

Banding of Intact Bacteriophage using the Thermo Scientific S100-AT6 Rotor and Sorvall Discovery SE Microultracentrifuges

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KEY WORDS

- Bacteriophage (Phage)
- Cesium Chloride
- Microultracentrifuge
- S100-AT6 Rotor
- Superspeed Centrifuge
- SLA-1500 Rotor
- Fiberlite F14S-6x250y mL Rotor

Abstract

With recent advances in molecular, environmental, and therapeutic biology, the isolation and characterization of bacteriophage continues to increase. Thermo Scientific Sorvall Discovery SE microultracentrifuges allow rapid concentration and purification of bacteriophage in a cesium chloride (CsCl) step gradient. The pure phage solution obtained via ultracentrifugation can be subsequently used for electron microscopy and protein or nucleic acid analysis.

Introduction

Bacteriophage, commonly referred to as phage, are bacterial viruses that are ubiquitous in nature and are likely to be found in the natural environment of their host organism(s). They have been extensively studied in the food fermentation industry since they were identified as a main source of fermentation failure, especially in applications based on starter cultures.^{1,2,3} As producers of biofilms and organic carbon, they have also been isolated from several natural environments, including soil,^{4,5,6,7} water, lakes, oceans,^{8,9,10,11,12} and plants. Furthermore, phage are suggested to have an important impact in biomedical fields as attractive therapeutic agents for bacterial infection when antibiotic resistance is rampant.¹³

In order to characterize a particular bacteriophage, it must first be isolated and purified. It is therefore necessary to develop

and optimize protocols that enable a rapid and efficient phage extraction. Centrifugation has long been considered the standard method to purify and concentrate bacteriophage. In this brief, we describe a centrifugation protocol which can be used to purify phage in a timely manner. The phage concentrate obtained can be used directly for electron microscopy and nucleic acid purification.

Procedures¹⁵

A 100 mL phage lysate was prepared prior to phage purification by challenging 100 mL of a mid-log *Pediococcus* culture grown in MRS medium containing 10 mM CaCl₂ and incubated until clearing. The phage supernatant was collected by centrifugation in a Thermo Scientific Sorvall RC6 Plus superspeed centrifuge with a Thermo Scientific Fiberlite F14S-6x250y mL or equivalent rotor for 20 minutes at 8,000 rpm (10,400 x g) and filter sterilized using a 0.45 µm filtration membrane. The phage lysate was incubated overnight with 2.9% NaCl and 10% PEG at 4 °C to promote phage precipitation. Phage were subsequently pelleted by superspeed centrifugation as before (8,000 rpm, 20 minutes, Fiberlite® F14S-6x250y mL rotor), and the pellet was resuspended in 2 mL of TE buffer (100 mM Tris, pH 7.6, 50 mM EDTA).

The 2 mL concentrated phage suspension was overlaid onto a three-step CsCl gradient containing 1 mL of 1.7 g/mL CsCl,



Thermo Scientific Sorvall Discovery M120 SE Microultracentrifuge

1 mL of 1.5 g/mL CsCl, and 1 mL of 1.4 g/mL CsCl in a 5.1 mL polyallomer Re-Seal™ ultracentrifuge tube. Phage were centrifuged for 7 hours at 100,000 rpm (approximately 600,000 x g) using the Thermo Scientific S100-AT6 rotor in a Sorvall® Discovery™ M150 SE microultracentrifuge (The Sorvall Discovery M120 SE microultracentrifuge will also suffice.) Phage-containing bands (translucent white/gray) were extracted through the wall of the centrifuge tube by puncturing with a needle, and the CsCl was subsequently removed by dialysis using a 6,000 – 8,000 dalton membrane (Baxter Diagnostics Inc., McGaw Park, IL) for 15 hours with three changes of de-ionized water. Phage purification methods were mostly adapted from Maniatis *et al.* 1982.¹⁴ The resulting phage samples can be further used for electron microscopy or nucleic acid purification.

For electron microscopy, the concentrated phage were negatively stained with 2% uranyl acetate (pH 4.0) and electron microscopy was carried out using a JEOL Model 100S electron microscope (JEOL USA, Peabody, MA) at 80 KV.

Results

Phage purification and concentration using the CsCl step centrifugation yielded highly concentrated samples (over 10^{11} phage/mL) of pure bacteriophage. The main advantage of this centrifugation step is time efficiency. In this case, only 7 hours of centrifugation with a microultra-centrifuge were necessary, whereas 24 hours of centrifugation are typically required with a standard ultracentrifuge. Also, the samples obtained were highly concentrated and very clean, enabling direct use for electron microscopy and protein or nucleic acid purification. Electron micrographs obtained with *Pediococcus* phage isolated from an industrial vegetable fermentation are shown in Figure 1.

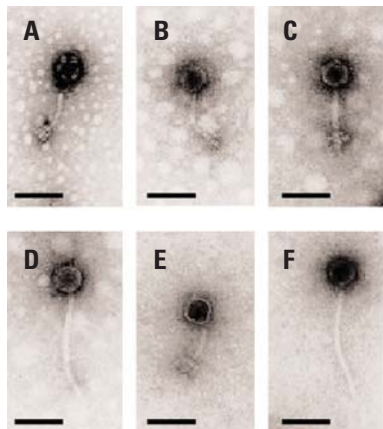


Figure 1. Electron micrographs of *Pediococcus* bacteriophages. The phage particles are negatively stained with uranyl acetate at a magnification of 85,000 x. A, ϕ Ps05a; B, ϕ Ps05b; C, ϕ Ps08a; D, Ps08b; E, Ps10a; F, Ps10b. Each bar denotes 100 nm.

Conclusion

The centrifugation procedure described in this study is designed for rapid and convenient phage purification and concentration. The suggested protocol for this application is 7 hours at 100,000 rpm (approximately 600,000 x g) using the S100-AT6 rotor in a Sorvall Discovery M150 SE or M120 SE microultracentrifuge. The phage samples prepared using this method can be used for electron microscopy and protein or nucleic acid purification.

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