Titration of lytic bacteriophage t2

A plaque essay demonstrating the lytic activity of bacteriophage T2 was performed to determine the titre (concentration) of bacteriophage T2. Serial dilutions (ten-fold) of the phage stock were plated onto an excess of sensitive bacteria on a nutrient agar plate.

One phage particle produces one plaque (zone of lysed cells) and the plaque count is directly correlated to the colony count for bacterial titration. The experiment was performed thrice and the data analysed for statistical variation and significance.

0. 9 ml of pre-diluted (10â ‍» 7) phage stock was dispensed into 3 microfuge tubes and tenfold dilutions of the phage (10â ‍» 1 to 10â ‍» 3) were made. A mixture of 100 ul E. coli and 100 ul of phage dilution 10â ‍» 1 in a tube of layer agar was poured onto the labelled plates and repeated for a second plate. The same procedure was repeated for the 10â ‍» 2 and 10â ‍» 3 dilutions with two plates for each sample. All plates were incubated overnight. The plates with valid and countable numbers of plaque (i. e. between 30 & 300) were selected and the number of plaques for all the replicates for each dilution (10â ‍» 1, 10â ‍» 2, 10â ‍» 3) was counted. TNTC (Too Numerous To Count) was recorded for plates with more than 300 plaques. The titre (pfu/ml) was determined using the original suspension and appropriate dilutions used and statistics done for all the replicates.

Results

Table 1. The number of plaques was counted for those plates which contained 30-300 plaques. The plaque count is equivalent to the bacteria colony count for bacterial titration as each plaque is derived from an

individual isolated virus. The plaque count values for dilution factor 10â » 2 were used to determine the overall titre values for the assay. The titre value (pfu/ml) represents the number of phage particles/plaque forming units produced per ml of the original phage diluent.

When the values of the plaque count were measured for the plates containing 10â » 2 and 10â » 3 dilutions of the phage, there was a considerable drop in the number of plaques counted for phage dilution of 10â » 3 (table. 1). Hence, a ten-fold reduction is sufficient to reduce the virus titre.

Generally, the experiment was pretty reliable as similar titre values were obtained and reproduced for each dilution factor when the experiment was repeated thrice.

The phage dilution factor of 10â » 2 was chosen for the statistical analysis because only accurate values of the plaque counts can be obtained at lower concentrations. If the phage concentration is too high (i. e. dilution factor of 10â » 1), the measurement of plaque formation would be inaccurate due to the huge numbers of plaque present.

Overall, there was considerable statistical variation between the same plaque assay when conducted on three different occasions. There was a significant drop in the phage titre from the first to the third attempt. There was a slight but significant reduction (P <) from the first to the second attempt and the greatest reduction (P <) was seen from the second to the third attempt.

The graph shows that there was quite a significant reduction (P < 0. 05) in the phage titre based on the plaque assay that was conducted on three separate days. There seems to be a gradual decline in the concentration of phage particles from the first to the second and finally to the third attempt. Thus, there appears to be a non-linear relationship between the phage titre and the number of bacteriophage titrations attempted.

In other words, the phage titre decreases as the number of attempts of bacteriophage titration increases. This could be due to a drop in virus replication during storage of the agar plates or inactivation of the virus during purification. Another possibility could be the variability in the plaque forming efficiency of the bacteriophage when the assay was conducted on three different days.

Discussion

T2 bacteriophage is only able to infect and replicate in rapidly dividing bacterial cells (i. e. mostly E coli.) that are undergoing the exponential phase of bacterial growth (Madigan, M. T. & Martinko, J. M., 2006). When the infected bacterial cell lyses, it releases large amounts of newly synthesized phage particles which go on to infect adjacent cells (Madigan, M. T. & Martinko, J. M., 2006). The process is repeated at regular intervals until bacterial growth ceases due to nutrient depletion and accretion of toxic products. One plaque (zone of lysis) on the nutrient agar plate is produced from one phage particle when managed properly (Madigan, M. T. & Martinko, J. M., 2006). Thus, a suspension of lytic T2 bacteriophage can be titrated through suitable serial dilutions and the virus titre determined.

As the bacteriophage only infects specific bacterial species, it may be useful in therapeutic applications such as phage therapy and molecular biology (Clercq E. D., 2004).

High peak virus titre (e. g. dengue fever) has been associated with increased disease severity (Vaughn et al., 2000). A 90% drop in the level of virus load in patient serum is significant. At peak viremia (3-4 days after initial infection), the patient can have 109 genome equivalence /ml of virus. Thus, it is possible to statistically separate out those patients that have severe disease due to the viral infection & those that don’t. Those patients that don’t suffer from disease have on average 107 to 108 genome equivalence/ml of virus titre. Therefore, there appears to be a relationship between disease severity and virus titre or load (Vaughn et al., 2000).

The significance of determining virus titres during a viral infection is crucial in developing new and specific anti-viral strategies that can suppress virus titre during the early phase of viral replication (Clercq E. D., 2004). This is likely to have an impact on disease control. There is no need to develop an anti-viral drug that completely knocks out virus infectivity as a small (ten-fold) reduction in virus titre is sufficient to prevent the subsequent onset of severe disease.

This experiment was designed for the purpose of determining the level of virus titre that is significant enough to cause infection and which may contribute to subsequent disease consequences.

The phage titre this experiment was determined by quantitative plaque-forming assay. The titre (pfu/ml) can also be used to measure the infectivity

ratio. The measurement of infectivity was not performed due to limited resources. Although there is a substantial amount of plaque formation on the agar plates at higher phage concentrations (i. e. less diluted), not all the virus particles are infectious due to mutations in the viral genome. Furthermore, not every single bacteriophage will form a plaque due to structural defects/flaws in the virus (Mahichi et al., 2009). One improvement that can be made to the experiment is to determine the infectious virus titre as compared to the total virus titre.

There were more than 300 plaques (TMTC) present for the plates containing 10â ‍» 1 dilution of phage particles (table. 1). This indicates that the phage concentration is too high for the wide dispersion of virus particles in the agar medium and prevents the accurate measurement of plaque formation. Thus, the phage dilution factor of 10â ‍» 1 is sufficient for plaque formation but too low (i. e. phage solution is too concentrated) for accurate plaque measurement. This also suggests that at higher concentrations of phage, there may be a greater chance of an overlap of plaques on the agar medium, which would lead to an underestimation of the actual number of viruses present. Another possible reason for the high number of plaques formed could be the aggregation of phage particles in the viral preparation leading to widespread lysis of sensitive bacteria on the agar medium.

As dilution increased from 10â ‍» 2 to 10â ‍» 3 (i. e. ten-fold decrease in phage concentration), there was significantly less plaque formation on all three attempts of the plaque assay (table. 1). This indicates a distinct reduction in virus titre when the concentration of phage particles is reduced over the course of the assay.

To get more accurate statistical data, more replicates (i. e. preferably more than two) can be performed to show the range of statistical variation between each sample of different dilutions. Another method would be to analyse multiple sets of data generated from the same starting solution.

Resources:

Madigan, M. T. and Martinko, J. M. Brock Biology of Microorganisms. 11th Edition, (Prentice Hall 2006) pp 236-249.

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Clercq E. D. (2004) Antivirals and antiviral strategies. Nature reviews 2, 704-720

Vaughn D. W., Sharone Green S., Kalayanarooj S., Innis B. L., Nimmannitya S., Suntayakorn S., Endy T. P., Raengsakulrach B., Rothman A. L., Ennis F. A. & Nisalak A. (2000) Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. The Journal of Infectious Diseases 181, 2-9