Investigating Extreme Life

Optimizing concentration of SSV1; a virus of *Sulfolobus*

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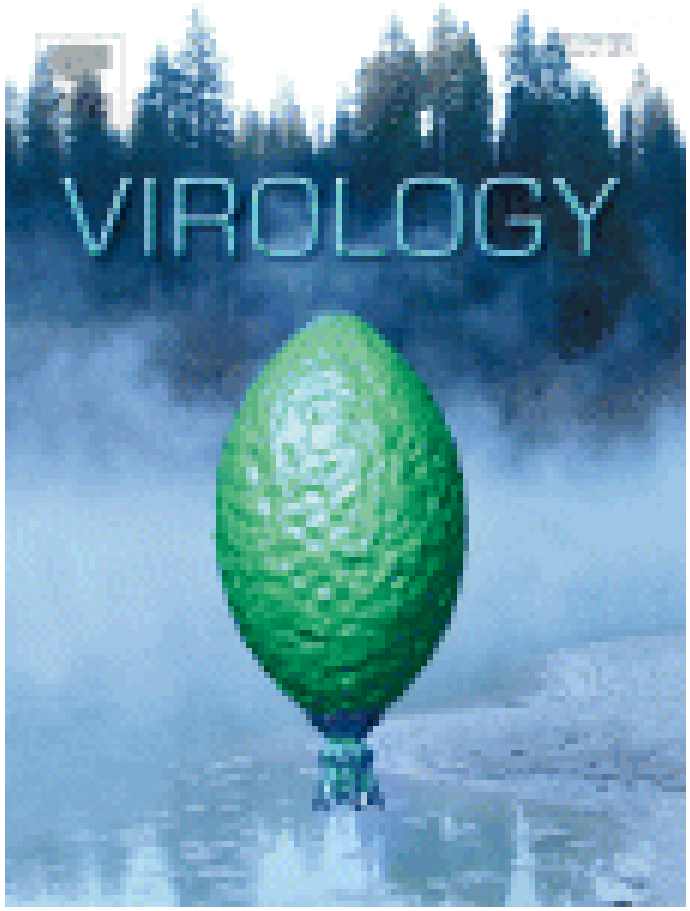
REU Program 2016

Presentation Overview

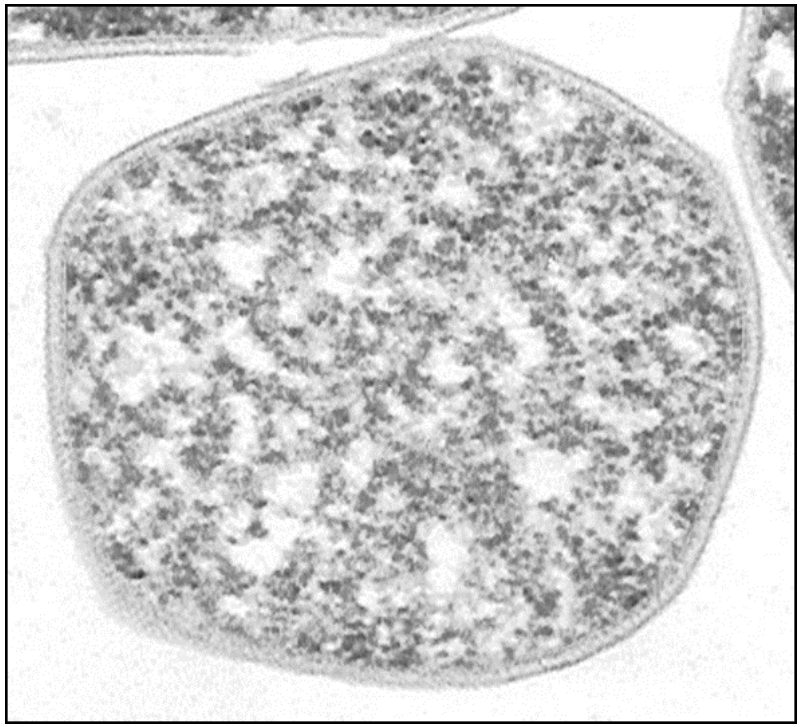
- Host
- Virus
- Reasons for increasing concentration
- How to calculate concentration
- Methods
- Results
- Conclusion and further research



Cover from *Virology* Volume 474,
1 January 2015

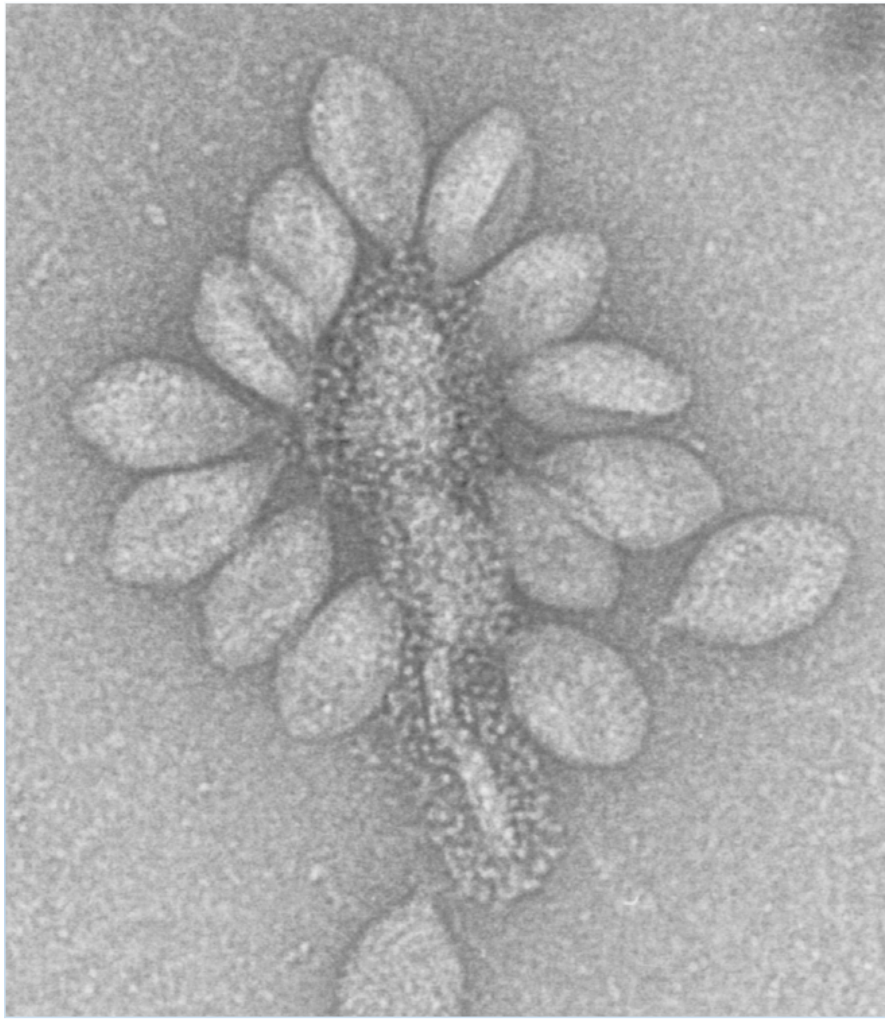
Sulfolobus solfataricus

- Thermoacidophilic archaeon
  - 80°C, pH^+ 3.0
- Simple to culture
- Interesting viruses



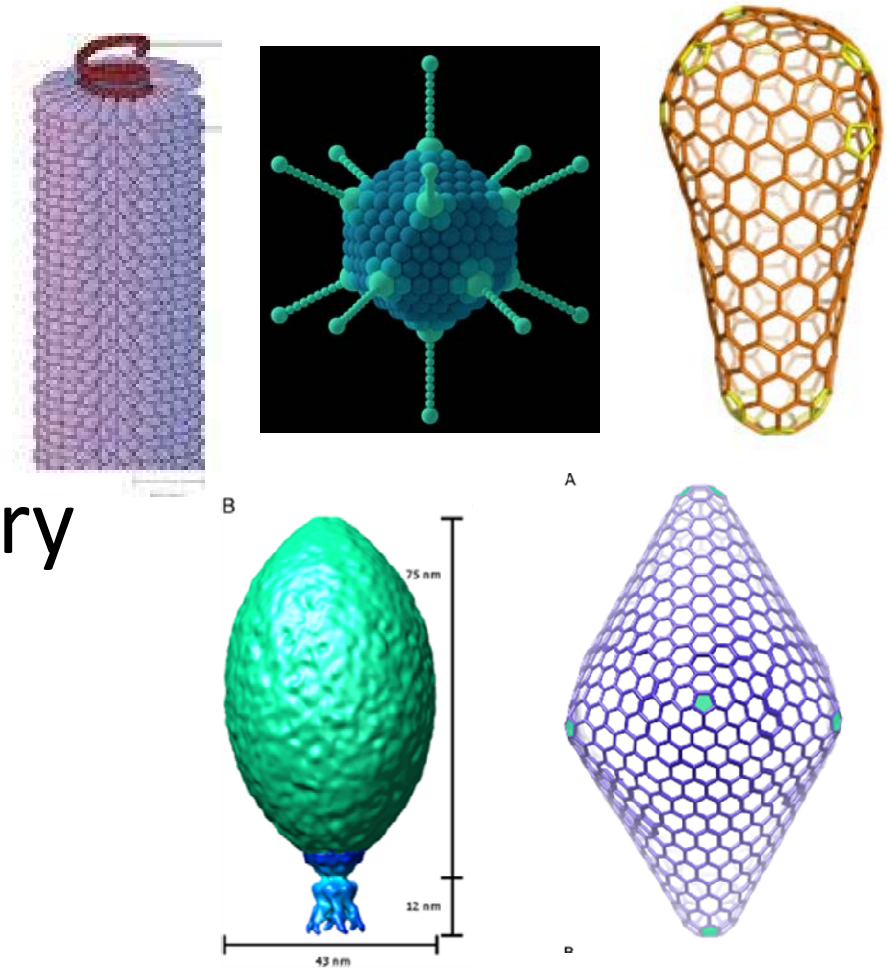
SSV1

- *Sulfolobus* spindle-shaped virus 1
- Fusellovirus
  - SSV's found globally
- Circular dsDNA
  - Positively supercoiled genome
  - 15.4 kbp
- Unique shape



Structural modeling

- Viral structures
  - Icosahedral
  - Helix
  - Potential third category
    - HIV, SSV1 (?)



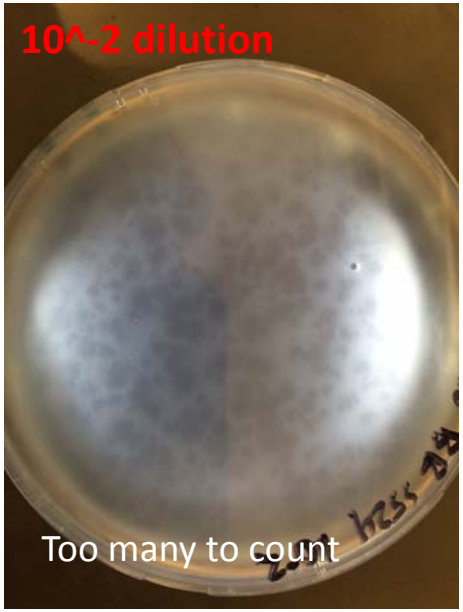
Morais et al., 2015

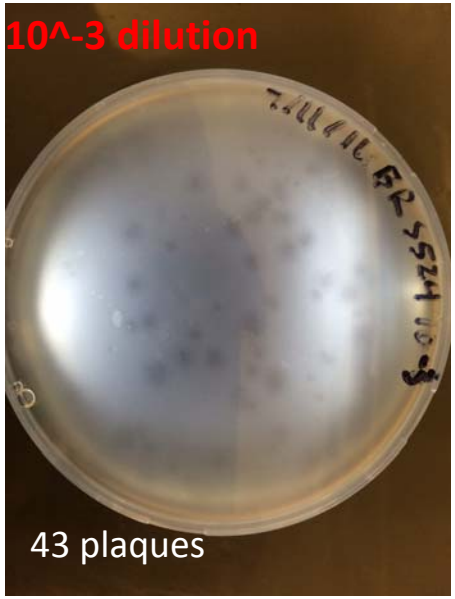
Plaque Assay: Finding concentration of virus

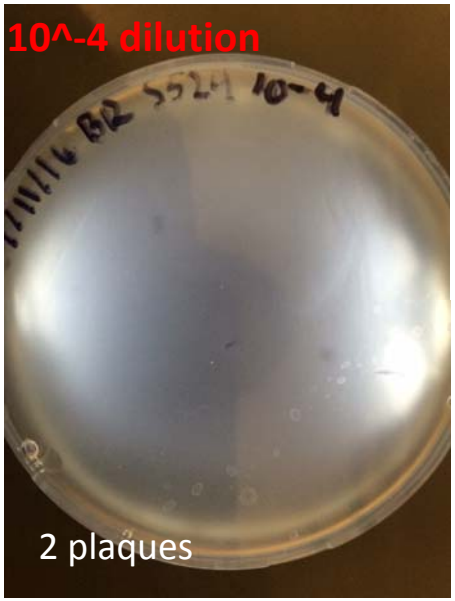
Plaque forming units/mL=(Original Dilution)*(dilution of plate)*(# of plaques on plate)

ex: pfu/mL=(10)*(10^3)*(43)

= 4.3*10^5 plaque forming units/mL

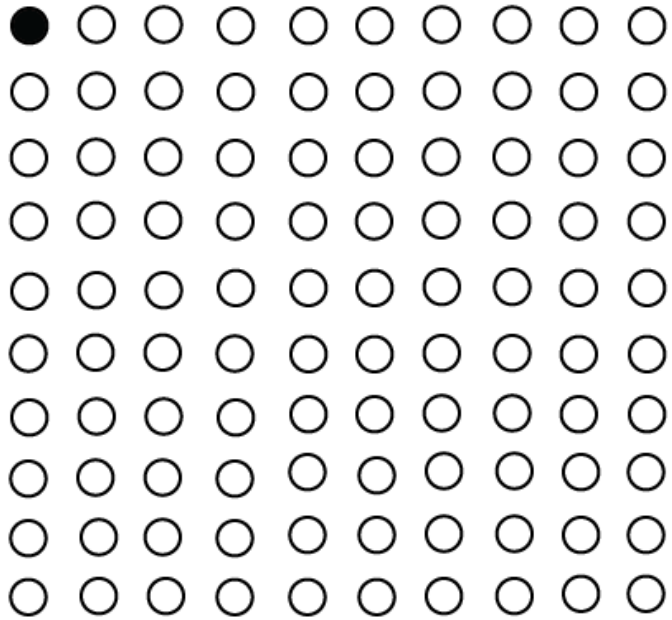






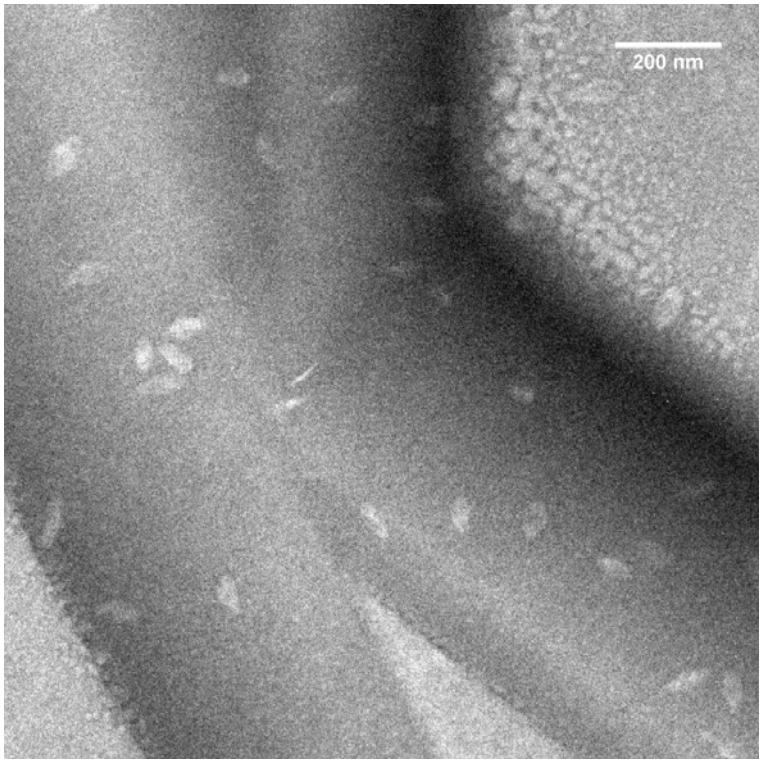
Low Multiplicity of Infection (MOI)

- Ratio of host to viral particles
- 1 virus particles for every 100 host cells (MOI of .01)
- Allows for multiple rounds of infection



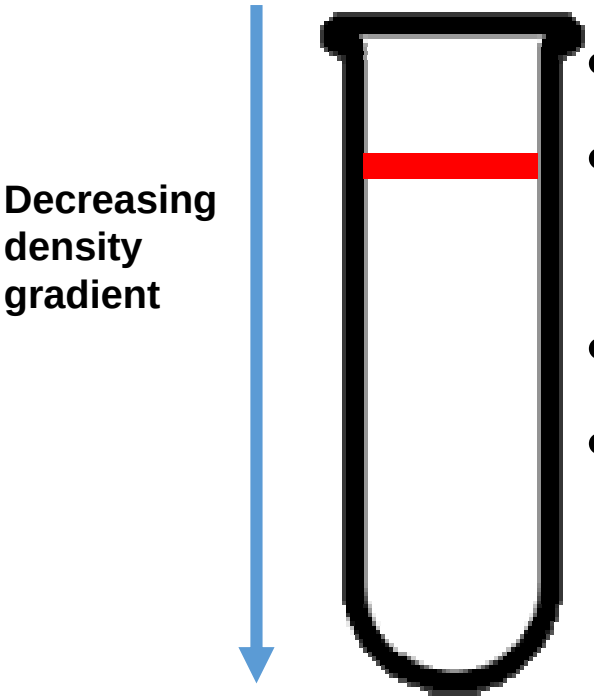
Low MOI Results

Benefits
- Simple!
- High yields
- Maintain shape



Potential Problems
- Takes several days
- Cell debris

Cesium Chloride Centrifugation

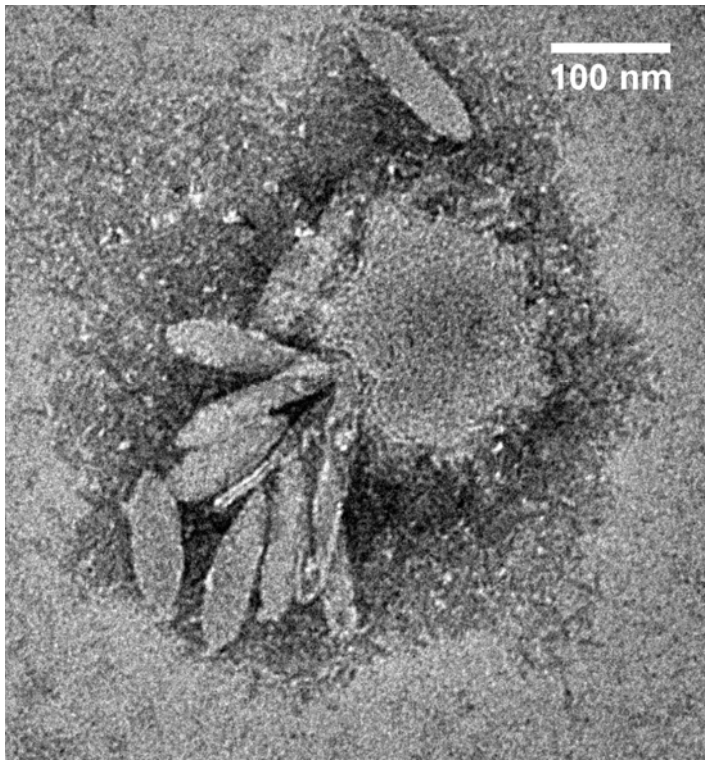


- High salt concentration
- High speed (30,000 rpm) for many hours (22 hours)
- Creates density gradient
- Band visible using light dispersion

Cesium Chloride Centrifugation Results

Benefits

- Purification
- low man-hours required
- Potentially concentrate lots of virus at once



Potential Problems

- Causes structural abnormalities
- Created hollow viral particles

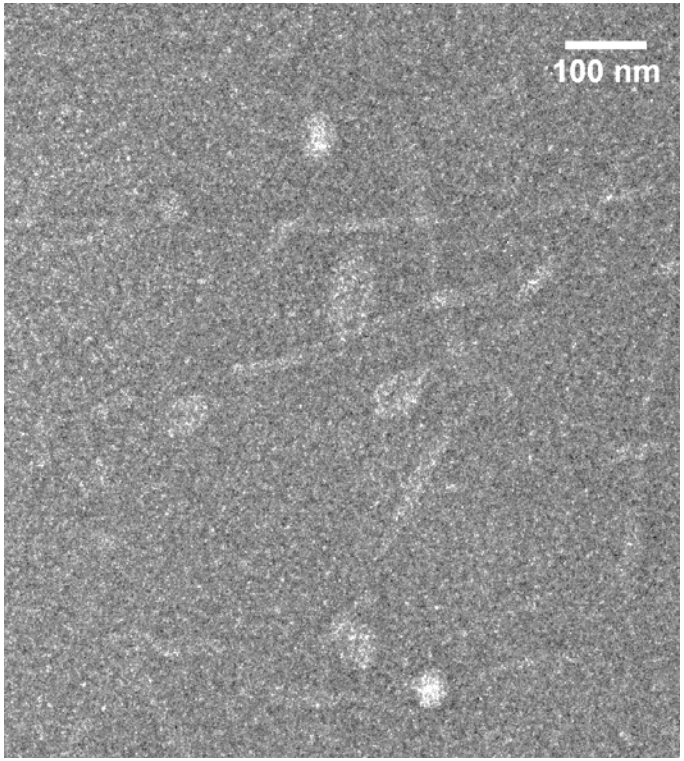
“Soft-layer” Isolation

- Originally described in Quemin et al., 2015
- Removing soft-layer from high concentration plaque assays
- Uses shaking to suspend virus from gel
- Multiple high speed spins to attempt to purify

“Soft-layer” Isolation Results

Benefits

- High concentration
- Short timeline
- High volumes produced



Potential Problems

- “Dirty”
- Could require more steps
- Requires lots of plaque assays

Conclusions and Further Research

- Soft-layer isolation and MOI infection yield high concentration
- Purification methods for soft-layer isolation
- Optimal collection time from low MOI infection


Thank you
Dr. Kenneth Stedman
David Goodman
Stedman lab members
NSF