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Amino-terminal Sequencing of SSV1 Major Capsid Protein VP1 Mutants

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Extreme Viruses

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ABSTRACT

Unique lemon-shaped viruses, the *Fuselloviridae,* or *Sulfolobus* Spindle-Shaped Viruses (SSVs) of hyperthermophilic archaea are characterized by both their unusual structure and extreme thermal tolerance. 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. The major capsid protein, VP1 forms the unique structure of the virus and provides protection for the genome. The amino terminus of the VP1 protein purified from wild-type SSV1 is glutamate 66 (E66) indicating proteolytic cleavage. E66 is universally conserved in all SSVs. Interestingly, the N-terminus of VP1 upstream of E66 which is cleaved off is required for infectivity, indicating that VP1 may be made or assembled as a precursor protein. Virus mutants made by single point mutations at E66 in VP1 in SSV1, changing the glutamate to either alanine or glutamine produces infectious virus which displays the traditional lemon-shape morphology that varies slightly in structure from wild-type. This work aims to determine whether these mutant viruses are cleaved at the same location (66) as the wild-type or cleaved elsewhere. To determine the location these mutant viruses are proteolytically processed, whole virus purification of mutant SSV1 virus and N-terminus sequencing will be performed. Ultimately, the role of proteolytic cleavage in virus assembly and structure will be determined.

RESEARCH QUESTION

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

REFERENCES

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INTRODUCTION – THE SSV1 VIRUS

- Thrives in boiling acid (Figure 1)
- Unique morphology (Figure 3)
- Major capsid protein cleaved at E66 (Figure 4)
- Tolerates many mutations, including E66.

Figure 1. Boiling Springs Lake – Lassen Volcanic National Park, CA. *Sulfolobus* habitat 50-94℃, pH 2.5

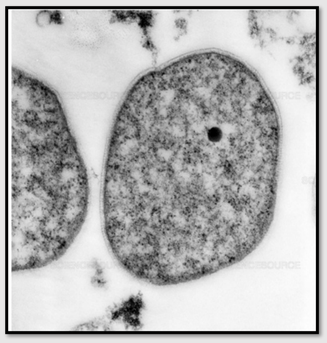


Figure 2. *Saccharolobus* SSV1 Host. Cell = ca. 1 µm diameter

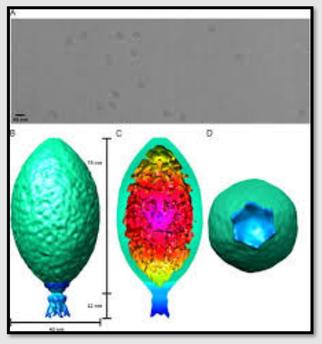


Figure 3. SSV1 structure from Cryo-EM. (Stedman et. al. 2015)

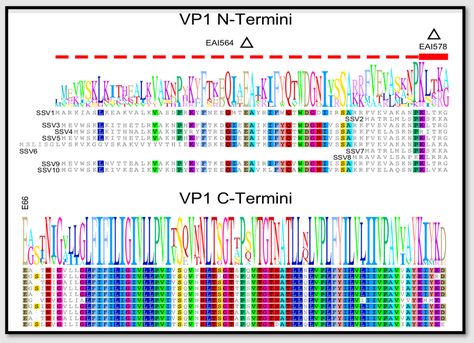


Figure 4. SSV VP1 amino acid alignment. Alignment split at conserved glutamate (E66) cleavage site (Iverson et al. 2017).

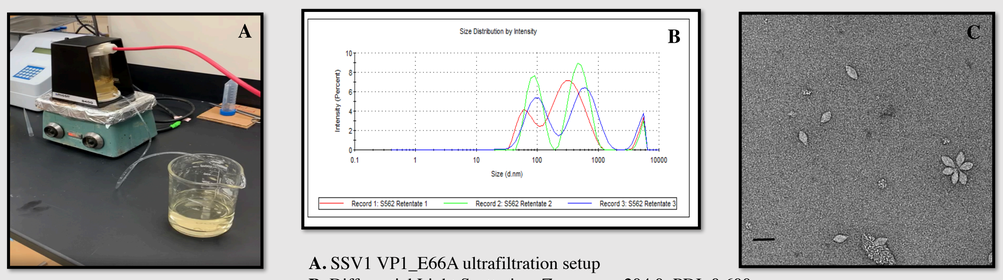
ONGOING RESEARCH

- Diafiltration to further concentrate virus particles
- Electroblotting 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 *Saccharolobus** 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)

Figure 5 – Crude SSV Preparation

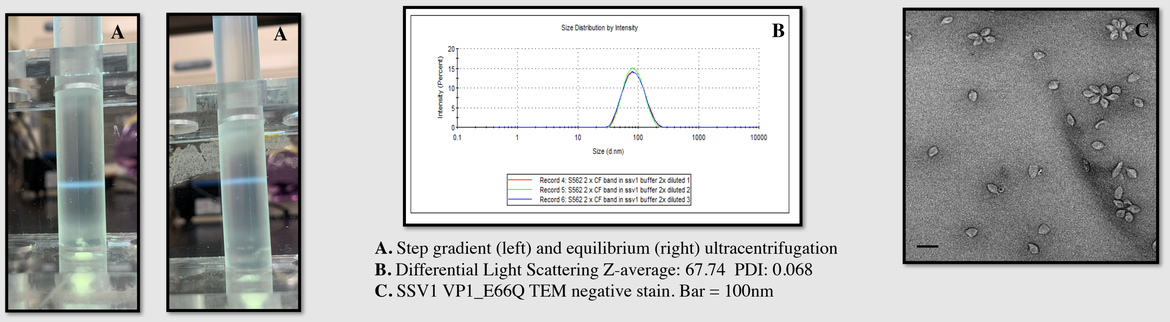


A. SSV1 VP1_E66A ultrafiltration setup
B. Differential Light Scattering. Z-average: 204.0 PDI: 0.600
C. SSV1 VP1_E66A. TEM negative stain. Bar = 100nm

RESULTS – VIRUS PURIFICATION

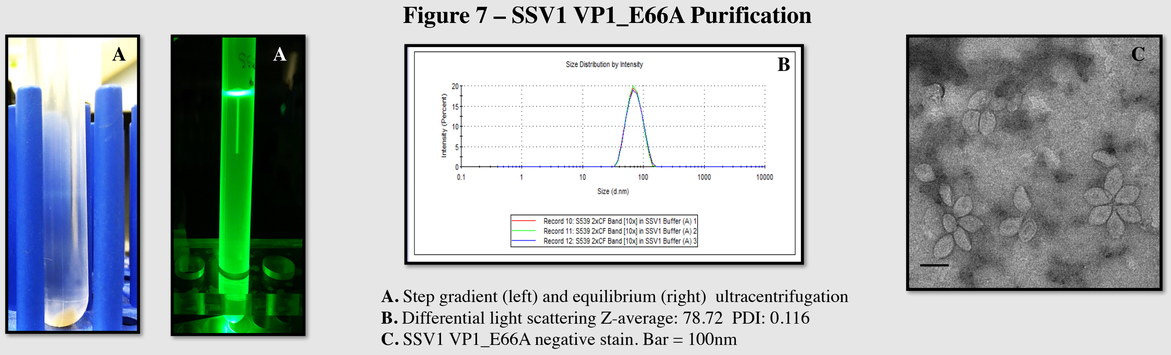
- Ultrafiltration concentrates virus particles for downstream analysis (Figure 5A) but they are insufficiently pure (Figure 5B)
- SSV1 viral particles including mutated VP1 protein band in CsCl at ~ρ1.3g/mL during ultracentrifugation (Figures 6A, and 7A)
- A step gradient followed by equilibrium ultracentrifugation 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)

Figure 6 – SSV1 VP1_E66Q Purification



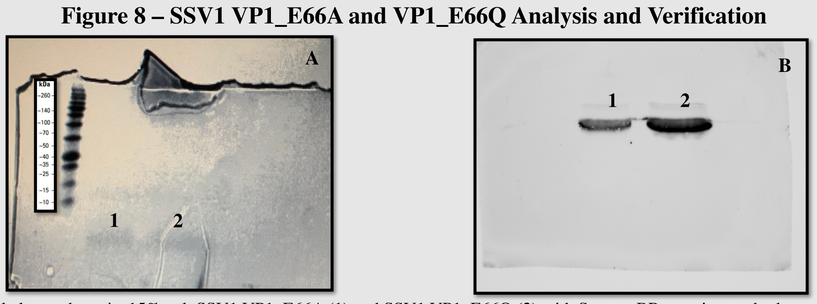
A. Step gradient (left) and equilibrium (right) ultracentrifugation
B. Differential Light Scattering Z-average: 67.74 PDI: 0.068
C. SSV1 VP1_E66Q TEM negative stain. Bar = 100nm

Figure 7 – SSV1 VP1_E66A Purification



A. Step gradient (left) and equilibrium (right) ultracentrifugation
B. Differential light scattering Z-average: 78.72 PDI: 0.116
C. SSV1 VP1_E66A negative stain. Bar = 100nm

Figure 8 – SSV1 VP1_E66A and VP1_E66Q Analysis and Verification



A. SDS-PAGE gel electrophoresis. 15% gel. SSV1 VP1_E66A (1) and SSV1 VP1_E66Q (2) with Spectra BR protein standard.
B. Expected VP1 band ~10.3kDa. Western blot of gel from Figure 8 using Anti-VP1 antibody. SSV1 VP1_E66A (1) and SSV1 VP1_E66Q (2)