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DIFFERENT PHASE ESCHERICHIA COLI EFFECT ON T4 BACTERIOPHAGE LYSIS AND PRODUCTION

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ABSTRACT

The environment within which the micro-organisms live is constantly evolving. Under such evolving conditions, the Escherichia coli in nature face unfavorable growth conditions. This causes physiological and genetic changes with a dramatic effect on the growth of Escherichia coli and its susceptibility to bacteriophage infection.The studies of Escherichia coli using various phase culture confirm properties of its cells as in nature. In different medium the interaction between E. coli host cells and T4 bacteriophage has been different. The T4 bacteriophage has the ability to adjust according to the various growth parameters of Escherichia coli. During different phase Escherichia coli has been reported regulating up the expression of specific proteins while regulating down the others thus affecting its binding with the bacteriophage. A standard plaque assay has been used in this study in order to inspect the effects of Escherichia coli culture on formation of infective centers by T4 bacteriophage.

This study characterizes the influences of well-defined physiological conditions on Escherichia coli growth and its interactions with T4 bacteriophage. In our present study we observed that the maximum growth and lysis of T4 bacteriophage was in stationary phase. T4 bacteriophage production and lysis was also good in log phase but in lag phase and death phase production and lysis activity was less as compared to other mentioned phase.

KEYWORDS: T4 Bacteriophage, E. coli, Phase, Lysis, Production

INTRODUCTION

Escherichia coli is the most extensively studied bacterium in microbiology. However, the majority of Escherichia coli studies are performed using fresh or log phase cultures and may not accurately represent the characteristics of such cells in nature [1]. Studies have shown that at different phase of cell the general biosynthetic processes, metabolism and protein production are not same [2]. These dramatic physiological differences lead to an interest in performing studies on different phase Escherichia coli, as well the relationship between Escherichia coli and other organisms.

The T4 bacteriophage is a most commonly used and well studied virus that infects Escherichia coli [3]. However, T4 bacteriophage is capable to adjust its growth to the physiological state of the host cell [4]. This modification depends not only on the capability of the bacterial cell to produce progeny phage particles and lysis proteins but also on other factors and processes [5]. In particular, the development of T4 bacteriophage is usually longer than the time necessary to form a sufficient number of progeny phages. Interestingly, under conditions of a very slow bacterial growth, T4 bacteriophage development may even be stopped [5]. It also stops at the early phases of development if the bacterial host is

not growing at all [6]. When host bacteria are starved, T4 bacteriophage does not form progeny virions. Instead, its genome can be maintained in the infected host cell until the host's growth resumes and then a small burst of progeny phage is produced and the cell is killed by lysis [6].

However, as the physiology of Escherichia coli constantly changes as the culture ages, there is a possibility that infective center production may increase or decrease at different time points. The effect of different phase Escherichia coli on the lysis activity of T4 bacteriophage was checked and this is the first report on the lysis activity of T4 bacteriophage in these above conditions.

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) [7]. For phage-plaque formation, LB-based solid medium containing 1.5 and 0.5% agar was used for the lower and upper layer, respectively [8]. The phage stock was stored at -80 ^{0}C in LB broth containing 50% glycerol. Phage titer was determined as plaque-forming units (pfu/ml) using the double layer agar plate method similar to that of Adams [9]. Progress of lysis and production were recorded by plaque count assays to check the effect of different phase E.coli (1 OD at 600nm) on the lysis activity of T4 phage (ATCC11303-B4) ($4x10^9$ pfu/ml).

Statistical Analysis

Statistical analysis included t test for the comparison of change in outcome variables in response to different phase E.coli with methods described by sigma stat (Figure 1). The analysis was carried out with Graph Pad Prism 5 software.

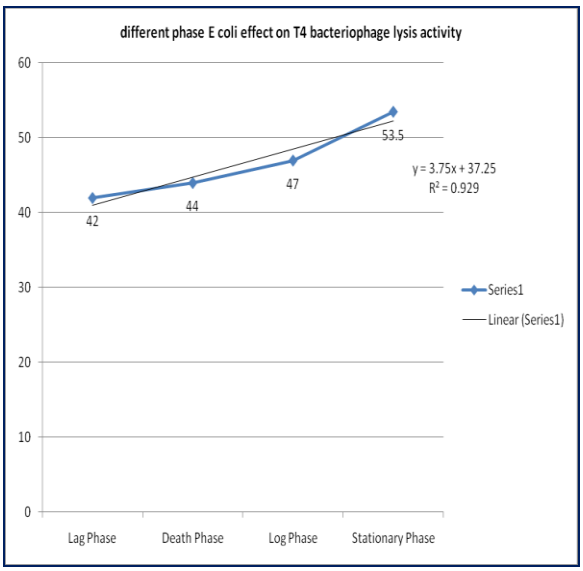

Figure 1: Different Phase E. coli Effect on T4 Bacteriophage Lysis Activity and Production

RESULTS

Production of phages with lowered affinity to bacterial cells, or phages which can bind only to growing bacterial cells seems to be an effective strategy for phages which are constantly endangered by adsorption to cells which are starving or are suspected to be excreted outside of the relatively safe environment of the mammalian gut The ability of phage T4 to produce a fraction of virions unable to infect starved cells is linked to the functions of genes rI and rIII, as well as rIIA. This may represent the adaptation of bacteriophage T4 in order to persist in unfavorable environmental conditions [10].

In our experiments we observed that the maximum growth and lysis of T4 bacteriophage was in stationary phase. T4 bacteriophage production and lysis was also good in log phase but in lag phase and death phase production and lysis activity was less as compared to other mentioned phase.

DISCUSSIONS

Earlier work on the bacterium –bacteriophage reaction has stressed the importance of bacteria growth as a condition factor for phage production. Our result showed that bacterial growth to be primer condition factor for phage production and in the present study we observed that the maximum growth and lysis of T4 bacteriophage was in stationary phase. T4 bacteriophage production and lysis was also good in log phase but in log phase and death phase production and lysis activity was less as compared to other mentioned phase as Golec *et al*., [10] reported that T4 bacteriophage is able to adjust its development to the growth parameters of the host cell.

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REFERENCES

1. M. T. Madigan, J.M. Martinko, and J. Parker, "Brock Biology of Microorganisms", 9th Edition. Prentice Hall Upper Saddle River, NJ, pp. 991, 2000.
2. J. Ou, L. Wang, X. Ding, J. Du, Y. Zhang, H. Chen, and A. Xu, "Stationary phase protein overproduction is a fundamental capability of Escherichia coli". *Biochem Biophys Res Commun*, vol, 314, n^0 1, pp. 174-180, January 2004.
3. C. Chan, J. Chan, G. Lee, and J. Lee, "Culture Age and Extrinsic Factors Affect Formation of T4 Bacteriophage Infective Centers in Stationary Phase *Escherichia coli* ZK126", *Journal of Experimental Microbiology and Immunology (JEMI)*, vol, 5, pp. 50-60, April 2004.
4. H. Hadas, M. Einav, I. Fishov, and A. Zaritsky, "Bacteriophage T4 development depends on the physiology of its host *Escherichia coli," Microbiology*, vol 143, pp. 179–185, 1997.
5. M. Łos, G. Wegrzyn, and P. Neubauer, "A role for bacteriophage T4 rI gene function in the control of phage development during pseudolysogeny and in slowly growing host cells", *Research in Microbiology*, vol, 154, n^0 8, pp. 547–552, October 2003.

6. E. Kutter, E. Kellenberger, K. Carlson, S. Eddy, J. Neitzel, L. Messinger, J. North, and B. Guttman, Effects of bacterial growth condition and physiology on T4 infection, in: J.D. Karam (Eds), In Molecular Biology of Bacteriophage T4. Washington, DC: American Society for Microbiology, pp. 406–418, 1994.

7. G**.** D. Sezonov, P. Joseleau, and D.R. Ari, "Escherichia coli Physiology in Luria-Bertani Broth. *Journal of Bacteriology"*, vol, 189, n^0 23, pp. 8746-8749, September 2007.

8. J. Sambrook and D.W. Russell, "Molecular Cloning, a Laboratory Manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001.

9. M.K. Adams, "Bacteriophage. Inter science Publishers", New York, pp. 450– 456, 1959.

10. P. Golec, A. Wiczk, J.M. Łos, G. Konopa, G. Wegrzyn, and M. Łos, "Persistence of bacteriophage T4 in a starved Escherichia coli culture: evidence for the presence of phage subpopulations", *Journal of General Virology*, vol, 92, **,** n^0 4, pp. 997–1003, April 2011.