

Free bacteriophage plaque assay report sample

[Literature](#), [Russian Literature](#)



\n[toc title="Table of Contents"]\n

\n \t

1. [Microbiology laboratory report](#) \n \t
2. [Introduction](#) \n \t
3. [Method](#) \n \t
4. [Results](#) \n \t
5. [Discussion](#) \n \t
6. [References](#) \n

\n[/toc]\n \n

Microbiology laboratory report

Abstract

Bacteriophages are intracellular parasitic viruses that infect bacteria to carry out their multiplication. During the lytic infection cycles, phages lyse the host cells forming a clear zone called plaque on the agar media. The experiment is aimed to measure the number of plaques created when the virus T4 infects the host bacteria and their genetic material integrates into the genetic material of the host cell. This happens because the E. coli becomes resistant to T4 bacteriophage. The T4 phage was diluted in a test tube and then E. coli bacteria was added in the tubes. The mixture was poured with soft agar in the petri dishes. After one week of incubation, the concentration of T4 in the original phage density was calculated that is expressed in Plaque Forming Units per mL (pfu/ml). A Plaque Forming Unit is the single infectious virus particle that forms a plaque.

Introduction

In this lab report is described the completed procedure that has been followed in order to perform the “ Titration of bacteriophages culture”.

Bacteriophage is the type of extensively studied class of viruses that can infect different bacteria. In general, viruses are composed of a single strand of genetic material (RNA/DNA, single or double stranded) packed within a protein capsule. They do not possess the ability of self-reproduction as they lack the machinery needed for biosynthesis rather they depend on their host bacterium for multiplication. While the virus is extracellular i. e. not infecting, it is in dormant state with the nucleoprotein intact and known as virion. On infection in the suitable host cell, virion changes to virus (the active reproductive form with only the genetic material inside the infected cell). On infection, Bacteriophage can replicate in the host cells via lytic or lysogenic replication cycles. In the experiment, Bacteriophage T4 and E. coli bacteria were used to study the behaviour of T4 when it comes in contact with E. Coli bacterial cells. The analysis was done by studying the virulent property of phage on the host and identifying the T4's titration by calculating the plaque forming units (PFU) on a nutrient agar medium.

T4 phage is one of the largest bacteriophages that measures 200 nm in length and 80-100 nm in width (Mayer, 2010). It is structurally composed of a protein head or Capsid that protects the nucleic acid inside. The tail covered with a contractile sheath provides the nucleic acid a path to pass in the host cell on infection and the tail fibres at the end helps in binding the virus to the cell surface. In its genome, it contains approximately 168, 000 base pairs of double stranded DNA that codes for around 300 gene products.

Once injected, the host mechanism comes to a standstill and the virus' nucleic acid utilizes the host biosynthetic machinery to replicate itself and ultimately lysing the host cell releasing the virus particles that appears as plaque.

Method

The E. coli bacterial culture was spin at 3000 rpm for 10 minutes. The supernatant was then tipped out using a Pasteur pipette and re-suspended in 1 ml nutrient broth, by means of a well mixing for several times. The bacterial culture was kept for later use.

Results

The table above show the results that were obtained according to the plate count. These results refer to the concentration of bacteriophage in the original stock culture, after the necessary calculations. The concentration is given in terms of PFUs per mL. As 0.5ml of the mixture was used, it is multiplied by 2 to find the exact concentration per ml (Reynolds, 2011). For example, the calculation for the first group on 10⁻⁷ dilution plate with 24 plaques will be:

$$24 \times 2 \times 10^7 = 4,8 \times 10^8 \text{ pfu/ml.}$$

Discussion

After performing all the appropriate stages required for the experiment, results were obtained and recorded in the table as given above. Irrespective of the dilution concentrations of the virus, T4 succeeded to replicate in the E. coli bacterial host cells. This proves that T4 was able to infect E. coli, in some cases with bigger success compared to others, depending on the dilution

densities (Lenski, 1984). The interpretation of the above statement could be that some of the E. coli strains were resistant to T4 phage infection not because of the exposure of the bacteria to specific virus, but because the bacteria could present resistance to the virus due to introduction of mutations in its genome.

On observing the table, it is evident that the different groups gave similar results on same dilutions irrespective of being distinct strains. This means that different groups concluded to the same interpretation (Rabinovitch. et al, 2002). Numerical variations between the groups are acceptable and this can be attributed to the experimental errors that might occur during the procedure. Here, it is worth noticing that Group 3 obtained a different trend in the replication of T4. Despite the fact that the rest of the groups recorded fewer plaques in a less dense viral dilution; Group 3 recorded more in the same environment (Panec and Katz, 2013). For example, Groups 1, 2 and 4 for a dilution factor of 10^{-7} obtained plaques in the order of 10^8 multiplied with a slightly different factor, whereas Group 3 for the same dilution factor obtained plaques of one order higher, 10^9 . This comes to a contradiction with rest of the groups and can be subjected to experimental errors.

A correlation of the observed results with the already published literature reviews of similarly conducted experiments can only amount to the success of the conducted experiment (Thomas and Wineland, 2010). Based on the known successful attempts, the conducted experiment can be characterized successful as the culture contained in the agar turned from cloudy to clean in some areas. It indicates that the bacteriophage was able to infect and subsequently replicate inside the potential host cell (Nerney et al, 2003).

Therefore, it can be considered that this experiment was consistently successful, as similar characteristics were obtained with the ones described in the theory.

The undertaken experiment must have undergone several experimental errors that need to be accounted in the observations. The observed inaccuracies and inconsistencies appearing in the results of an experiment can be due to these limitations. The origin of these errors may vary, so here it is considered one by one. The most common ones are the human errors that are due to limited experimental skills; in case of students who are inexperienced. For instance, human error could be possibly the main reason affecting the numerical results of Group 3. A way to minimise the errors in this case is to repeat the experiment more carefully and compare the previous results with the one's obtained in the fresh experiment. Another is experimental errors whereby a mistake can occur during pipetting (instrumental limitation) or contamination. This might occur either due to wrong instrumental calibrations (factorial or from the experimenter) and thus leading to incorrect experimentation, or accidental contamination of the instrument from previously conducted experiments. A good way to prevent these errors is to be careful of post experimental procedures whereby the instruments must be placed into UV light; a recommended solution of avoiding any contamination.

Conclusion

References

Mayer Dr. Gene, 2010, Chapter seven, Bacteriophage. [Online] Available at:
Last accessed 22 December 2013.

<https://assignbuster.com/free-bacteriophage-plaque-assay-report-sample/>

Nerney Mc B, Kambashi, Kinkese. J, Tembwe. R and Godfrey. P, 2003.

Development of a Bacteriophage Phage Replication Assay for Diagnosis of Pulmonary Tuberculosis. [Online] Available at: Last accessed 4 January 2014.

Panec D Marie, Katz Sue, 2013. Plaque Assay Protocols. [Online] Available at: Last accessed 26 December 2013.

n/n. 2011. Bacteriophage T4 Virus. [Online] Available at: Last accessed 22th Dec 2013.

n/n. 2013. Experimental Error . Available: http://academics.wellesley.edu/Chemistry/chem211lab/Orgo_Lab_Manual/Appendix/experimental_error.html. Last accessed 3th Jan 2014.

Rabinovitch. A, Fishovw. I, Hadasw. H, Einavw. M and Zaritsky. A, 2002.

Bacteriophage T4 Development in Escherichia coli is Growth Rate Dependent. [Online] Available at: Last accessed 4 January 2014.

Reynolds Jackie, Richland College, 2011, Bacteriophages. [Online] Available at: Last accessed 26 December 2013.

Richard E. Lenski, 1984. Two-step Resistance by Escherichia Coli B to Bacteriophage T2. [Online] Available at: < <http://www.genetics.org/content/107/1/1.full.pdf>. Last accessed 4 January 2014.

Thomas Brett, Wineland M Jordan, 2010. Lab report. [Online] Available at: Last accessed 3 January 2014.