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LYSIS INHIBITION EFFECT T4 BACTERIOPHAGE BURST SIZE

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ABSTRACT

At least six T4 genes are required for lysis inhibition phenotype which is basically a very complex mechanism. Lysis inhibition is induced by super infection and in a superinfected cell, the concentration of endolysin exceeds the final concentration in a nonsuperinfected cell. In a lysing culture superinfection induces lysis inhibition immediately (Hadas *et al*., 1997).

The present study firstly explains the basic biology of lysis inhibition and also describes lysis of T4-infected cells at high culture densities. Secondly we presented that when the lysis inhibition is initiated there is sudden increase in the size of plaque which is caused by adsorption of T4 bacteriophage (secondary adsorption). It is a novel mechanism through which the lytic nature of T4 phage particle has evolved and our study provides logical arguments to conclude that lysis inhibition increase the burst size.

KEYWORDS: Molecular Cloning, Laboratory Manual, Lysis Inhibition, Bacteriophage

INTRODUCTION

There is still a gap in our understanding of the lysis mechanism of T4 bacteriophage despite the level to which T4 bacteriophage is otherwise understood physiologically, genetically and molecularly. On the other hand, the lysis mechanism of phages such as 4X174, MS2 and Lambda has been well understood and characterized genetically molecularly (young, 1992). The reason for which there had been lack of efforts made toward the understanding of T4 lysis mechanism over the past 20 years could be that this is a result of its complexity and especially a consequence of the existence of a phenomenon known as lysis inhibition (LIN) (young, 1992).

Basically lysis inhibition is a continuation of the latent period of T4 phage infected cell and an amplification of the T4 phage burst size. Lysis inhibition occurs by the adsorption of a second T4 bacteriophage and this lysis inhibition induction requires at least six genes functions which are rI, rIL4, rnIB, rIII, rIV, and rV (Mosiq, 1983). So cells, which are infected by T4 phage may lyse at the end of a normal latent period even if lysis inhibition is not induced. This occurs in a way that is most probably similar to that of phage lambda infected cells (young, 1992). However this lysis occurs at the end of a considerably longer lysis inhibition latent period.

T4 particles have multiple complications during lytic cycle (young, 1992). In the induction of the lysis inhibition state there is secondary T4 phage adsorption which causes collapse of lysis inhibition. There is loss of plaque-forming ability (secondary trauma) due to multiple T4 secondary adsorptions. This association between adsorption and lysis inhibition suggests a mechanism of extracellular induction of lysis inhibition collapse which could be related to lysis from

without or secondary trauma. Surprisingly, lysis from without and secondary trauma are distinguishable genetically i.e different T4 genes code for the bulk of resistance to each phenomenon, gene imm for resistance to secondary trauma and gene sp for resistance to lysis from without trauma (Cornett, 1974). The study of this inhibition was considered to be the most promising point of attack as lysis inhibition seems to be the cause of the difference in plaque morphology. Our study deals with experiments which are designed to give additional information about the effect of lysis inhibition on phage plaque size.

MATERIALS AND METHODS

Bacteria, Phage and Culture Media

For conducting the study, the E.coli BL21 strain was used as the primary host for lysis activity of the bacteriophage named Escherichia coli bacteriophage (ATCC11303-B4). The E. coli BL21 was obtained from the American Type Culture Collection (ATCC). All bacterial stock cultures prepared/obtained were stored at -80 ^{0}C in Luria-Bertani broth (Oxiod) containing 50% (v/v) glycerol. The frozen cultures were plated onto LB agar (Oxiod) on the need basis. For looking the effect of lysis inhibition on T4 phage burst size, first an overnight culture of E. coli BL21 was prepared by inoculating LB broth with a single isolated E. coli BL21 colony from an LB plate and incubating it in a 37°C until the OD_{600} reached 1. Bacteria and phages were propagated in LB broth.

LB medium consisted of 10 g tryptone (BD), 5 g yeast extract (BD) and 10 g sodium chloride per 1,000 ml of water (pH 7) (Sezono *et al*., 2007). For phage-plaque formation, LB-based solid medium containing 1.5 and 0.5% agar was used for the lower and upper layer, respectively (Sambrook and Russell, 2001; Murray *et al.,* 1994).

Standard Procedure for Experiments on Lysis Inhibition

The lysis inhibition with T4 phage (10^9 pfu/ml) against E.coli BL21 was checked by double agar overlay method similar to that of Adams (1959) and the MOI was 3. We have noticed that particles of phage which get adsorbed secondarily are enough to effect inhibition. Since after ten minutes there were still 5.1 particles to be adsorbed in the context of 10 fold multiplicity and no inhibition occurred, we can accomplish that primary and secondary infections must be separated by more than one minute, however, the primary infection could be followed by secondary infection by as little as 4 or 5 minutes.

RESULTS

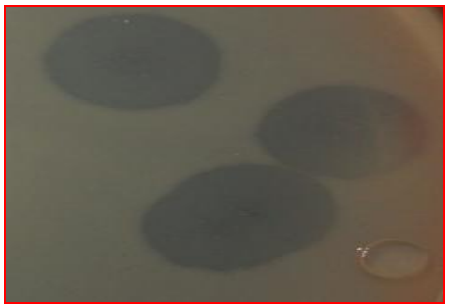

Figure 1: Plaque after Lysis Inhibition

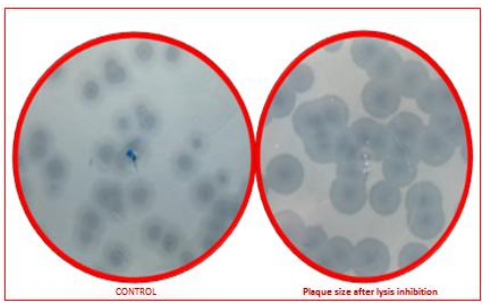

Figure 2: Effect of Lysis Inhibition on Burst Size

The results described the following picture of the actual events occurring under conditions of lysis inhibition. Bacteriophages T4 infect bacteria that reproduce the phage during the latent period, at the end of which lysis begins, first in a few bacteria, but increasing rapidly. Immediately after liberation of the first new phage particles they become reabsorbed on those bacteria which have not lysed. The reactions involved in and following this secondary adsorption of T4 phage delay the lysis of the secondarily infected bacteria.

One further point that deserves mention is the fact that there is a decided increase in the number of phage particles liberated per bacterium after lysis inhibition has been effected. Figure 1 showed that the step size is higher when T4 infected bacteria are inhibited than when they are not inhibited. The burst size of plaque is larger in secondary infected bacteria as compared to control one as showed in figure-2.

DISCUSSIONS

The cellular content of PSS dictate burst size which is a measure of rate of phage synthesis. Burst size is extensively affected by cell lysis time as well as delay in cell lysis; this delay occurs due to superinfection and in turn yields much more phages. Lysis time is related to lysozyme synthesis i.e. its synthesis time, rate and effective concentration. These all parameters depend on cell dimensions (volume and surface area) (Hadas *et al*., 1997). Superinfection maximizes lysis inhibition which in turn increases burst size.

Present study findings showed that lysis inhibition increased the plaque size of phage and support the results of Hadas *et al*. (1997), who reported that lysis inhibition delay the lysis time and increased the plaque size of bacteriophage.

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