

## Various Culture Media Effect on T4 Phage Lysis and Production

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**ABSTRACT:** Studies on bacteriophage growth and its development played a vital role in the history of molecular biology which in turn helped in clarification of many points. Most of the previous studies on bacteriophage development and growth have been performed under optimal conditions for the host cell. While On the other hand these conditions may not be optimal for the T4 bacteriophage. As a matter of fact in nature E. coli faces many unfavorable growth conditions, good example are those conditions prevailing in the human gut in which E.coli manages to survive well. This study characterizes the effects and influences of well-defined physiological conditions on T4 bacteriophage growth and development. In addition to this, T4 bacteriophage interactions with its bacterial host have also been demonstrated.

In our present study we observed that the maximum growth and lysis of T4 bacteriophage was on luria-bertani (LB) and nutrient media (NM). Moreover the T4 bacteriophage production and lysis was also good in luria-bertani plus glucose (LB+G) media but when compared with its production in luria-bertani (LB) and nutrient media it was found to be less than these medium. Our study results also showed that in minimal media (MM) rate of growth and lysis activity of T4 bacteriophage was lowest as compared to other mentioned medium.

**KEYWORDS:** T4 Bacteriophage, E.Coli, Media, Lysis, Production.

### 1 INTRODUCTION

Studies about growth and development of bacteriophage played important role in the molecular biological history [1]. The information, particularly with the model species T4 bacteriophage was accumulated during the 1940s and this placed the base of the evolving field [2]. Ellis & Delbruck [3] performed classical one-step growth experiment which defined the latent period, rise time, burst size and the eclipse period. Eclipse period was discovered by the effective procedure planned to disrupt infected bacteria before their lysis to occur spontaneously and the mature phages were not damaged [4].

There is a highly specific binding of phage to one of the cell envelope layers of bacteria which initiates phage-host interaction. Fibers of the phage tail attach to specific receptors present in the bacterial envelope [5]. This process is called as adsorption and depends on various factors present in the environment [6].

Lysis of the bacterial host is the last event in lytic bacteriophage infection cycle [7] but standardization of media is very important factor for phage lytic cycle and its production. The ultimate solution to this issue would be to have a synthetic medium which is chemically defined but preserving the sensitivity [8]. The culture media composition is also very important [9]. Apart from the nature of the culture medium, plaque formation also depends on the medium from which the culture is obtained [10]. Plaque formation depends on solid media while the lysis in liquid media is even more dependent on the cultures history [10].

## **2 MATERIALS AND METHODS**

### **2.1 BACTERIA AND PHAGE**

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 °C in various broths containing 50% (v/v) glycerol. The frozen cultures were plated onto various agars on the need basis. For looking at the effect of various culture media, first an overnight culture of E. coli BL21 was prepared by inoculating broth with a single isolated E. coli BL21 colony from an plate and incubating it in a 37°C until the OD<sub>600</sub> reached 1.

### **2.2 CULTURE MEDIA**

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

Luria-Bertani (LB+G) pulse 0.8% glucose medium consisted of 10 g tryptone, 5 g yeast extract and 10 g sodium chloride per 1,000 ml of water (pH 7).

Minimal Media contains M9 minimal salts solution (5X concentrate) (64g sodium phosphate, penta-hydrate -- Na<sub>2</sub>HPO<sub>4</sub>-7H<sub>2</sub>O ,15g potassium phosphate (dibasic) -- KH<sub>2</sub>PO<sub>4</sub>, 2.5g table salt – NaCl and 5.0g ammonium chloride -- NH<sub>4</sub>Cl per 1,000 ml of distilled water) 1M solution of magnesium sulfate (MgSO<sub>4</sub>), 20% solution wt/wt of glucose and 1M solution of calcium chloride (CaCl<sub>2</sub>) (pH 7).

Nutrient medium consisted of peptone 5 g, sodium chloride 5 g, beef extract 1.5 g, yeast extract 1.5 g per 1,000 ml of water (pH 7).

For phage-plaque formation semi solid medium containing 1.5 and 0.5% agar was used for the upper layer, respectively [11], [12].

The various medium effect on the lysis activity and production of T4 phage (10<sup>9</sup> pfu/ml) against E.coli BL21 was checked by plaque count assays and the MOI was 3. The pH of media was obtained by using HCl and NaOH and then the lysis activity and production were checked against E.coli BL21 by double agar overlay method similar to that of Adams [13].

### **2.3 STATISTICAL ANALYSIS**

Statistical analysis included t test for the comparison of change in outcome variables in response to various culture medium with methods described by sigma stat (Fig. 1). The analysis was carried out with Graph Pad Prism 5 software.

## **3 RESULT**

An ideal medium would be one on which maximal bacterial growth occurs and on which the phage has optimal activity. Some factors favoring growth of the bacteria may have a tendency to inhibit phage action and some factors favoring phage activity may tend to limit growth of the bacterial host. A balance may be reached however in which both activities are maintained at a high level [14].

E. coli cells grow faster and larger cells are obtained in richer media, because a higher proportion of their mass is included in the protein-synthesizing system (PSS) [15]-[16].

In present study we observed that the maximum growth and lysis of T4 phage was on LB and nutrient media. T4 phage production and lysis was also good in LB plus glucose media but a little less than LB and nutrient media while in minimal media rate of growth and lysis activity was lowest as compared to other mentioned medium as showed in fig. 1.

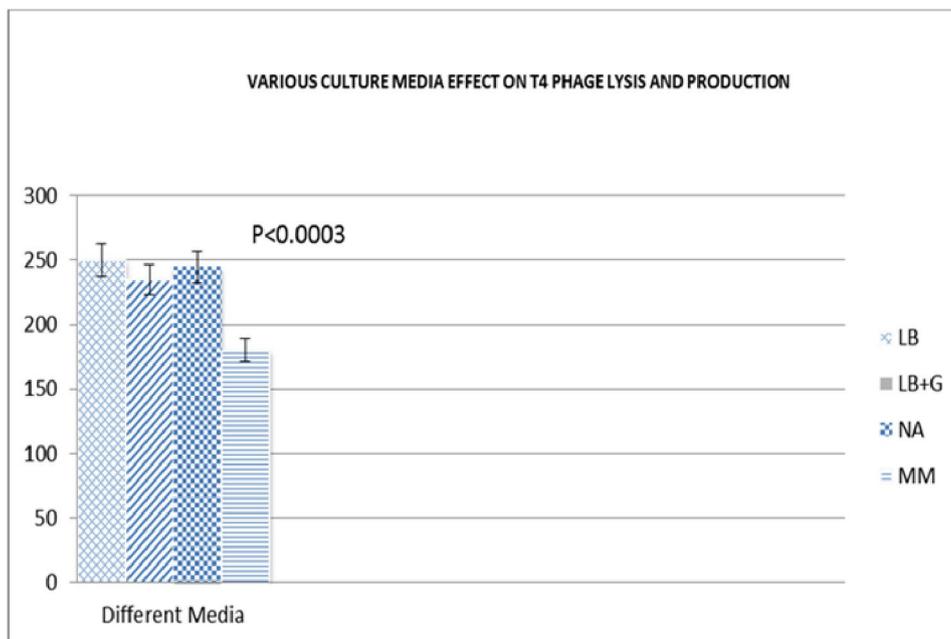


Fig. 1. Various media effect on T4 phage lysis and production

#### 4 DISCUSSION

Most of the previous studies on bacteriophage development have been performed under optimal conditions for the host cell but these conditions may not be optimal for the phage. In nature *E. coli* faces unfavorable growth conditions such as those prevailing in the human gut [17]. The rate of phage production is proportional to the amount per cell of the PSS at the time of infection and the increased rate of phage production results in larger burst sizes in the bigger cells [18].

This study characterizes the effects of well-defined physiological conditions on T4 phage growth and also its interactions with the bacterial host. In the present study we observed that the maximum growth and lysis of T4 phage was on LB and nutrient media. T4 phage production and lysis was also good in LB plus glucose media but a little less than LB and nutrient media while in minimal media rate of growth and lysis activity was lowest as compared to other mentioned medium as Sula and Sulova [8] reported that media can affect phage lysis activity.

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