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ORIGINAL ARTICLE

Cyanidin inhibits quorum signalling pathway of a food borne opportunistic pathogen

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Abstract Quorum sensing (QS) is the process of population dependent cell to cell communication used by bacteria to regulate their phenotypic characteristics. Key virulence factors that determine the bacterial pathogenicity and food spoilage were found to be regulated by QS mechanism. Hence, disrupting the QS signaling pathway could be an attractive strategy to manage food borne pathogens. In the current study, QS inhibitory activity of a naturally occurring anthocyanin-cyanidin and its anti-biofilm property were assessed against an opportunistic pathogen *Klebsiella pneumoniae*, using a biosensor strain. Further, QS inhibitory property of a naturally occurring anthocyanin cyanidin was further confirmed using *in-silico* techniques like molecular docking and molecular dynamics simulation studies. Cyanidin at sub-lethal dose significantly inhibited QS-dependent phenotypes like violacein production (73.96 %), biofilm formation (72.43 %), and exopolysaccharide production (68.65) in a concentration-dependent manner. Cyanidin enhanced the sensitivity of test pathogen to conventional antibiotics in a synergistic manner. Molecular docking analysis revealed that cyanidin binds more rigidly with LasR receptor protein than the signaling compound with a docking score of −9.13 Kcal/mol. Molecular dynamics simulation predicted that QS inhibitory activity occurs through the conformational changes between the receptor and cyanidin complex. Our results indicate that cyanidin, can be a potential QS based antibiofilm and antibacterial agent for food borne pathogens.

Keywords Quorum sensing · Anthocyanin · Molecular docking · Dynamics Simulation · *K. pneumoniae*

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Introduction

Many pathogenic bacteria are known to regulate their virulence factors by releasing signaling molecules called autoinducers through a mechanism called quorum sensing (QS). It is the cell to cell communication mechanism triggered in a population dependent manner for coordinated gene regulation (Fuqua et al. 2001). In Gram positive bacteria peptide based compounds act as autoinducers whereas, in Gram negative bacteria N-acyl homoserine lactones (AHLs) acts as a signaling compound. Quorum sensing mechanism of Gram negative bacteria has been studied extensively for its role in production and regulation of virulence factors (Williams et al. 2007; Dong et al. 2007). It has been well documented fact that there has been an increased resistance to conventional antibiotics by various bacterial pathogens. Therefore interfering QS mechanism could be the novel target to manage bacterial pathogens without the development of antibiotic resistance. So far, several natural and chemically synthesized compounds have been demonstrated to have the QSI activity. This includes malvidin (Gopu et al. 2015), aspirin (Mowafy et al. 2014), eugenol (Zhou et al. 2013), ellagic acid (Sarabhai et al. 2013) and, rutin (Vikram et al. 2010). On the other hand, most of these compounds have not been used in the mammalian system because of their adverse physiological activity and toxicity. Hence, novel plant based bioactive compounds would be of immense potential in this regard.

Klebsiella pneumoniae is a Gram-negative opportunistic pathogen, ubiquitous in nature, mainly associated with nosocomial and urinary tract infections (UTIs) leading to complexity like pneumoniae and septicemia. The number of food-

borne disease outbreaks has been reported attributing to *K. pneumoniae* in various foods (Hamilton et al. 2006). Colonization and biofilm formation are the two main physiological process used by *K. pneumoniae* for its pathogenicity (Costerton et al. 1999). In *K. pneumoniae*, LuxS dependent QS mechanism involving type-2 QS regulatory molecules and AI-2 transport genes were found to be involved in biofilm formation (Stoodley et al. 2002).

The present study is designed to investigate the QSI activity of cyanidin, which has been selected based on the molecular docking analysis with LasR receptor protein. At its sub-lethal dose cyanidin is screened for its activity against QS-regulated phenotypes of *K. pneumoniae* particularly biofilm formation and EPS production. Further to understand the mechanism of QS inhibitory activity, the *in-silico* analysis including molecular docking and simulation studies are conducted to study the conformational changes in the LasR receptor protein.

Materials and methods

Bacterial strains and culture conditions

Strain of *Chromobacterium violaceum* CV026 (CECT5999) procured from Spanish type culture collection, a derivative of wild strain unable to produce its AHLs was used as reporter strain. *K. pneumoniae* strain PUFST23 (GenBank: KF817575; MTCC 12202) dry fish isolate from the departmental culture collection was used as an experimental organism and *C. violaceum* MTCC 2656 was used as a positive control. Bacterial strains were selected on the basis of QS dependent phenotypes. Unless otherwise stated all the cultures were grown in Luria Bertani (LB) broth at 30 °C supplemented with kanamycin (20 μg/ml) for CV026. N-octanoyl-DL-homoserine lactone (10 μM) was added to induce the violacein production in CV026 when required.

Selection of active component

The phytochemical compound cyanidin screened for its QS inhibitory activity was selected based on the molecular docking analysis of 43 active components against LasR receptor protein in our earlier report (Gopu et al. 2015). Briefly, compound structure of LasR receptor protein (PDB ID 2UV0) was obtained from protein data bank, which was docked with the three-dimensional structures of active components obtained from Pubchem database (http: // pubchem.ncbi.nlm.nih.gov). PDB 2UV0 structure contains four chains (E, F, G, and H) whose confirmation was similar which was analyzed by superimposing with chimera. Since, the H chain is longest and contained the preferred binding site for the natural ligand N-octanoyl DL-homoserine lactone, H chain was used for docking. All the water molecules and other chains were removed from the LasR receptor protein for the analysis to select the potential QSI compound. Docking studies were performed with the Schrodinger 2012.

Minimal inhibitory concentration of cyanidin

The MIC for cyanidin (Sigma-Aldrich, India) was determined as per the guidelines of Clinical and Laboratory Standards Institute, USA (CLSI). The stock solution was prepared by dissolving 1 mg of cyanidin in 1 ml of 70 % methanol. One percent overnight culture test pathogens were added to appropriate growth medium supplemented with cyanidin to attain the final concentration ranging from (1 to 250 μg/ml). Microtiter plates were then incubated for 24 h. MIC was recorded as the lowest concentration that showed complete inhibition of visible growth. All further experiments in the present study were performed only at sub-MIC concentrations of cyanidin.

QSI bioassay for QS inhibitory activity

Quorum sensing inhibitory activity of cyanidin was qualitatively determined by using violacein-negative mutant *C. violaceum* CV026, which requires exogenous OHL to induce violacein production. Briefly, LB broth supplemented with OHL (10 μM) and cyanidin at different concentration (10–80 μg/ml) was inoculated with 1 % of overnight culture (adjusted to 0.4 OD at 600 nm) and incubated at 30 °C overnight. Flasks were observed for the diminution in violacein production (Choo et al. 2006).

Violacein produced was precipitated by centrifuging 1 ml of culture broth from each flask at 13,000 rpm for 5 min. The pellet was dissolved in 1 ml of DMSO and vortexed robustly to solubilize the violacein completely and centrifuged again to remove the cells. QSI activity was quantified at 585 nm using microplate reader (Biotek, USA) and the percentage of inhibition was calculated by the formula

$$\frac{control\ OD585nm - test\ OD585nm}{control\ OD585nm} \times 100$$

Reduction in exopolysaccharide

LB broth with and without cyanidin (50–150 μg/ml) were inoculated with 1 % of test bacterial cultures and incubated at 30 °C. Biofilms adhered to the walls of test tubes were harvested to obtain the crude EPS. Briefly, late log phase cells were removed by centrifugation at 5000 g for 30 min at 2 °C. Filtered supernatant was added to three volumes of chilled ethanol and incubated overnight at 2 °C to precipitate the dislodged EPS. Precipitated EPS was collected by centrifugation at 5000 g for 30 min which was then dissolved in 1 ml of

deionized water, and stored at −40 °C until further use. Total carbohydrate content in the EPS was quantified by the phenol-sulfuric acid method using glucose as standard (Huston et al. 2004).

Inhibition of biofilm formation by Microtiter plate assay

Microtiter plate assay was performed to quantify the effect of cyanidin on the biofilm formation of test bacterial pathogens (Limsuwan and Voravuthikunchai 2009). LB broth with and without cyanidin (50–150 μg/ml) were inoculated with 1 % of bacterial cultures and incubated at 30 °C for 24 h. After incubation, wells were carefully rinsed with double-distilled water to remove loosely attached cells. Adhered cell on the walls were stained with 100 μl of 0.2 % crystal violet solution (HiMedia, India) for 10 min. Excess stain was removed by rinsing with distilled water and washed with 100 μl of 95 % ethanol. Intensity was measured at OD_{650nm} by using microplate reader (Biotek, USA), for quantification of biofilm biomass.

In-situ visualization of biofilm

One milliliter of sterilized LB broth containing glass slide 1 × 1 cm in 12 well microtiter plate with and without the extracts were inoculated with 1 % of overnight culture. After 24 h of incubation, slides were carefully washed with distilled water to remove loosely attached cells. Biofilm in the glass slides were tainted by crystal violet and visualized under light microscope at 100× magnification (Nikon, Japan).

Bacterial biofilms were allowed to develop on the glass slides 1 × 1 cm with and without the plant extract for confocal laser scanning microscopy (CLSM). After 24 h of incubation, glass slides were stained with acridine orange (1 %) for 1 min. Stained glass slides were rinsed with distilled water to remove the excess stain. Glass slides were then dried and visualized under the advanced confocal microscope at 10× (LSM 710, Zeiss, Germany).

Synergistic effects of cyanidin with antibiotics

Twelve well microtiter plate containing 1 ml LB broth along with cyanidin at different concentration (50–150 μg/ml) was added with 1 % test pathogens and antibiotics, wells without cyanidin was maintained as control (Rogers et al. 2010). Antibiotics that were tested include erythromycin (10 μg), tetracycline (30 μg), and kanamycin (30 μg). Plates were incubated at 37 °C overnight was measured at OD_{600} using microplate reader (Biotek, USA).

Fractional inhibitory concentration

Synergistic effect resulted from combining cyanidin with antibiotics were assessed by determining fractional inhibitory concentration (FIC) index. FIC was determined by the formula: FIC index = FIC A+ FIC B = [A]/MIC A + [B]/MIC B, where [A] is the concentration of drug A, MICA is it's MIC and FICA is the FIC of drug A for the organism, while [B], MICB, and FICB are defined in the same fashion for drug B. The FIC index thus obtained was interpreted as follows: <0.5, synergy; 0.5 to 0.75, partial synergy; 0.76 to 1.0, additive effect; >1.0 to 4.0, indifference; and >4.0, antagonism (Timurkaynak et al. 2006). Finally, the varying rates of synergy between the agents were determined.

Molecular dynamics simulation

Followed by the docking analysis, molecular dynamics simulation studies were performed to study the conformational changes in the receptor due to binding of signaling molecule or cyanidin. Protein-signaling molecule and protein-active compound complex were simulated by Gromacs4.5.3 (Pronk et al. 2013) simulation package using gromos force field (Gunsteren and Berendsen 1987). All the complexes were placed into a cubic box with the size of 1.2 Å along with SPCE water model as a solvent. The system was equilibrated well before final simulation of 20 ns with the time step of 2 ps.

Statistical analysis

All the experimental data represents the mean of triplicate values. Differences between control and test samples were analyzed by one-way ANOVA.

Results and discussion

Type-2 QS regulatory molecules and AI-2 transport genes were found to be involved in biofilm formation by *K.*

Table 1 Docking scores of cyanidin and OHL with LasR receptor protein

Pubchem ID	Compound	Docking Score	Glide Emodel Score
3474204	N-octanoyl-DL-homoserine lactone	−4.28	−42.2
128861	Cyanidin	−7.76	−66.5

pneumoniae, and the role of LuxS dependent signal molecule in the earlier stages of biofilm formation was also elucidated. Therefore, interrupting the QS mechanism would be a great strategy to manage food borne pathogens. In the present study QS inhibitory activity of cyanidin was screened to hamper the QS regulated phenotypes in an opportunistic pathogen *K. pneumoniae*.

Selection of active component

Molecular docking of 43 active components with LasR receptor protein showed that anthocyanins like petunidin, malvidin and cyanidin ranked top most in the list with docking score more than −7.7 most comparable with that of the natural ligand (Table 1). As the anti-quorum activity of malvidin has been already published by our group (Gopu et al. 2015), in the present work, cyanidin with docking score −7.76 was selected for screening of its quorum inhibitory activity against reporter strain and opportunistic pathogen *K. pneumoniae*. In this study, structure based virtual designing was used to identify novel quorum sensing inhibitors. Computer-aided identification of quorum sensing inhibitors against *Pseudomonas aeruginosa* was reported earlier by Yang et al. (2009).

Minimum inhibitory concentration

Minimum inhibitory concentration of cyanidin was determined against bio-sensor strain *C. violaceum* and *K. pneumoniae*. MIC was determined as the lowest concentration which showed complete inhibition of visible growth. The MIC of cyanidin was found to be 150 μg/ml for *C. violaceum*, and 250 μg/ml against *K. pneumoniae*. Hence, sub-MIC concentrations were fixed at 0–80 μg/ml for the experiments with *C. violaceum* and 0–150 μg/ml for experiments with *K. pneumoniae*. As all the experiments conducted were at the sub-MIC level as it is not expected to impose pressure on test pathogens to develop resistance. In our earlier report malvidin exhibited minimum inhibitory concentration in the range of 40 μg/ml against *K. pneumoniae* (Gopu et al. 2015).

QSI bioassay

Quorum sensing inhibitory activity of cyanidin was screened by flask incubation method. Results revealed that cyanidin significantly reduced violacein production in *C. violaceum* CV026 at all tested concentration (20–80 μg/ml). At the concentration of 20 μg/ml, 13.17 % inhibition was observed when compared with the control ($P < 0.05$). The gradual increase in the inhibitory activity was observed with increasing concentration of cyanidin and maximum of 73.96 % inhibition was observed at the concentration of 80 μg/ml (Fig. 1a). Our

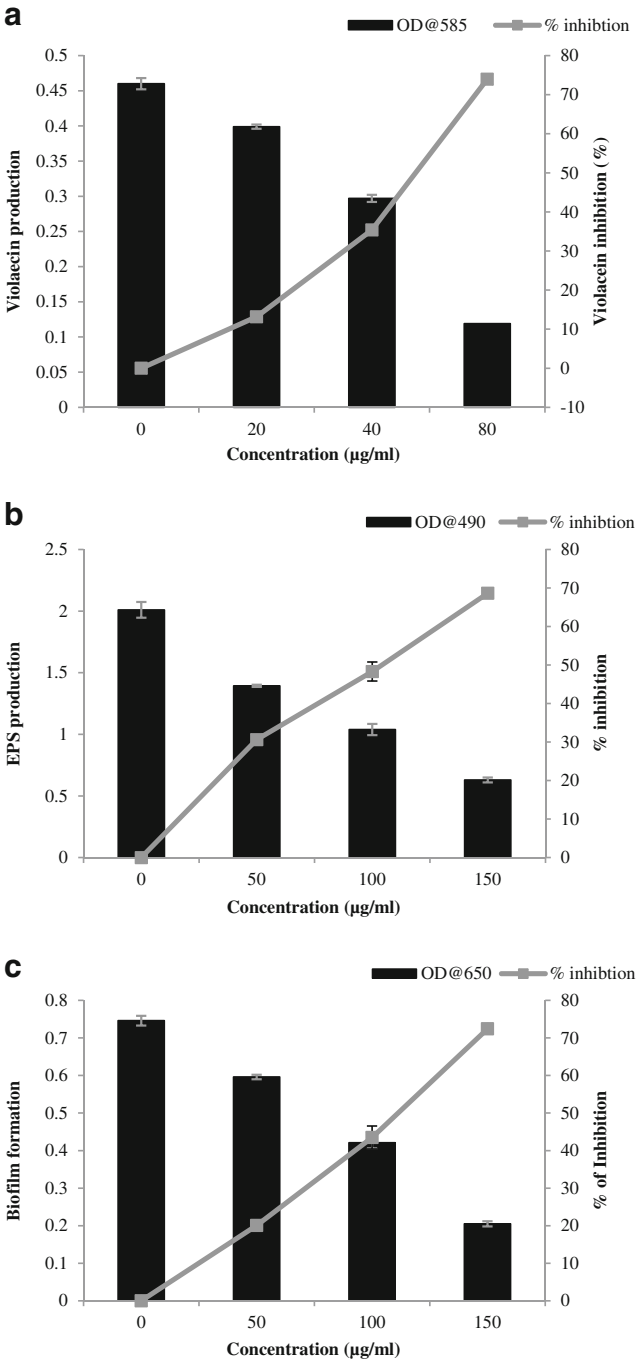

Fig. 1 Quantitative analysis violacein inhibition in *C.violaceum* (**a**), EPS reducing activity (**b**) and, anti-biofilm activity (**c**) by cyanidin at different concentration (0–80 μg/ml). Line graph represents the percentage inhibition. Vertical bars represented the mean of triplicates values with a standard deviation

results are comparable with those of Zhou et al. (2013) who reported that 200 μM of eugenol inhibited violacein production up to 56.5 % in CV026. Similarly, inhibition of violacein production in CV026 by glycosyl flavonoids from *Cecropia pachystachya* was reported by Vanegas et al. (2014).

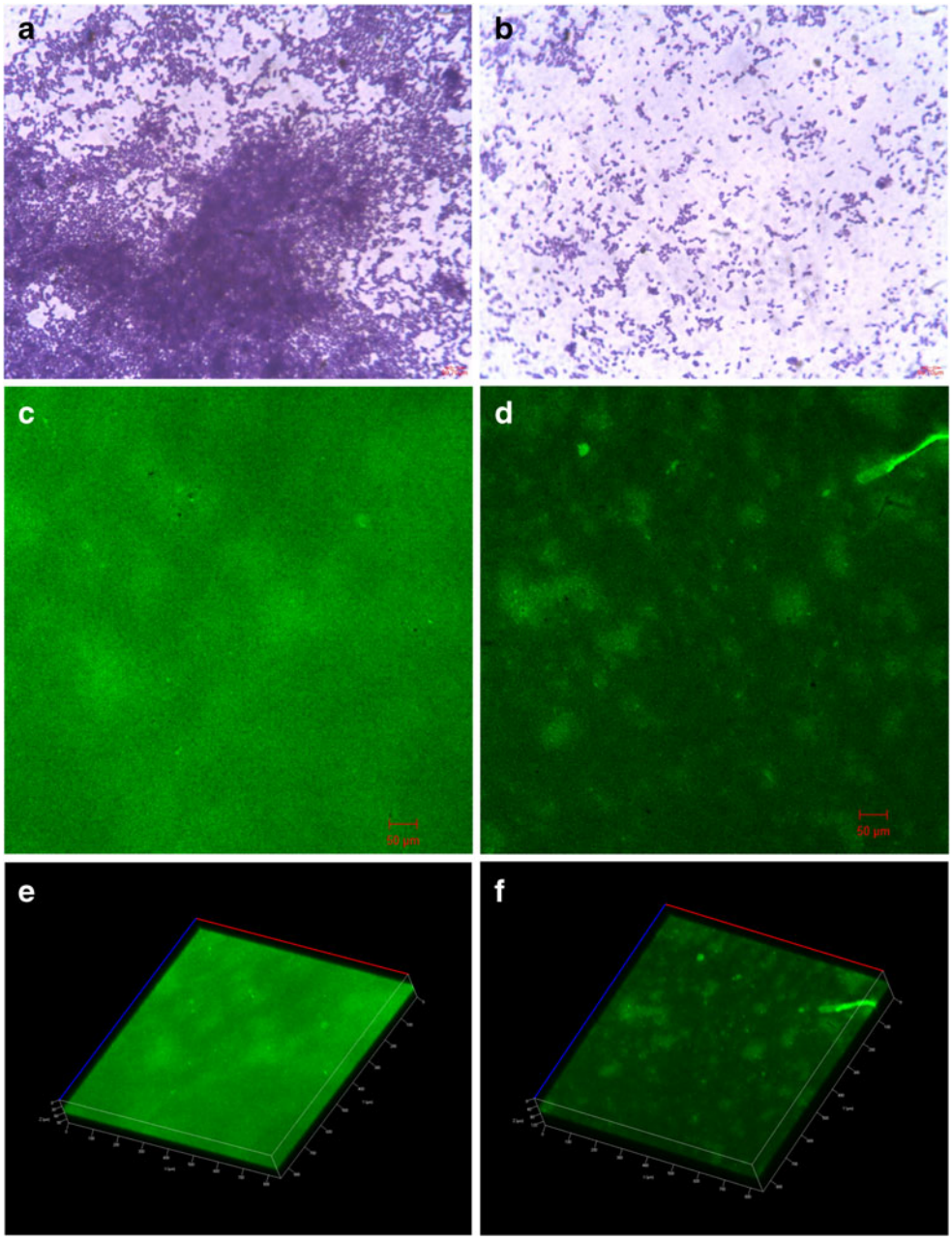

Fig. 2 Light microscopic and confocal laser scanning microscopic (CLSM) images of bacterial biofilm grown in the presence and/or absence of cyanidin (150 μg/ml). Images **a**, **c**, and **e** – Untreated slide; Images **b**, **d**, and **f** - treated slide

Table 2 Synergistic effect of cyanidin with antibiotics against *K. pneumoniae*

Bacterial strain	Antibiotics	Growth inhibition (%)	Growth inhibition with cyanidin (%)					
			50 μg	FICI	100 μg	FICI	150 μg	FICI
K. pneumoniae	Erythromycin	25.51 ± 1.80	27.98 ± 2.20	0.45	41.40 ± 1.12	0.65	65.05 ± 1.09	0.85
	Tetracycline	42.7 ± 0.49	52.75 ± 1.23	0.63	69.95 ± 1.34	0.83	83.29 ± 1.80	1.03
	Kanamycin	60.02 ± 0.51	67.84 ± 0.54	0.80	78.26 ± 1.09	1.00	86.45 ± 1.01	1.20

FICI fractional Inhibitory concentration index

<0.5 Synergy; 0.5–0.75 Partial synergy; 0.76–1.0 additive effect; 1–4 indifference; >4 Antagonism

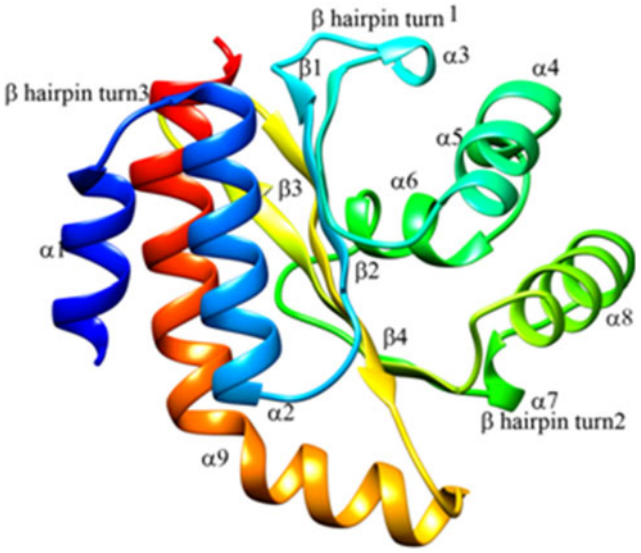

Fig. 3 Showing three-dimensional structure of LasR receptor protein [Reproduced with permission from Microbial Pathogenesis 79 (2015) 61–69]

Reduction in EPS production

Biofilm formation is characterized in part by the production of extensive EPS network. EPS confers facilitation of initial attachment of bacteria enhanced resistance to antimicrobial agents, also to environmental stress and formation of microcolony structure (Costerton et al. 1999). Thus, inhibition of EPS production may facilitate the direct exposure of food borne pathogens to the antibiotics, may in turn facilitate the eradication of biofilm. Here, cyanidin at the concentration of 150 μg/ml exhibited 68.65 % of reduction in the exopolysaccharide production by test pathogen. A steady increase in the inhibition rate was observed with the increasing concentration of cyanidin (Fig. 1b). Quantification of precipitated EPS revealed 30.64 % reduction in the EPS production in *K. pneumoniae* treated with 50 μg/ml of cyanidin compared to control ($P < 0.05$). Our results are in accordance with Abraham et al. (2011) reported that *Capparis spinosa* reduced EPS production up to 67 % in *P. mirabilis*.

Inhibition of biofilm formation

Quorum sensing mechanism plays a crucial role in the process of biofilm formation. Type-2 QS regulatory molecules and AI-2 transport genes were found to be involved in biofilm formation by *K. pneumonia*, and the role of LuxS dependent signal molecule in the earlier stages of biofilm formation was also elucidated (González Barrios et al. 2006). Cyanidin at the concentration of 150 μg/ml exhibited 72.43 % inhibition of biofilm formation in *K. pneumoniae* when compared with the untreated control ($P < 0.05$). It was observed that there seem to be concentration dependent decrease in the inhibition with decreasing concentration (Fig. 1c). The obtained results are comparable with that of Vikram et al. (2010) who reported that 100 μg/ml of quercetin exhibited up to 90 % inhibition of biofilm formation of *Vibrio harveyi*. It was also reported that rutin at the concentration of 100 μg/ml inhibited around 40 % of biofilm formation in *Escherichia coli*.

In-situ visualization of biofilm developed with or without cyanidin was carried out using light microscopy, scanning electron microscopy and confocal laser microscopy (Fig. 2). Light microscopic images showed a thick biofilm biomass on the control slide; whereas slide treated cyanidin at 150 μg/ml concentration exhibited the dislodged biofilm. SEM analysis also revealed that there was deterioration in the biofilm architecture and cells were loosely attached to the surface in the slides treated with test compound. Three-dimensional images of CLSM clearly showed a reduction in the thickness of the

Table 3 Details LasR receptor protein docked with N-octanoyl-DL-homoserine lactone (1), cyanidin (2) and, cyanidin along with N-octanoyl-DL-homoserine lactone (OHL)

S.No.	Molecules (Drug Bank ID)	Hydrogen Bonding interactions			Dock score (Kcal/mol)	Glide- Emodel score	Hydrophobic interactions
		H-Bond donor	H-Bond Acceptor	length (Å)			
1.	N-octanoyl-DL-homoserine lactone (OHL) - 3474204	Lig::O2	Trp 60:NH1	3.01	−4.28	−42.2	Leu 110, Phe 101, Tyr 93, Ala 105, Trp 88, Tyr 56, Ser 129, Tyr 64, Leu 36, Ala 127, Tyr 47, Ala 50, Val 57, Ile52
		Thr 75:OG1	Lig::N1	3.30			
		Asp 73:OD1	Lig:: N1	3.00			
2.	Cyanidin - 128861	Tyr47:O	Lig::O6	3.03	−7.76	−66.5	Leu :40, Ala :50, Leu :125, Leu :39, Ile :52, Gly :38, Gly :126, Ala :127, Leu :36, Val :76, Tyr :56, Thr :75, Tyr :64, Asp :73, Trp :88, Ser :129
		Trp60:NE1	Lig::O4	2.83			
		Lig::O3	Thr75:OG1	2.70			
		Lig::O3	Thr115:OG1	3.09			
3.	Cyanidin - 128861 (Third iteration)	Lig::O6	Tyr:47 O	2.85	−9.13	−61.1	Ile :52, Ala :50, Gly :38, Tyr :64, Val :76, Leu :36, Ala :127, Tyr :56, Ser :129, Thr :115, Thr :75, Leu :110, Asp :73, Trp :88
		Lig::O4	Trp:60 NE1	2.93			

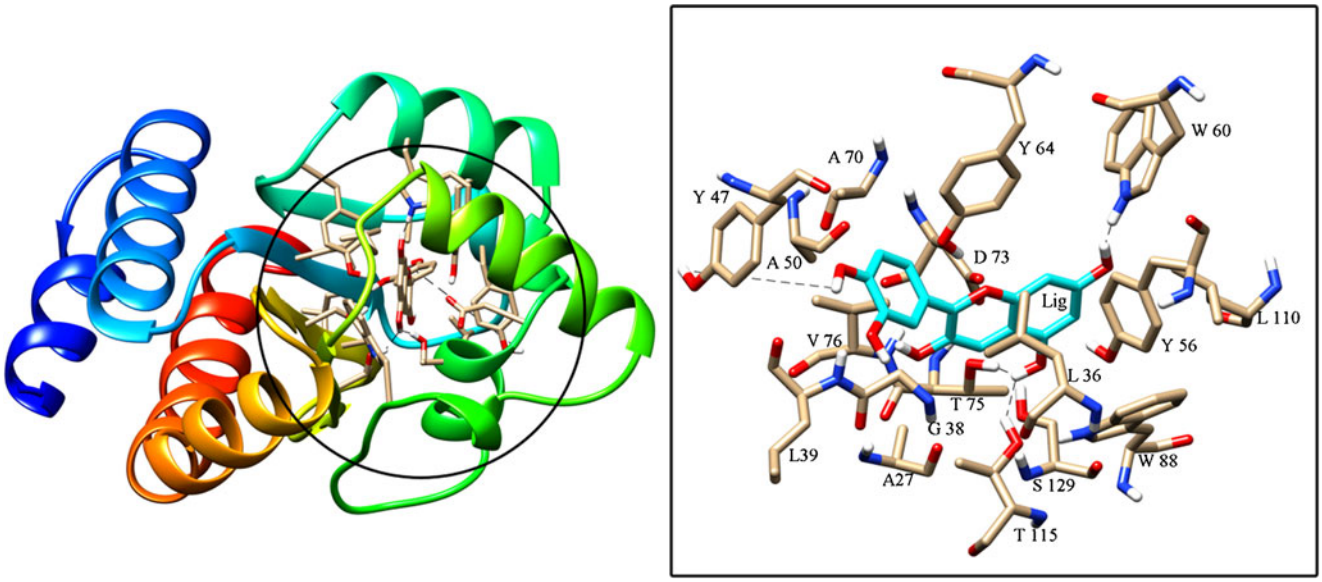

Fig. 4 Docked conformation of signaling molecule into the active site of LasR receptor protein. H-Bonds are displayed in the dashed line. Residues, which are forming hydrophobic interaction, are also labeled

biofilm in the treated slides when compared with untreated one. Results obtained are in accordance with Watnick and Kolter (1999) who reported a similar decrease in EPS production and biofilm formation.

Synergistic effect of cyanidin

This assay was performed to examine the synergistic activity of cyanidin with selected antibiotics against test pathogen. On testing the pathogens growth inhibition with antibiotics, *K. pneumoniae* showed the maximum of 86.45 % inhibition against kanamycin. Enhanced susceptibility was observed towards all tested antibiotics when treated with cyanidin at different concentration (50–150 μg/ml). It was also revealed that increasing concentration of cyanidin with antibiotics enhanced the sensitivity of *K. pneumoniae* towards relevant antibiotics in a dose-dependent manner. Upon treatment with 150 μg/ml of cyanidin, *K. pneumoniae* showed 65.05, 83.29 and 86.45 % increase in sensitivity towards Erythromycin, tetracycline, and kanamycin respectively. Earlier, Bjarnsholt et al. (2005) showed that the mechanism of enhanced susceptibility to antibiotics relies upon signaling mechanism. Up on treatment with added cyanidin, enhanced susceptibility was observed towards all tested antibiotics. Fractional inhibitory concentration (FIC) index were also calculated for each dose of treatment. Obtained values indicated that most of the combinations were synergistic and partial synergistic except treatment with kanamycin at the concentration of 100 μg/ml; tetracycline and kanamycin at the concentration of 150 μg/ml (Table 2). Our results revealed that anti-quorum compound at sub-MIC level increases the antibacterial action of conventional antibiotics in a synergistic manner. It was reported that *Vibrio vulnificus* showed enhanced sensitivity towards doxycycline, in a report by Brackman et al. (2008).

Molecular docking

The crystal structure of LasR receptor protein at 1.80 Å was taken from PDB database which was already reported by Bottomley et al. (2007). Auto inducing domains for the transcriptional process at the domains 20 to 60 was revealed from the database (Bauer et al. 2011). Secondary structure

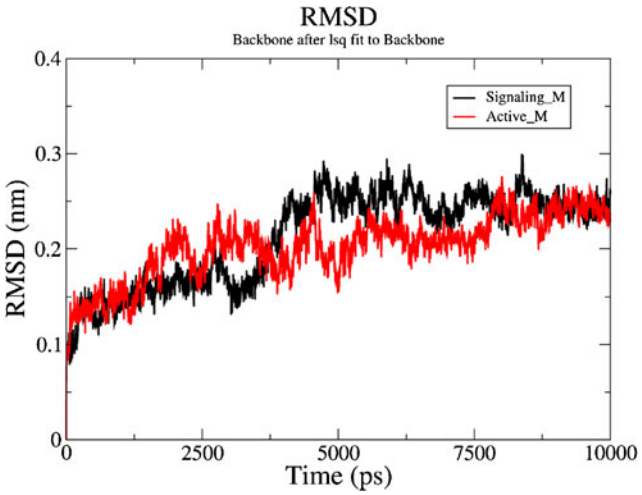

Fig. 5 RMSD profile of both complexes black color indicates the protein and signaling molecules while red color is showing protein-active molecule. The constant nature of red line is showing that the structure has achieved the minimum energy conformation. While the inconsistency in black line is showing that the protein-signaling molecule has not reached the minimum state

component of the receptor protein was analyzed using PDBSum database (Beer et al. 2014). The LigPlot analysis revealed that the receptor protein contains 4 β strands with 3 β hairpin turns and nine α helices (Fig. 3). The active site cavity of the protein was formed by the second β hairpin turn which is crucial for the docking analysis. The hotspot residues of the protein were revealed by performing molecular docking analysis. The docked complex was submitted to PDBSum database to visualize H-bond interaction between the residues. Here the numbers of H-bonds are 3 along with 14 hydrophobic interactions with the doc score of −4.28Kcal/mol. The information of interacting atoms along with the docking score is in Table 3.

The grid parameters used for signaling molecule was reused for the second iteration with cyanidin. After completion of docking, complex with the maximum dock score −7.76 Kcal/mol was used for further in vitro analysis. This complex was submitted to PDBSum database for further analysis. Three H-bonds was formed between protein and active compound along with 16 hydrophobic interactions. The information of interacting atom of protein and active compounds is given in Table 3.

The competitive nature of the test compound against signaling molecule was screened by third iteration of docking. In this attempt, the docking score of cyanidin was −9.13 Kcal/mol. All the ligands including signaling molecules docked to the same site that was used in earlier docking. In the presence of signaling molecule, the docking score of docked complexes was changed. Complex submitted to PDBSum database revealed that there were 2 H-bonds formed between LasR and cyanidin along with 14 hydrophobic interactions, which provided additional strength to this complex. The information on all interacting atoms of protein and cyanidin along with H-bond direction and distances is given in Table 3. The pose of cyanidin in LasR receptor protein is shown in Fig. 4. Black dashed line represents the H-bonds.

In-silico analysis may prove the mode of action by which the test compounds exhibit the quorum quenching potential. Molecular docking analysis of LasR receptor protein showed that cyanidin binds rigidly to the receptor with high docking score when compared with signaling molecule in both docking conditions (signaling molecule docked with and without cyanidin). A strong interaction between the compounds may be due to the binding of particular specific groups, which mediates conformational changes in the receptor protein.

Molecular dynamics simulation

Molecular dynamics simulation was performed to predict the conformational changes for activation and deactivation of LasR receptor protein in the presence of signaling molecule and cyanidin respectively. The simulations were performed with two complexes, LasR-OHL, and LasR-cyanidin. The simulations were run for 20 ns with the time step of 2 ps.

The RMSD profile was generated to screen the behavior of protein throughout simulation with signaling molecule and cyanidin. It can be seen in the RMSD profile (Fig. 5) that the protein-signaling molecule is unstable as compared to protein-test compound complex. This instability is caused because the signaling handles the activation of LasR protein.

This opening and closing of the pocket was validated by submitting both complex protein-signaling molecule and protein-test compound to CASTp server, which calculates the accessibility of protein pocket by using water molecule as a probe. RMSD profile showed that throughout the simulation, LasR-cyanidin complex is more stable than the LasR-signaling molecule complex. In a recent study Mowafy et al. (2014) evidenced that docking analysis may suggest the quorum quenching efficiency of test compounds. It was proved through molecular docking studies that aspirin can act as an anti-quorum agent against *P. aeruginosa*.

Conclusion

To conclude the present study evidenced the quorum sensing inhibitory potential of cyanidin against opportunistic pathogen *K. pneumoniae* by means of in-vitro and *in-silico* evidence. It was proved that cyanidin efficiently interrupted the QS regulatory mechanisms like biofilm formation, EPS production in test pathogen and violacein production in biosensor strain used. *In-silico* studies evidenced that cyanidin binds more rigidly with LasR receptor protein than the signaling molecule which, proves that cyanidin may act as a potential competitive inhibitor of signaling molecules towards LasR protein activity. Hence, cyanidin could serve as a cost-effective source for developing novel QS-based antibacterial strategies, for the management of food-borne pathogens in ensuring food safety.

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