. Glycopeptides of human immunoglobulins. 3. The use and preparation of specific glycosidases. *Immunochemistry.* 9, 217–228 (1972).

75. Thomsen, R. & Christensen, M. H. MolDock: a new technique for high-accuracy molecular docking. *J Med Chem.* 49, 3315–3321 (2006).

76. Liang, J., Edelsbrunner, H. & Woodward, C. Anatomy of protein pockets and cavities: measurement of binding site geometry and implications for ligand design. *Protein Sci.* 7, 1884–1897 (1998).

77. Pettersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem.* 25, 1605–1612 (2004).

**Acknowledgements**

We thank Christina Khoo (Ocean Spray Cranberries Inc.) for providing the cerPAC sample, Marie-Christine Groleau for technical assistance and helpful discussions, Sylvain Milot for technical assistance in the LC-MS analysis, and Mike Mitzel for assistance in manuscript editing. N.T. acknowledges Howard Lam and Che O’May for preliminary work and helpful discussions. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Wisconsin Cranberry Board and the Cranberry Institute. N.T. holds the Canada Research Chair in Biocolloids and Surfaces and E.D. holds the Canada Research Chair in Sociomicrobiology.

**Author Contributions**

V.B.M., N.T. and E.D. conceived the experiments. V.B.M. conducted the *in vitro* and *in vivo* experiments and analysed the results. Y.L.-D.L.S. contributed by performing *in silico* docking analysis. V.B.M. and Y.L.-D.L.S. performed molecular graphics and structural analysis. All authors contributed to writing of the manuscript.

**Additional Information**

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

**How to cite this article:** Maisuria, V. B. et al. Cranberry-derived proanthocyanidins impair virulence and inhibit quorum sensing of *Pseudomonas aeruginosa*. *Sci. Rep.* 6, 30169; doi: 10.1038/srep30169 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/