Application Note

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Enhancing Phage Clarification Efficiency Using Diatomaceous Earth as Filter Aid

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Abstract

Phages are viruses that infect and lyse bacteria, offering a promising alternative to antibiotics. Clarifying phage cultures from *Escherichia coli* (*E. coli*) is crucial, traditionally achieved through centrifugation, which is time-consuming and labor-intensive. This study explores the use of diatomaceous earth (DE) as a filter aid combined with PES 0.22 µm bottle top filters (Sartoclear Dynamics® Lab) as an alternative to centrifugation. We compared three methods: centrifugation, filtration with DE, and filtration without DE. Filtration with DE significantly reduced clarification time to approximately two minutes and increased phage yield compared to centrifugation. Filtration without DE faced blockage issues. The results indicate that using DE filter aid with bottle top filters (Sartoclear Dynamics® Lab) is the most efficient method, simplifying the workflow and enhancing overall efficiency.

Introduction

Phages are viruses that specifically infect and lyse bacteria. In biotechnological research and industry, the clarification of phage cultures from *E. coli* is becoming increasingly important, as phages represent a promising alternative to traditional antibiotics for treating bacterial infections. One of the critical steps in this process is the removal of bacterial debris and other contaminants to obtain pure phage lysate. Traditionally, centrifugation has been the standard method for this clarification process.[1,2] Centrifugation effectively separates phage particles from bacterial cells and debris by exploiting differences in density, but it can be time-consuming and labor-intensive.

An effective alternative to centrifugation is utilizing diatomaceous earth (DE) as a filter aid. DE has demonstrated the ability to extend the filtration process before filter blockage occurs, while also maintaining a high yield of the target molecule.[3,4,5] DE works by providing a highly porous matrix that traps debris while allowing the liquid and target molecule to pass through. This method offers advantages such as reduced processing time and labor, while still achieving effective clarification of the phage lysate. The use of DE in filtration can thus be a viable and efficient alternative to centrifugation, particularly in high-throughput settings where time and efficiency are critical.

The aims of this study are to examine and prove the increased efficiency of DE filter aid when combined with bottle top filters (Sartoclear Dynamics® Lab), specifically in terms of time consumption and the retention of the targeted phage yield in the final filtrate. Moreover, the objective is to present an alternative, time-saving filtration method to standard protocol measures such as centrifugation. In this study, phage lysate was filtered through Sartolab® PES 0.22 µm bottle top filters both with and without the addition of DE to compare their efficiency in time consumption and phage titer to the standard method of centrifugation.

Material and Methods

Clarification Methods

The experiments were designed to first determine the optimal amount of DE filter aid (Sartoclear Dynamics® Lab) necessary for the filtration of *E. coli* phage lysate. Three different DE concentrations were tested: 2.5 g/L, 5 g/L, and 10 g/L. DE filter aid was weighed out under sterile conditions. The weighed DE filter aid was then added to 50 mL of *E. coli* phage lysate, mixed briefly by gentle shaking, and the suspension was filtered using a Sartolab Bottle-Top Filter (Sartolab RF50, 0.22 µm PES membrane) under vacuum. The volume of the collected filtrate was determined by gravimetry, and the filtration time was measured using a stopwatch. A 1.5 mL sample of the filtrate was used for quantification via a plaque assay and subsequently stored at 4.0 ± 2.0 °C.

In a second step, three methods for clarifying 125 mL samples of *E. coli* phage lysate were compared in terms of process time and phage yield. These methods included filtration with the optimized amount of DE filter aid, filtration without DE, and the reference method of centrifugation at 3076g for 30 minutes, where the supernatant was collected after centrifugation. A 1.5 mL sample was taken from each of the filtrates and supernatants and stored at 4.0 ± 2.0 °C for further quantification.

Preparation *E. coli* phi X174 Phage Lysate

In preparation of a bacterial culture, *E. coli* (ATCC 13706) is inoculated into trypticase soy broth (TSB) and incubated overnight. From this culture, 2 mL are transferred into a cultivation flask containing 200 mL of TSB. The bacterial culture is incubated at 37.0 ± 2.0 °C until an OD of 0.5 is reached. Subsequently, phi X174 (ATCC 13607-B1) at a multiplicity of infection (MOI) of 0.15 is added and the culture is incubated for an additional 22 hours. An amount of 2 liters of *E. coli* phi X174 phage lysate were prepared, frozen, and subsequently thawed for the experiments.

Results and Discussion

Plaque Assay for Phage Quantification

In preparation of the quantification, an overnight culture of *E. coli* (ATCC 13706) grown in TSB with a targeted OD of 2 to 6 was diluted in sterile tubes. For the dilution, 300 µL from the overnight culture was added to 30 mL of TSB in tubes, resulting in a 1:100 dilution.

For the preparation of sample dilutions, serial dilutions with TSB to achieve concentrations of 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} were prepared. From these dilution series, 150 µL of the solution were added to the incubation tubes. Next, 150 µL of the 1:100 diluted bacterial culture is added to all the tubes. All samples are then incubated for at least 10 minutes at room temperature. After the incubation period, 2.5 mL of top agar is added to each tube, and the contents are poured onto the agar plate. The plate is briefly swirled to ensure even distribution of the top agar. The plates were incubated for 18 – 24 h at 37.0 ± 2.0 °C in the incubator. At the end of the incubation period plaques were counted and calculated considering the dilution levels of the phage titers.

In addition, controls were carried out to show that media and bacterial culture are not contaminated by phages.

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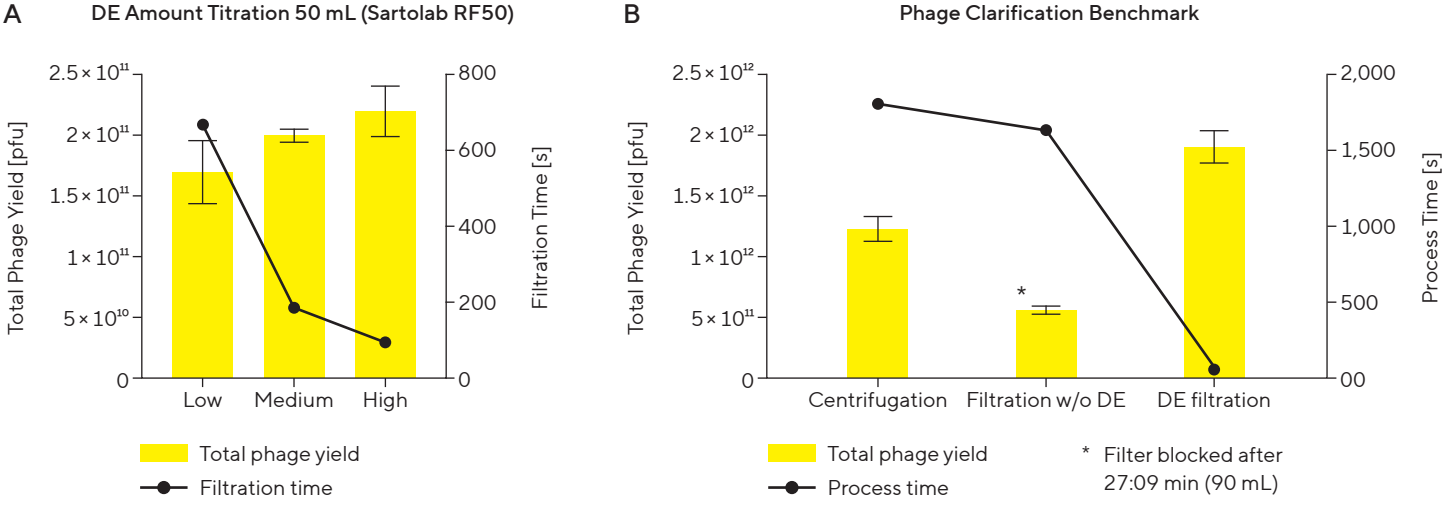
Optimal Amount of DE Filter Aid

Three different DE concentrations were tested with a threefold repetition (n = 3) to determine the optimal conditions for the comparative filtration test: Low = 2.5 g/L, Medium = 5 g/L, and High = 10 g/L. The results (Figure 1) indicate that the filtration time decreased asymptotically with increasing DE concentration, while the phage yield showed a general increasing trend with higher DE concentrations. Based on this trial, we selected the DE quantity level High for the benchmarking test to combine fast filtration with a high yield in relative terms.

Benchmarking Different Methods for Clarification

We evaluated three methods for clarifying an *E. coli* phage suspension: centrifugation, filtration using diatomaceous earth (Sartoclear Dynamics® Lab), and filtration without diatomaceous earth. The performance of each method was assessed based on the yield of phages and the time required for the clarification process. Centrifugation required 30 minutes to clarify the suspension. However, the yield of phages was lower compared to the filtration method using DE. Filtration with DE demonstrated a significantly faster clarification time of 2.03 minutes. Additionally, this method resulted in a significantly higher yield of phages compared to centrifugation. Filtration without DE encountered issues due to filter blockage. Consequently, only 90 mL of the initial 125 mL sample could be processed. These results indicate that Sartoclear Dynamics® Lab is the most efficient method in terms of both time and phage yield, while filtration without DE is not suitable due to blockage issues.

Figure 1: *Evaluating DE Filter Aid and Clarification Methods for Optimal Phage Yield and Efficiency*



Note. A) Filtration of 50 mL *E. coli* phage lysate using DE filter aid in three different quantities: Low = 2.5 g/L, Medium = 5 g/L and High = 10 g/L. To characterise the filtration performance, the filtration time and the yield of phages in the filtrate were determined. B) Clarification of 125 mL *E. coli* phage lysate using three different methods: Centrifugation, filtration with and without DE filter aid. To characterize the clarification performance the process time and the yield of phages in the filtrate | supernatant were determined.

Conclusion

The results of the study demonstrate that using Sartoclear Dynamics® Lab with DE filter aid significantly improves the clarification of an *E. coli* phi X174 phage lysate, both in terms of time efficiency and yield. This method eliminates the need for centrifugation, thereby simplifying laboratory workflows and improving overall efficiency. It is also advisable to conduct a preliminary trial to determine the optimal amount of DE required for the specific lysate.

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