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QUORUM SENSING EFFECT THE LYSIS MECHANISM OF T4 BACTERIOPHAGE

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ABSTRACT: According to the cell density bacteria can regulate their gene expression. Gene regulation is initiated by the release of signaling molecules into the environment which are called autoinducers. When the population density increases, these autoinducers accumulate extracellularly and these can be detected by the bacteria through quorum sensing.
We found that *E. coli* make use of indole quorum-sensing signals to prevent it from infection by T4 phage. This has been acknowledged for the first time that *E. coli* utilizes antiphage defense mechanism regulated by quorum sensing. We proposed that through quorum sensing *E. coli* is protected in conditions where there is increased risk of infection as for example during growth in high cellular density in which there is mixed species environments. In microbial communities, quorum-sensing is a general phenomenon which controls *E. coli* susceptibility to phage.

KEYWORDS: Quorum, Sensing. Lysis, Mechanism, T4 Bacteriophage

1 INTRODUCTION

Quorum sensing (QS) is an important way to communicate information between bacteria. Bacteria used different quorum sensing molecule for communication and these signals molecules are called autoinducer (AI). These autoinducers are produced and released by the quorum sensing bacteria to levels dominating the increasing cell-population density. The attainment of minimal threshold stimulatory concentration of an autoinducer leads to an alteration in gene expression. Both Gram-positive and Gram-negative bacteria are capable of using quorum sensing communication circuits for regulating a diverse array of physiological activities. These activities include symbiosis, competence, virulence, conjugation, antibiotic production, sporulation, motility and biofilm formation. In the field of quorum sensing revealed, cell to cell communication via autoinducers both within and between bacterial species. The establishment of enormous data in this field suggests autoinducers acquiring specific responses from host organisms. Despite the difference in chemical signals, signal relay mechanisms and the target genes controlled by the bacterial quorum sensing systems, the ability to communicate with one another allows bacteria to coordinate the gene expression as well as the behavior of the entire community. This process presumably confers upon the bacteria some of the qualities of higher organisms. The evolution of quorum sensing systems in bacteria thus could have been one of the early steps in the development of multicellularity [1].

This study was influenced by the hypothesis that quorum sensing could additionally be used as a mean of regulating phage-bacterium interactions. Bacteriophages are viruses that attack their respective bacteria. Under natural environments the phages exert around 10 fold predation pressure which might result in outstripping of bacterial cells [2]. Therefore host bacteria have developed very strong antiphage mechanisms, such as blocking phage initial attachment, degrading phage genome, or host suicide there by preventing the phage progeny spread in the population [3]. As bacterial host is always required for the proliferation of phages, therefore in thickly populated mixed-species environments the phages are always

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more abundant and diverse than in sparsely populated environments, so the risk of suffering phage attacks is generally higher in microbial cell densities. The costs associated with phage resistance mechanisms are considerable and help as a key factor in shaping the evolutionary dynamics between the phage and host [4-5].We assume that if bacteria used quorum sensing to adjust their antiphage activities, they could accurately regulate their defense mechanisms to elude infection during growth under high-risk conditions, while saving the metabolic load of maintaining constantly raised antiphage tactics.

The initial effort to test whether quorum sensing is used to regulate phage-bacterium interactions, we explored the role of quorum sensing in the standard model system of T4 phage and its host *Escherichia coli* BL21. Since the discovery of T4 phage about 60 years back, it has been studied extensively and by this way phage represents the most widely understood biological entity. The investigations upon T4 phage and its interactions with *E. coli* have served as a bridge in molecular studies and paved the way to understand the key mechanisms of quorum sensing [6].

However many researchers have explained that *E.coli* use multiple systems of quorum-sensing [7] and one of the most important molecule for inter-species quorum sensing is indole [8], which acts as a signaling molecule and activates transcription of astD, tnaAB and gabT genes. Among these genes, tnaAB operon activation induces indole production, whereas the remaining two genes i.e. gabT and astD activation leads to the degradation of amino acids to either succinate or pyruvate. Signaling pathway, which utilizes indole, plays an important role in adaptation of bacteria in poorly nourished environment, where degradation of amino acids is an important energy source [9].

The goal of our research was to clarify the role of bacterial quorum sensing, in shaping the interactions between bacteria and the bacteriophage that prey on them. I presented evidence for this hypothesis by using model system of *Escherichia coli* and T4 bacteriophage.

2 MATERIALS AND METHODS

2.1 BACTERIA AND BACTERIOPHAGE

In order to conduct the study, the *E. coli* BL21 (DE3) 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°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.

2.2 LURIA-BERTANI MEDIA

LB medium containing 10 g tryptone, 5 g yeast extract and 10 g sodium chloride per 1,000 ml of water (pH 7).

2.3 SEMI SOLID MEDIA

For phage-plaque formation semi solid medium containing 1.5 and 0.5% agar was used for the upper layer, respectively.

2.4 CHEMICALS

Indole C_8H_7N (>99%) was obtained from Sigma–Aldrich (USA) and indole working solution was diluted in water to receive a final concentration of 1%.

2.5 PLAQUE COUNT ASSAYS

The progress of lysis was recorded by plaque count assays to check the effect of indole on the lysis activity of T4 bacteriophage ($4x10^9$ pfu/ml) against *E. coli* BL 21(DE3) (1 OD at 600nm). 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.

2.6 PLATING OF T4 BACTERIOPHAGE

For indole experiment 20 µl of 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 5 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°C.

2.7 STATISTICAL ANALYSIS

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

3 RESULT

In nature, bacteria normally occur in polymicrobial communities. Interactions between community members typically involve several mechanisms, including responses to antimicrobial compounds, nutritional interactions, and signaling. Chemical signaling is widespread in bacteria, and in *E. coli* it involves several compounds, including indole. A common diagnostic marker for *E. coli* identification is indole, which is formed by tryptophanase enzyme from tryptophan. Indole can also act as an extracellular signaling molecule. In this study, we have demonstrated that quorum sensing molecule indole can reduce the infection activity and production up to 45% of T4 phage as shown in Figure-1.

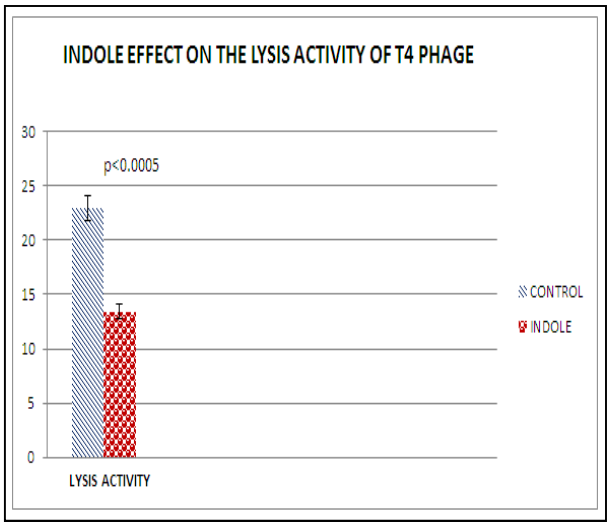

Figure-1: Indole signaling pathway has its role in adaptation of bacteria in a poor nutrient environment, where amino acids degradation is a very important source of energy while in our experiment we checked the effect of indole on the lysis activity of T4 phage and result showed that indole decreased the lysis activity of T4 bacteriophage. Reported differences were evaluated using t test. (P<0.0005)

4 CONCLUSION

Quorum sensing plays an important role in both symbiotic and pathogenic bacteria-host interactions. 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.

The present study was an effort to look at the effect of quorum sensing molecule which is indole on production and infection activity of T4 bacteriophage. In this study, we have identified a unique antiphage defense mechanism regulated by quorum sensing in *E. coli*. Results obtained during the study showed that indole which can also act as a signaling molecule and is a common diagnostic marker for the identification of *Escherichia coli* reduced the infection activity and production of T4 bacteriophage as Mahyar, [10] reported that indole can reduced virus attachment to host.

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