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Amino-terminal Sequencing of SSV1 Major Capsid Protein VP1 Mutants

Nichole D. Procter  
*Portland State University*

Kenneth M. Stedman  
*Portland State University*

Ignacio de la Higuera  
*Portland State University*

George Kasun  
*Portland State University*

Ajay Sapre  
*Oregon Health and Science University*

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Correspondence:
Department of Biology, Center for Life in Extreme Environments (CLEE)
Portland State University, OHSU - Knight Cancer Institute
Correspondence: procter@pdx.edu, extremeviruses.org

ABSTRACT
Unique archaeal-A type viruses, the *Parvoviridae* or *Sulfolobus* Spindle-Shaped Virus (SSV) family, are unique taxa in hyperthermophiles and extreme environments. The major capsid protein, VP1, forms the unique virus structure and provides protection for the genome. The amino acid alignment of the SSV1 major capsid protein, VP1, from wild-type SSV1, changing the glutamate to either alanine or glutamine produces proteolytic cleavage at the same position as in wild-type SSV1. The most well characterized of the SSVs is SSV1. Wild-type SSV1 tolerates multiple mutations to its genome while still remaining infectious, making this virus ideal to study molecular processes in extreme environments.

RESEARCH QUESTION
Are mutated SSV1 VP1 major capsid proteins cut at the same position as in wild-type SSV1?

INTRODUCTION – THE SSV VIRUS
• Thrives in boiling acid (Figure 1)
• Unique morphology (Figure 3)
• Major capsid protein cleaved at E66 (Figure 4)
• Tolerates many mutations, including E66.

ONGOING RESEARCH
• Dialfiltration to further concentrate virus particles
• Electroblocting to PVDF membrane and staining
• Edman Degradation for N-terminal amino acid sequencing
• Determination of SSV1 VP1 cleavage site in mutants

METHODS – VIRUS PURIFICATION
• Grow 1L of *Sulfolobus* strain S441 (formerly *Sulfolobus* SSV1) host (Figure 2)
• Infect host culture with mutant viruses (SSV1 VP1_E66A, and SSV1 VP1_E66Q)
• Concentrate infected culture supernatants with ultrafiltration (Figure 5A)
• Analyze particles by differential light scattering (Figures 5B, 6B, 7B) and Transmission Electron Microscopy (Figures 5C, 6C, 7C)
• Further purify particles with step gradient followed by equilibrium ultracentrifugation in cesium-chloride (Figures 6A and 7A)
• Analyze SSV1 proteins with SDS-PAGE (Figure 8A)
• Confirm presence and identity of VP1 by Western Blot (Figure 8B)

RESULTS – VIRUS PURIFICATION
• Ultrafiltration concentrates virus particles for downstream analysis (Figure 5A) but they are insufficiently pure (Figure 5B)
• SSV1 viral particles including mutated VP1 protein band in CsCl at ~p1.3g/mL during ultracentrifugation (Figures 6A, and 7A)
• A step gradient followed by equilibrium ultracentrifugation produces a homogeneous preparation of mutant SSV1 particles (Figure 6B and 7B)
• SDS-PAGE and Western blot analysis with anti-VP1 antibody confirm virus purification, presence, and identity of VP1 (Figure 8A, and 8B)

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