

Targeting Acyl Homoserine Lactone (AHL) of *Pseudomonas aeruginosa* Responsible for Biofilm Formation using Plant Metabolites

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Abstract

AHL (acyl homoserine lactone) is a signaling molecule responsible for communication in gram negative bacteria, which is liable for bacterial virulence as well as biofilm formation. This study aims in targeting the AHL formation and AHL mediated virulence factor using plant extracts / plant metabolites. AHL plays a major role in pigment production, motility and rhamnolipid production in *Pseudomonas aeruginosa*. Inhibition of these mechanism by any mean implies that it is having impact in biofilm formation too. Plant derived metabolites/ extracts were subjected for swarming motility assay, the metabolite which inhibited the swarming motility was subjected for further study, where *P. aeruginosa* SU-3 was allowed to grow in the presence of the metabolite and its impact over the AHL production, pigment production and rhamnolipid production was evaluated. Curcumin was found to be effective against all the metabolites used. Thus curcumin subjected for molecular docking against ENR (enoyl carrier protein reductase), the enzyme involves in fatty acid synthesis and production of AHL.

Keywords: *Pseudomonas aeruginosa* SU-3; acyl homoserine lactone (AHL); rhamnolipid; pyocyanin pigment; enoyl reductase; molecular docking.

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INTRODUCTION

Biofilm is the gathering of microbes to form a consortium of cells within a polymeric matrix containing an assortment of substances enclosed inside the slimy mould based on the surroundings or surfaces it develops amidst¹. Costerton² elucidated how wild type bacteria plan their survival strategy through extreme environmental conditions, the wild strains form a substantial substratum around the cells through which the protracted flimsy fimbriae projected aids in precise attachment to inert surfaces. *Pseudomonas aeruginosa* is dextrous in skillfully subjugating the systemic immunity whenever there is a contingency and forms tenacious biofilms within individuals predominantly suffering from cystic fibrosis³. The biofilm formed by *Pseudomonas aeruginosa* is rich in glycoproteins called alginate, composed of continuous non repetitive units of glucuronic acid and mannuronic acid, imparting a mucoidal appearance³ that deteriorates the chances of mitigating cystic fibrosis^{4,5}. Biofilm of *Pseudomonas aeruginosa*, is regulated by a density dependent mechanism coordinated through the presence of specific signalling molecules such as Acyl Homoserine Lactone (AHL), this mechanism is termed as Quorum Sensing (QS) controlled by *las* and *rhl* signaling systems⁶ mostly by N (3-oxododecanoyl) homoserine lactone (3OC12-HSL) and N-butanoyl-L-homoserine lactone (C4-HSL)^{7,8}. The various virulence factors associated with *Pseudomonas aeruginosa* are also influenced depending upon the cell population density of the organism⁹. Cao et al.¹¹ explained the role of another definite transcriptional regulator *mvfr* influencing quorum sensing. The *mvfr* controls the quinolone signalling in *Pseudomonas aeruginosa* which regulates various virulence factors such as pyocyanin,

elastase and rhamnolipid¹⁰. Rhamnolipids are molecules consisting of a hydrophilic carbohydrate unit L-rhamnose and a hydrophobic lipid unit hydroxyalkanoloxy alcanoic acid (HAA) which are linked through a glycosidic bond¹².

Migration in *Pseudomonas aeruginosa* is facilitated by monotrichous flagellum through an aqueous medium provided with limited agar source and by the dilation and contraction of pilus in case of solid surfaces¹³. *Pseudomonas aeruginosa* produces a strikingly noxious blue green pigment that belongs to the phenazine family which competently induces reactive oxygen species¹⁴ and furthermore involved in the production of 1-hydroxy phenazine and phenazine-1-carboxylic acid other than pyocyanin¹⁵. According to Dietrich et al. (2006) pyocyanin is a terminal signaling molecule¹⁶. The biosynthesis of pyocyanin is carried out when LasR and RhIR binds to 3OC12-HSL and C4-HSL respectively¹⁷. Certain naturally occurring substances derived from plant sources are making progress as potential anti-quorum sensing compounds¹⁸. Curcumin is one such natural compound belonging to the *Zingiberaceae* family having anti-inflammatory, antioxidant, antibacterial, antifungal and very few reports addressing anti-quorum sensing properties¹⁹.

In this study, metabolites extracted from plant and commercially available curcumin were tested against swarming motility of *Pseudomonas aeruginosa*. The influence of bioactive compounds against the mechanisms regulated by quorum sensing such as the production of AHL, pigment production and rhamnolipid production was also studied along. The effective molecule was studied for its interaction against Enoyl acyl carrier protein reductase (ENR) enzyme responsible for the chain elongation that is involved in the fatty acid synthesis, which is highly responsible for AHL formation²⁰.

MATERIALS AND METHODS

Organism chosen

Pseudomonas aeruginosa SU-1 reported in our earlier study²⁶ was used in this study (Genbank Accession number -GU395985).

Various bioactive compounds used

Alkaloids²⁸, flavonoids²⁹, saponins^{27,30} and tannins²⁸ of *Cassia alata*, *Annona muricata*, *Mentha*

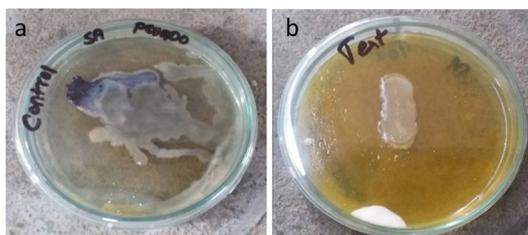


Fig. 1. Swarming motility of *Pseudomonas aeruginosa*
a) Control b) Against curcumin

spicata, *Cassia senna*, *Euphorbia hirta*, *Thespesia populnea* and *Wrightia tinctoria* were isolated and used in this study. Commercially available curcumin (Merck, India) were used in this study.

Swarming motility test

0.5% agar, 0.5% peptone and 0.2% beef extract were taken in 5 mL distilled water, autoclaved and was mixed with 5 mL of filter sterilized 1.0% w/v glucose. It was poured onto a sterile petri plate. The extracts/ curcumin with appropriate concentrations were seeded with 5 mL of 0.5% agar, melted and poured immediately onto the 10 mL of pre-poured agar plate as an overlay. 2 µL of overnight culture of *P. aeruginosa* was inoculated at the centre of the agar surface and the plates were incubated for 24 h at 37°C.

Extraction and Characterization of acyl homoserine lactone (AHL), pyocyanin and rhamnolipid

Pseudomonas aeruginosa inoculated into the 1 L of nutrient broth containing the effective compound/ extract (5 mg) (which showed effective inhibition of swarming motility alone was used here). Cell supernatants in the stationary phase cultures were extracted with equal two volume of ethyl acetate acidified with 0.5% acetic acid for three times. The solvent was removed by rotary evaporation (40–45°C) and the residue was resuspended in 1 mL of 20% ACN.

P. aeruginosa culture grown as above condition was used for pigment extraction²² and for rhamnolipid extraction. Rhamnolipid extract

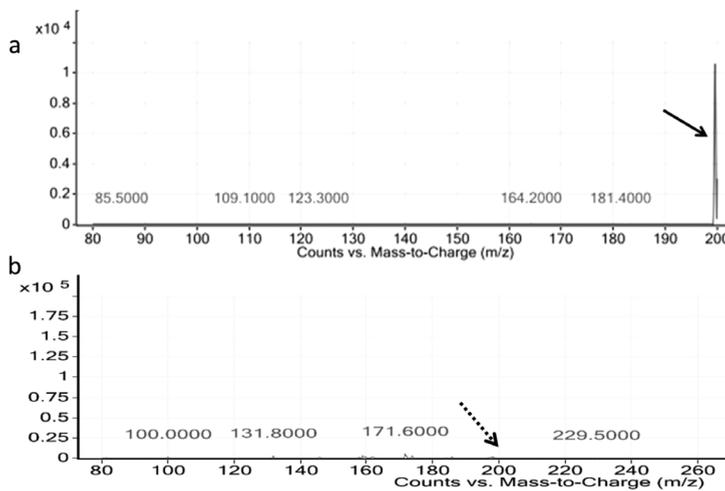


Fig. 2. LC-MS analysis of AHL of *Pseudomonas aeruginosa* a) Control b) grown with curcumin

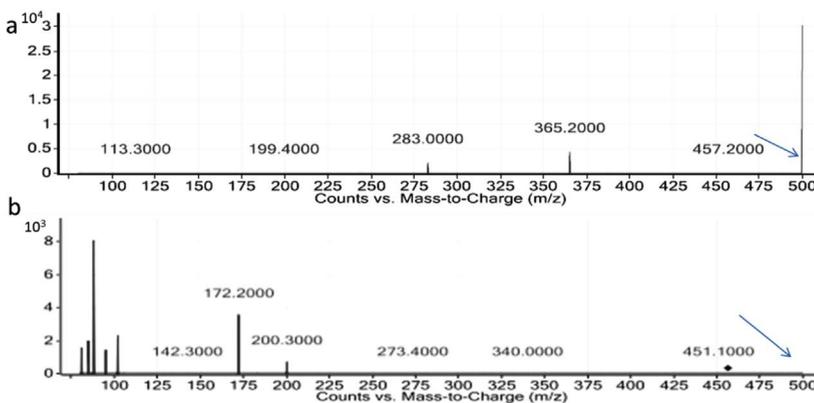


Fig. 3. LC-MS analysis of rhamnolipid of *Pseudomonas aeruginosa* a) Control b) grown with curcumin

was done as follows - the overnight culture of *Pseudomonas aeruginosa* was centrifuged at 10000 rpm for 20min at 4°C and the supernatant was adjusted to pH 2.0 by addition of 6M HCl. The solution was centrifuged at 10000 rpm for 20min at 4°C. Precipitate bound to the tubes were added with ethyl acetate. Ethyl acetate was allowed to evaporate.

Thus, obtained homoserine lactone, pyocyanin and rhamnolipid were subjected for LC-MS/MS analysis using Agilent 6400 Series Triple Quadra pole. The samples were eluted with an isocratic mobile phase of Acetonitrile: 5mM Ammonium formate (70:30)²¹.

Molecular Docking

Molecular docking was carried out between the receptor molecule enoyl acyl carrier protein reductase enzyme responsible for the chain elongation and this key enzyme involved in the fatty acid synthesis and curcumin using Discovery Studio version 2.1.

RESULTS AND DISCUSSION

Swarming motility test

The swarming motility plates were observed. There was effective inhibition for curcumin (Fig. 1b) but not for other metabolites (results not shown here) which was further taken to comprehend the effect towards the signaling molecule. Various plant extracts and plant derived materials have shown effective inhibition of swarming motility³¹.

Extraction and Characterization of acyl homoserine lactone (AHL), pyocyanin and rhamnolipid

LC-MS analysis of AHL was found to show a strong peak at m/z 200, where the peak is characteristic of 3-OH-C 5 -HSL (Homoserine lactone)^{23,24} (Fig. 2a), where the characteristic peak of 3-OH-C 5 -HSL was found missing in curcumin treated cells (Fig. 2b).

LC-MS analysis revealed the presence one major component detected at m/z 499 corresponding to Rha-C10-C8 (Fig. 3a)²⁵, curcumin treatment had a detrimental impact on the rhamnolipid production too (Fig. 3b).

Likewise, curcumin was showing impact on pigment too (Fig. 4b). rhamnolipid and pigments are influenced by AHL only. Inhibition of AHL has impact on virulence factors like rhamnolipid and pigments.

Molecular docking

Docking was performed using Accerlys Discovery studio version 2.1 where the receptor molecule was enoyl acyl carrier protein reductase and the ligand used was curcumin. The hydrogen in the 41st position of curcumin interacts with Asparagine in the 222nd position in the B chain of Enoyl acyl carrier protein reductase and the oxygen in the 3rd position of curcumin interacts with Tryptophan in the 55th position in the B chain of Enoyl acyl carrier protein reductase.

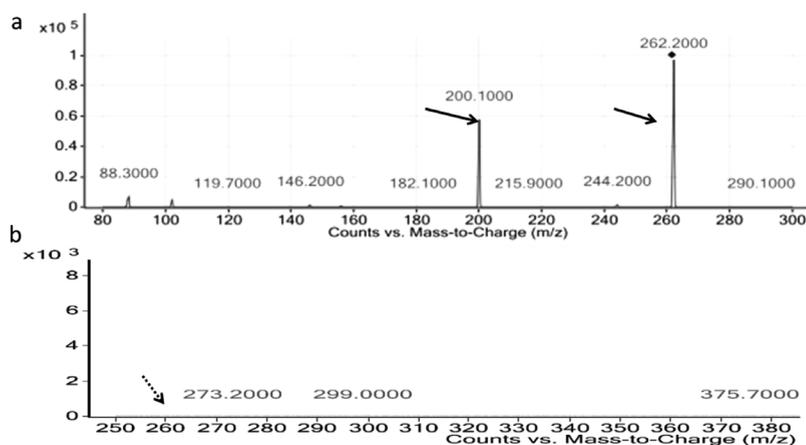


Fig. 4. LC-MS analysis of pyocyanin pigment of *Pseudomonas aeruginosa* a) Control b) grown with curcumin

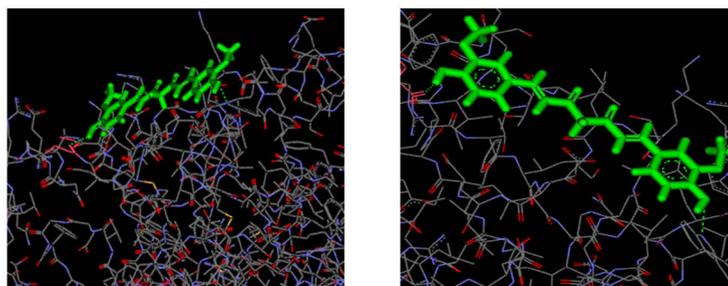


Fig. 5. Molecular docking of curcumin against ENR (enoyl carrier protein reductase)

CONCLUSION

Curcumin has shown effective inhibition of swarming motility and even showed impact on AHL production. It also showed negative impact against the quorum sensing molecules like pyocyanin pigment and rhamnolipid. Further, the molecular docking studies revealed that curcumin could interact with enoyl reductase enzyme responsible for AHL formation.

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None.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All the authors involved in idea creation. Ms. Samanvitha did all the work and supported by Mr. Sanjay Kumar. All the authors involved in manuscript preparation.

FUNDING

None.

DATA AVAILABILITY

All data generated during the study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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