Amino-terminal Sequencing of SSV1 Major Capsid Protein VP1 Mutants

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INTRODUCTION

Thrives in boiling acid (Figure 1)

Unique morphology (Figure 3)

Major capsid protein cleaved at E66 (Figure 4)

Tolerates many mutations, including E66.

METHODS – VIRUS PURIFICATION

Grow 1L of Saccharolobus strain SS41 (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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