



## SHORT COMMUNICATION

### Quorum Sensing Molecules Acyl-Homoserine Lactones and Indole Effect on T4 Bacteriophage Production and Lysis Activity

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#### ABSTRACT

The present study was an effort to look at the effect of quorum sensing molecules which are acyl-homoserine lactones and indole on production and lytic activity of T4 bacteriophage. A predator/prey system model was employed to conduct the experiment. Results obtained during the study showed that acyl-homoserine lactones (AHL), which are essential signaling molecules of quorum sensing in many Gram-negative bacteria, can increase T4 phage production and lysis against *Escherichia coli* BL21 (DE3) pLysS. In addition, our study showed that indole which can also act as a signaling molecule and is a common diagnostic marker for the identification of *E. coli* reduced the lysis activity and production of T4 bacteriophage.

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#### INTRODUCTION

It is well known that in infection cycle of a lytic bacteriophage, lysis of the bacterial host makes the last event (Wang *et al.*, 2000) and there are number of different agents which affect the lytic activity of bacteriophage like mitomycin, antibiotics, UV light and some chemicals (Little, 2005). There are certain chemical signals in the ecosystem that induce the lytic cycle under favorable conditions (Oppenheim *et al.*, 2005). It is concluded therefore, that in terrestrial ecosystems some induction mechanisms do exist. These chemical signaling molecules also known as autoinducers (Whitehead *et al.*, 2001) are synthesized and used by bacteria thus they play an important role in quorum sensing. Bacterial population use quorum sensing to coordinate their specific behaviors which are dependent on bacterial local density. Quorum sensing can take place within as well as between different species of bacteria, and thereby serve as a communication network. Common classes of molecules used as signals in quorum sensing are acyl homoserine lactones (AHL) in Gram-negative bacteria, oligopeptides in Gram-positive bacteria and a family of autoinducers i.e. autoinducer-2 (AI-2) in both Gram-positive and negative bacteria (Miller and Bassler, 2001).

However, quorum sensing has been a burning topic in Gram-negative *E. coli* research for very long period of time. In the recent last 8 years, many researchers have

elaborated that *E. coli* use many quorum-sensing systems (Walters and Sperandio, 2006) and indole is one of the most important inter-species quorum sensing molecule (Li and Young, 2013). But it is worth to note that *E. coli* do not produce AHL molecules which are commonly found in other Gram-negative bacteria and are important signaling molecules. However, *E. coli* has a receptor which detects AHLs formed by other bacteria (Ahmer, 2004).

This study was inspired by the hypothesis that quorum sensing can be used as a mean of regulating phage-bacterium interactions. We suppose that if bacteria make use of quorum sensing in order to regulate and govern their antiphage activities, they can enhance their defense mechanisms thus avoiding infection during their growth under high-risk conditions, besides saving the metabolic accountability and maintaining a steadily elevated antiphage strategy. As a first step, it was investigated the effect of AHL and Indole on the lysis activity of T4 phage against *E. coli* BL21 (DE3) pLysS. This is the first ever report on the lysis activity of T4 phage in the narrated above condition.

#### MATERIALS AND METHODS

The effect of AHL and Indole on the lysis activity and production of T4 bacteriophage against *E. coli* BL21 (DE3) pLysS was studied from January to May 2012.

**Bacteria and Phage:** In order to conduct the study, the *E. coli* BL21 (DE3) pLysS strain was used as the primary host for lysis activity of the bacteriophage named *E. coli* bacteriophage (ATCC11303-B4). The *E. coli* BL21 (DE3) pLysS was obtained from the American Type Culture Collection (ATCC). All bacterial and phage stock cultures prepared/obtained were stored at  $-80^{\circ}\text{C}$  in Luria-Bertani broth (Oxiod) containing 50% (v/v) glycerol. Phage titer was determined as plaque-forming units (pfu/ml) using the double layer agar plate method.

**Media, chemicals, and other reagents:** LB medium containing 10g tryptone, 5g yeast extract and 10g sodium chloride per 1,000ml of water (pH 7). *N*-Butyryl-DL-Homoserine lactone  $\text{C}_8\text{H}_{13}\text{NO}_3$  (>97%, HPLC) and Indole  $\text{C}_8\text{H}_7\text{N}$  (>99%) were obtained from Sigma-Aldrich (USA).  $\text{C}_8\text{H}_{13}\text{NO}_3$  (0.1%) concentration working solutions ( $1,000\text{ mg L}^{-1}$ ) of the analytes were prepared in acetonitrile (ACN). The solutions were kept at  $-20^{\circ}\text{C}$  and stored for four weeks, while to obtain indole working solution; it was diluted in water to receive a final concentration of 0.5%.

**Plaque count assays:** The progress of lysis and production were recorded by plaque count assays to check the effect of AHL and indole on the lysis activity of T4 bacteriophage ( $4 \times 10^9$  pfu/ml) against *E. coli* BL 21(DE3) pLysS (1 OD at 600nm). The effect of AHL and indole on diluted bacteriophage was also checked, for dilution test T4 bacteriophage was diluted in LB broth through a serial dilution. In order to avoid indole production by *E. coli*, 12 hours incubated *E. coli* cells in LB media were centrifuged (13000rpm/15min). By centrifugation all the indole produced during incubation in media was removed. The *E. coli* cells were taken and suspended in fresh LB media broth for experimental use.

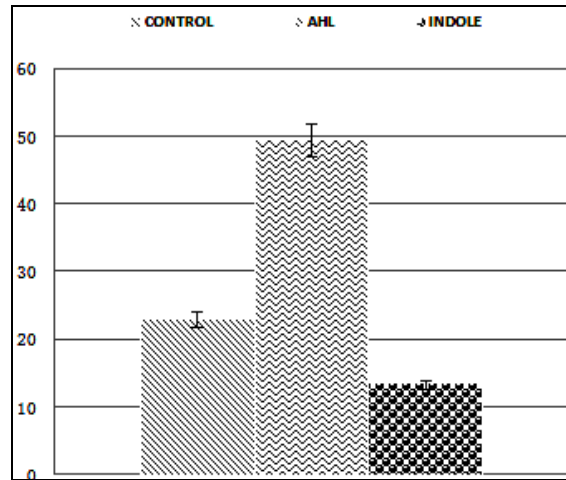
**Plating of phage:** For AHL and indole experiment 20  $\mu\text{l}$  of AHL/indole, was added to 0.2 ml of bacterial culture (*E. coli* cells from fresh LB media) and then allowed to adsorb to the *E. coli* for 3 minutes followed by addition of 0.1 ml of T4 bacteriophage to suspension. The suspension was then added to the soft agar and poured onto base plate. Agar tube was rolled between palms to mix for 2 or 3 seconds, and quickly poured onto agar surface of warm base plate. In order to disperse soft agar over the surface of the base plate agar they were gently moved in the pattern of figure eight. Soft agar was allowed to harden and then incubated at  $37^{\circ}\text{C}$ .

**Plaque size determination:** Pictures of plates with T4 bacteriophage plaques were taken using the gel documentation system and diameters of plaques were measured manually.

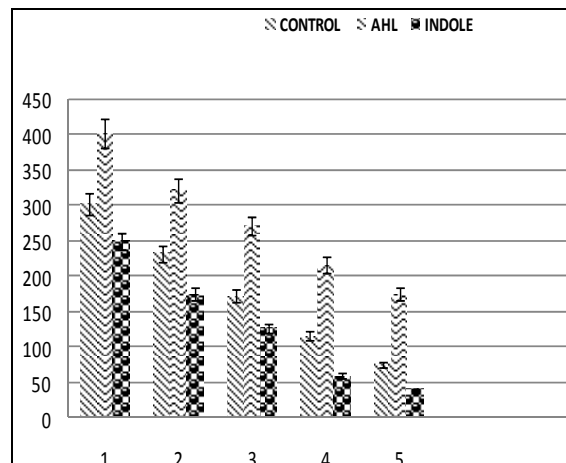
**Statistical analysis:** Statistical analysis included t test for the comparison of change in outcome variables in response to AHL and Indole with methods described by sigma stat. The analysis was carried out with Graph Pad Prism 5 software.

## RESULTS

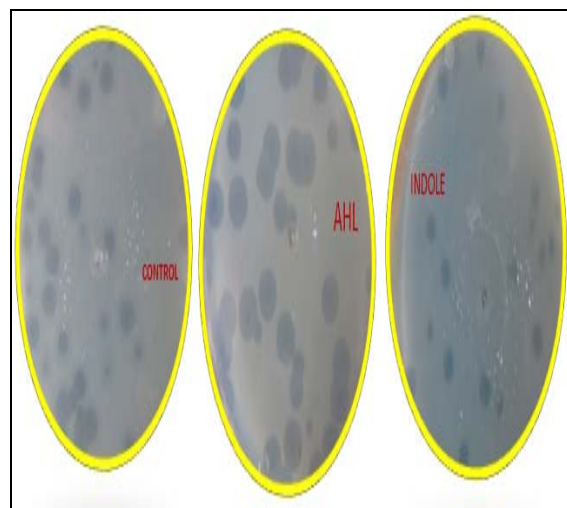
Relationship of quorum sensing molecules on lytic activity of T4 bacteriophage and its production experi-



**Fig. 1:** Relationship of quorum sensing molecules on lytic activity of T4 bacteriophage: AHL increased the lysis activity of T4 bacteriophage while indole decreased the lysis activity.



**Fig. 2:** AHL and indole effect the lysis activity of diluted T4 bacteriophage: the results showed that AHL increased the lysis activity in diluted T4 phage while indole decreased the lysis activity in diluted T4 phage ( $P < 0.004$ ).



**Fig. 3:** AHL and indole effect plaques size of T4 bacteriophage. AHL increased the plaque size and indole reduced the plaque size as compared to the control as shown in Fig.

mentally by using a model predator/prey system was tested. Bacteriophage T4, a viral predator, and its bacterial prey i.e. *E. coli* BL21 (DE3) pLysS were used. Through plaque count assays, the results exhibited that AHL (0.1%) increased the lysis activity and production by 115.2% of T4 bacteriophage (Fig. 1). It was demonstrated that indole (0.5%) can reduce the lysis activity and production up to 41.9% of T4 bacteriophage (Fig. 1). AHL increased the lysis activity by 54.4% in diluted bacteriophage while indole decreased the lysis activity by 27.4% in diluted bacteriophage (Fig. 2).

The effect of AHL and indole on plaque size was checked and result showed that AHL increased the plaque size by 100% as compared to the control and indole reduced the plaque size as compared to the control (Fig. 3). The plaque size of AHL was 10mm and indole was 5mm while the size of control plaque was 6mm after 16 hours.

### DISCUSSION

Quorum sensing plays an important role in both symbiotic and pathogenic bacteria-host interactions (Boyer and Wisniewski-Dye, 2009). In addition, bacteria have evolved to use quorum sensing as a way of regulating their interaction with their prey that is virus which is the most numerous biological entities on earth (Hoyland-Kroghsbo *et al.*, 2013).

AHLs are the most common class of autoinducer used by Gram-negative bacteria but *E. coli* does not produce AHL molecule commonly found in other Gram-negative bacteria (Ahmer, 2004). The present study findings showed that AHL increased the lysis activity and production of T4 phage as Ghosh *et al.* (2009), reported that acyl-homoserine lactones can induce virus production.

Indole is formed from tryptophan by the tryptophanase enzyme and indole can act as an extracellular signaling molecule (Li and Young, 2013). In

this study, we have found that indole can reduce the lysis activity and production of T4 phage as Mahyar *et al.* (2012) reported that indole can reduced virus attachment to host.

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