Bioprocess Strategies to Mitigate the Impact of Proteases on Adeno-Associated Viral (AAV) Vector VP1 Capsid Content

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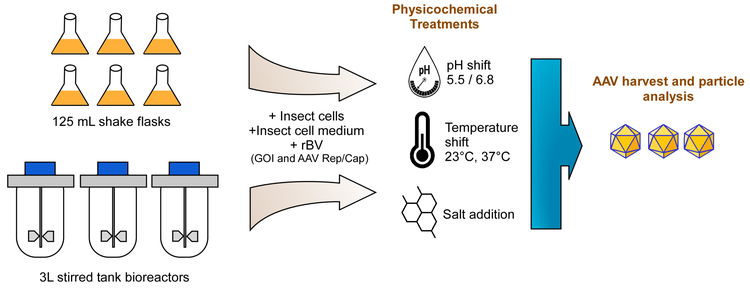
Background

- AAV vectors are currently being exploited as effective vehicles for gene therapy applications. The insect cell-baculovirus system is a major workhorse for their production at preclinical and clinical stages.
- A handful of studies (Galibert et al., 2018; Kaba et al., 2004) have suggested that baculovirus proteases may impact the nature of recombinant proteins (e.g, AAV capsids) and specifically cause degradation of the vector's VP1 protein, which has been determined to be important for virus infectivity.

Objective

- Design bioprocess strategies that may inhibit protease activity, and by extension prevent the reduction of AAV VP1 levels in purified capsids.

Methodology



AAV purification:
-Affinity chromatography
-Anion exchange chromatography

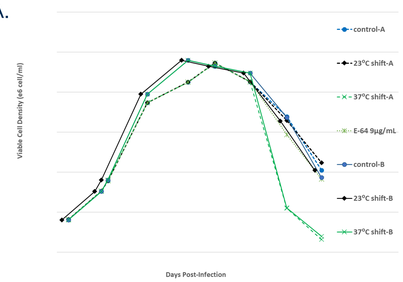
AAV analytics:
-Reverse-phase HPLC
-Capillary electrophoresis
-Mass spectrometry

Results

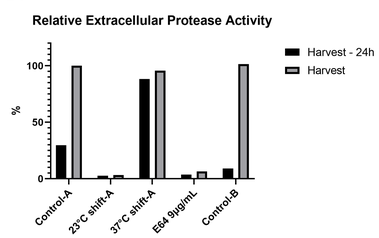
Low temperature change maintained protease levels low, and led to higher AAV VP1 levels

- Cell culture growth trends were comparable, with differences in final viable cell density (A)
- Addition of E64 protease inhibitor, as well as a late 23°C temp shift, effectively kept protease low (B) and showed the highest VP1 levels (C)

A.



B.



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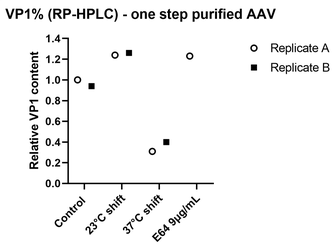
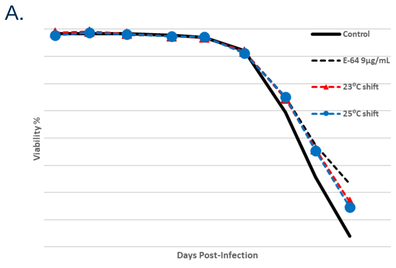


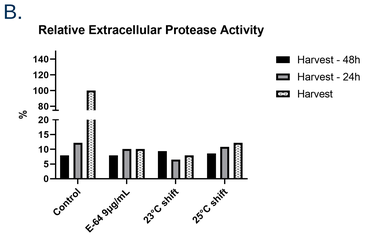
Bioreactor experiment confirmed temp shift prevented AAV VP1 degradation over time

- Comparable cell growth, slightly higher final cell viability % in testing conditions (A)
- All tested conditions prevented elevated protease levels (B, ~90% reduction)
- Both E64+ and 23°C temp shift conditions generated AAV particles with ~50% higher VP1 (C)

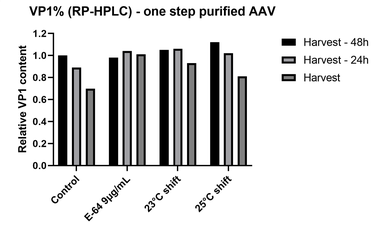
A.



B.



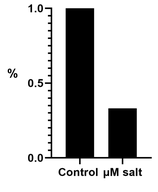
C.



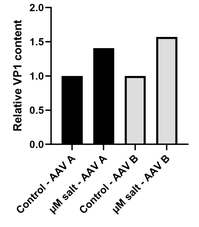
Salt addition at late timepoint contributed to higher VP1 profile

- Addition of an inorganic salt at micromolar concentration reduced protease levels by ~70% (A), and increased AAV VP1 levels by ~50% with one serotype X (B)
- Testing of 23°C shift and salt addition showed no additive effect on VP1 levels, AAV serotype Y (C)

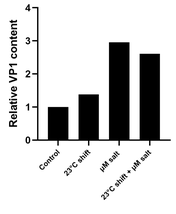
A. Relative Extracellular Protease Activity



B. VP1% - one step purified AAV



C. VP1% - two-step purified AAV



Conclusion

- 23°C temp shift and salt addition mitigated undesired effects of proteases released during insect cell culture, which appear to have a positive effect on the capsids of AAV vectors.

Questions ahead

- How does the improved VP1 profile translate into in vivo potency levels?
- How does the addition of these process changes impact process robustness and other product attributes?

Acknowledgments

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