Technical Note:
ANLECFGPHAGEMU 0112

Banding of Intact Bacteriophage using the Thermo Scientific S100-AT6 Ultracentrifuge Rotor

KEY WORDS

- Bacteriophage (Phage)
- Cesium Chloride Step Gradient
- S100-AT6 Rotor
- Sorvall MX Centrifuge
- Sorvall MTX Centrifuge
- Fiberlite F14-6x250y Rotor
- Sorvall RC 6 Plus Centrifuge

Abstract

With recent advances in molecular, environmental, and therapeutic biology, the isolation and characterization of bacteriophage continues to increase. Thermo Scientific Sorvall MTX and MX Micro-Ultracentrifuges allow for 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 the biomedical field as therapeutic agents for bacterial infections 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 rapid and efficient phage extraction. Centrifugation has long been considered the standard method to purify and concentrate bacteriophage. In this note, we describe a centrifugation protocol, adapted from Sambrook and Russell (2001)[14], 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.

Procedures[15]

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 $CaCl_2$ and incubated until clearing. The phage supernatant was collected by centrifugation in a Thermo Scientific Sorvall RC 6 Plus superspeed centrifuge with a Thermo Scientific Fiberlite F14-6x250y rotor for 20 minutes at 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 for 20 minutes at 10,400 x g in a Fiberlite® F14-6x250y rotor. 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, 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 (PN 45248). Phage were centrifuged for 7 hours at 603,000 x g using the Thermo Scientific S100-AT6 rotor in a Sorvall® MTX Micro-Ultracentrifuge; the Sorvall MX Micro-Ultracentrifuge can alternatively be used. The white-to-grey phage-containing bands 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 for 15 hours with three changes of deionized water.
The concentrated phage were negatively stained with 2% uranyl acetate (pH 4.0) and electron microscopy carried out using an electron microscope at 80 KV.

Sorvall MTX Micro-Ultracentrifuge

Results

Phage purification and concentration using CsCl step gradient centrifugation yielded highly concentrated samples of pure bacteriophage (over 10 phage/mL[11]). The main advantage of this centrifugation step is time efficiency. This protocol requires only 7 hours of centrifugation with a micro-ultracentrifuge, whereas 24 hours of centrifugation are typically required with a standard ultracentrifuge. In addition, 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 industrial vegetable fermentation are shown in Figure 1.

Conclusion

The centrifugation procedure described in this note is designed for rapid and convenient phage purification and concentration. The suggested protocol for this application is 7 hours at 603,000 x g using the S100-AT6 rotor in a Sorvall MTX or MX Micro-Ultracentrifuge.

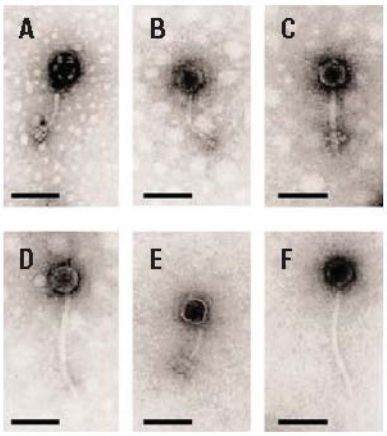

Figure 1. Electron micrographs of *Pediococcus* bacteriophages. The phage particles are negatively stained with uranyl acetate at a magnification of 85,000 x. A, ΦPs05a; B, ΦPs05b; C, ΦPs08a; D, Ps08b; E, Ps10a; F, Ps10b. Each bar denotes 100 nm.

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