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Patulin interference with ATP binding cassette transferring auto inducer −2 in *Salmonella typhi* and biofilm inhibition via quorum sensing

Princy Vijayababu [a,*,1], Gopinath Samykannu [a,1], Christian Bharathi Antonyraj [b], Jebastin Thomas [a], SundaraBaalaji Narayanan [a], Syed Ibrahim Basheer Ahamed [b], Shanmughavel Piramanayagam [c]

[a] Structural Biology Laboratory, Department of Bioinformatics, Bharathiar University, Coimbatore, Tamil Nadu, India
[b] Centre for Bioinformatics, Pondicherry University, Pondicherry, India
[c] Computational Biology Laboratory, Department of Bioinformatics, Bharathiar University, Coimbatore, Tamil Nadu, India

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ABSTRACT

Salmonella typhi are Gram-negative pathogens that infect many hosts including humans and animals and cause diseases ranging from gastroenteritis and diarrhea to life-threatening systemic syndrome. Owing to the metabolic versatility, they will colonize as multi cellular aggregates on various surfaces to enhance the virulence by forming a biofilm in which bacterial cells are more resistant to antibiotics than planktonic cells. Quorum Sensing (QS) is a cell-to-cell communication mechanism in the bacterial system to coordinate group behaviors such as biofilms formation and virulence factors production. In QS system ATP Binding Cassette (ABC) transporter component, LsrA, plays a key role to transport autoinducer-2 (AI-2) to increase the cell density. In order to reduce biofilm formation, patulin was selected as a natural QS inhibitor and its function was studied by biofilm inhibitory assays. Significant differences in the spectroscopic values were obtained between antibiotic resistance of kanamycin (30 μg/ml) and patulin (30 μg/ml). Furthermore, to distinguish the molecular level interaction of LsrA, patulin and AI-2 were docked. Both the compounds were interact in the same pose with Glide score of −4.237 kcal/mol and −7.126 kcal/mol respectively. These results will suggest that patulin is the efficient Quorum Sensing Inhibitor to control biofilm formation in *S.typhi*.

1. Introduction

In the microbial world, 99.9% of micro-organisms including bacteria have the ability to construct biofilm on a wide range of surfaces [1]. A biofilm is defined as inhabitants of one or more organisms involved to each other on the surface by means of a bacterium-initiated matrix [2,3]. Generally, these matrixes resource is very constant surface and more resistance to antimicrobial agents. These community or planktonic cells continuously shed to form biofilm, which in humans can lead to a systemic infection or release of the organism into the environment [4]. Gathering on the surface occurs when bacterial cells reproduce and form multi-layered cell clusters resulting a biofilm [5]. Based on the adhesion rate, biofilm formation will be divided into three substantial stages. They are (i) preliminary non-specific, reversible primary adhesion; (ii) exact irreversible adhesion (gathering at the surface involving biomolecular processes including quorum sensing) (iii) biofilm spreading and recolonization [6–8]. In this routine, pathologically relevant processes such as biofilm formation and bacterial virulence were regulated by an essential mechanism known as Quorum Sensing [9].

Quorum Sensing (QS) is a cell-to-cell communication mechanism in bacterial system for harmonizing group behaviors such as forming biofilms and producing virulence factors [10,11]. This intercellular communication is accomplished through the production, exchange and release of small chemical signaling molecules called autoinducers (AI) [12], that increase the concentration with population density and received by transcriptional regulators, can control gene expression [13]. Presently, autoinducers including acyl homoserine lactones (AHLs), autoinducer-2 (AI-2), autoinducer-3 (AI-3), 4-quinolone signal (4Qs) and autoinducing peptides (AIPs) have been identified in various bacteria [14]. A key compound in quorum sensing pathway is 4,5-dihydroxy-2, 3-pentanedione (DPD) [15], which can occur in several forms and termed as “AI-2” present in both Gram-positive and Gram-negative organisms.

* Corresponding author.
E-mail addresses: princymcc@gmail.com, princy.vijayababu@buc.edu.in (P. Vijayababu).
[1] Sharing equal contribution.

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Gram-negative bacteria include: *H. influenzae,Y. pestis,C. jejuni, H. pylori, N. meningitidis, V. cholera, V. harveyi, E. coli, S. typhimurium* and *S.typhi* [16,17]. These bacterial species can regulate pathologically significant cellular processes such as virulence factor production and biofilm formation, in an AI-2-dependent manner [18]. Quorum sensing provides an essential mechanism for the collective regulation of pathologically relevant processes such as biofilm formation and bacterial virulence [23].

LsrA is an ATP-binding protein responsible for energy coupling in ABC transport of quorum sensing system [19]. ATP-binding cassette (ABC) transporters are a large superfamily of membrane proteins with diverse functions. They convert the energy gained from ATP hydrolysis into trans-bilayer movement of substrates either into the cytoplasm (import) or out of the cytoplasm (export) [20]. ABC transporter must be a potential target for drug development against pathogenic bacteria [21]. In *S.typhi* use AI-2, a small diffusible signaling molecule as communication mediator, which is transported to the cells through ABC transporter [22]. Therefore, blocking *S.typhi* QS system from the transporter has considered as an alternative and noticeable approach instead of traditional antibiotics to control biofilm formation [23–25].

Formerly, many natural and synthetic AI-2 analogues were reported as potential agonist and antagonist [26,27]. Natural Quorum Sensing Inhibitors (QSIs) such as iberin [28], baicalein [29], quercetin [30], 6-Gingerol [31], penicillic acid and patulin [32] were evidenced the biofilm inhibition in various bacterial species. Only a limited number of investigations were conceded to quorum sensing and their effect on biofilm formations [33].

Therefore, we interested in Patulin, which has structural analogue of AI-2 [34], atagonistic nature, and natural QSI present in eatable fruits. This a natural QSI ready to inhibit the biofilm formation with minimal concentration. Molecular interaction studies were carried out toward to understand the molecular functions, structural and inhibitory insights of *S.typhi* biofilm formation. These study may useful in drug development against typhoid fever and other systematic diseases which caused by *S.typhi* biofilm.

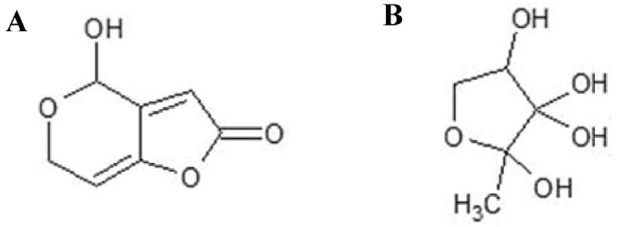

Fig. 1. 2D structure of A) Patulin and B) AutoInducer-2.

2. Methodology

2.1. Optimization of S.typhi biofilm formation

A sterile 96-well flat-bottomed microtiter plate with a lid was filled with 180 μl of LB broth (Tryptone 1%, Yeast Extract 0.5% and NaCl 1%) and 20 μl of overnight growth culture of bacterial suspension in each

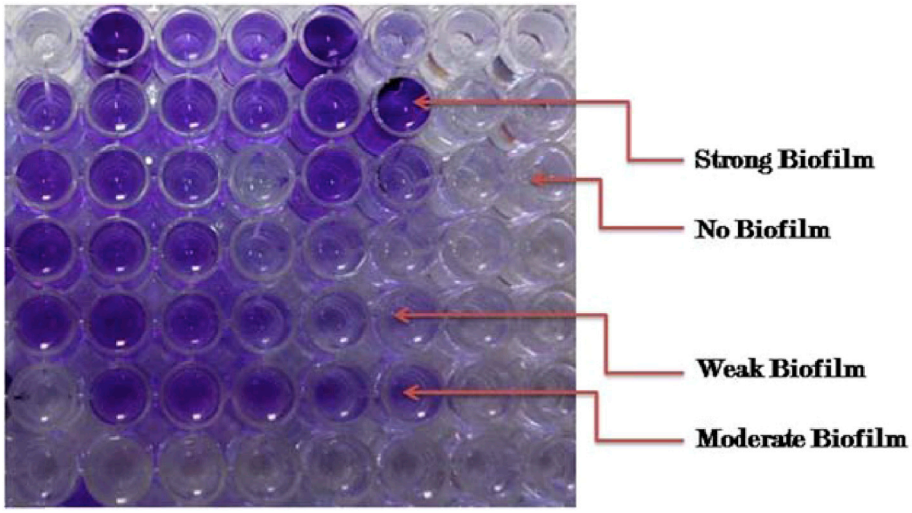

Fig. 2. 96-well flat-bottom microtiter plate-crystal violet adsorbed Biofilm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

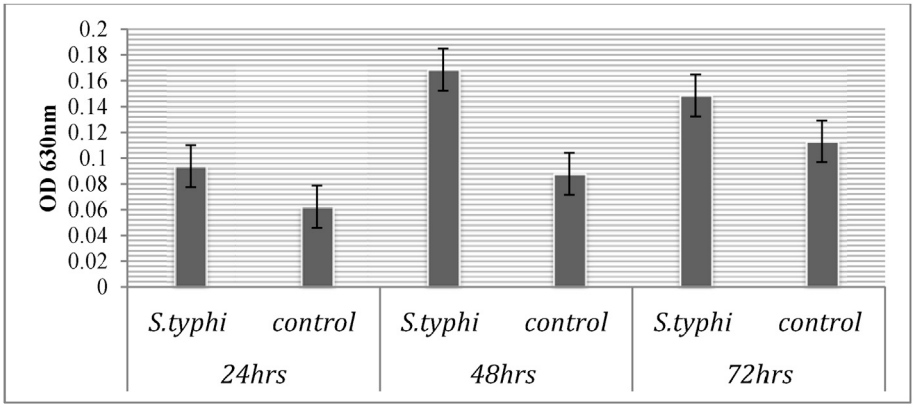

Fig. 3. Histogram shows biofilm formation in 24hrs, 48hrs and 72hrs. Error bars indicate the standard deviation (SD).