

Evaluation of culture media for the growth of *Flavobacterium covae*: toward standardization for inoculation studies

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Introduction

Flavobacterium covae is a significant pathogen in the channel catfish industry, as it is a primary bacterium responsible for causing columnaris disease.

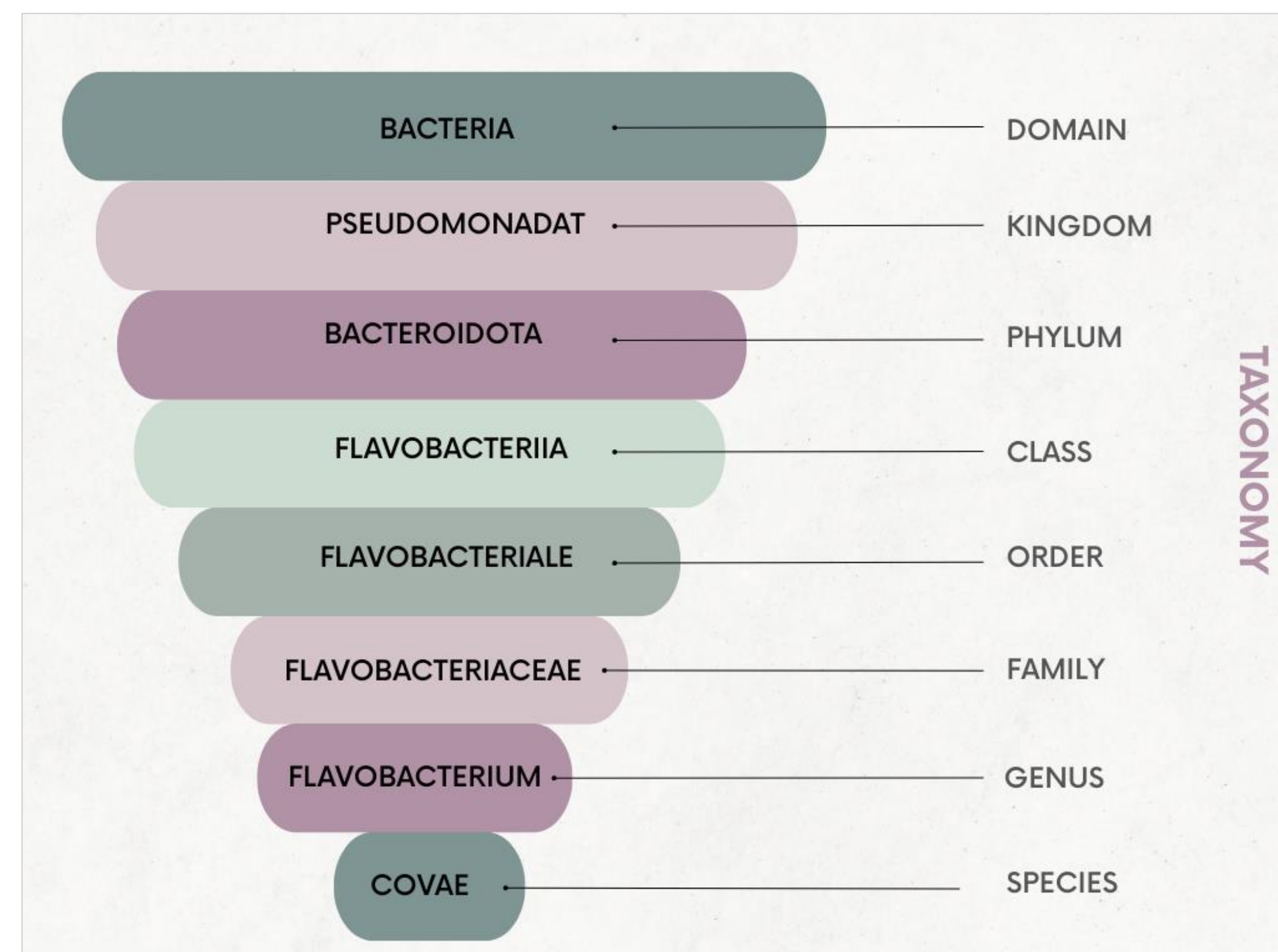


Figure 1. Taxonomic Classification of *Flavobacterium covae*¹

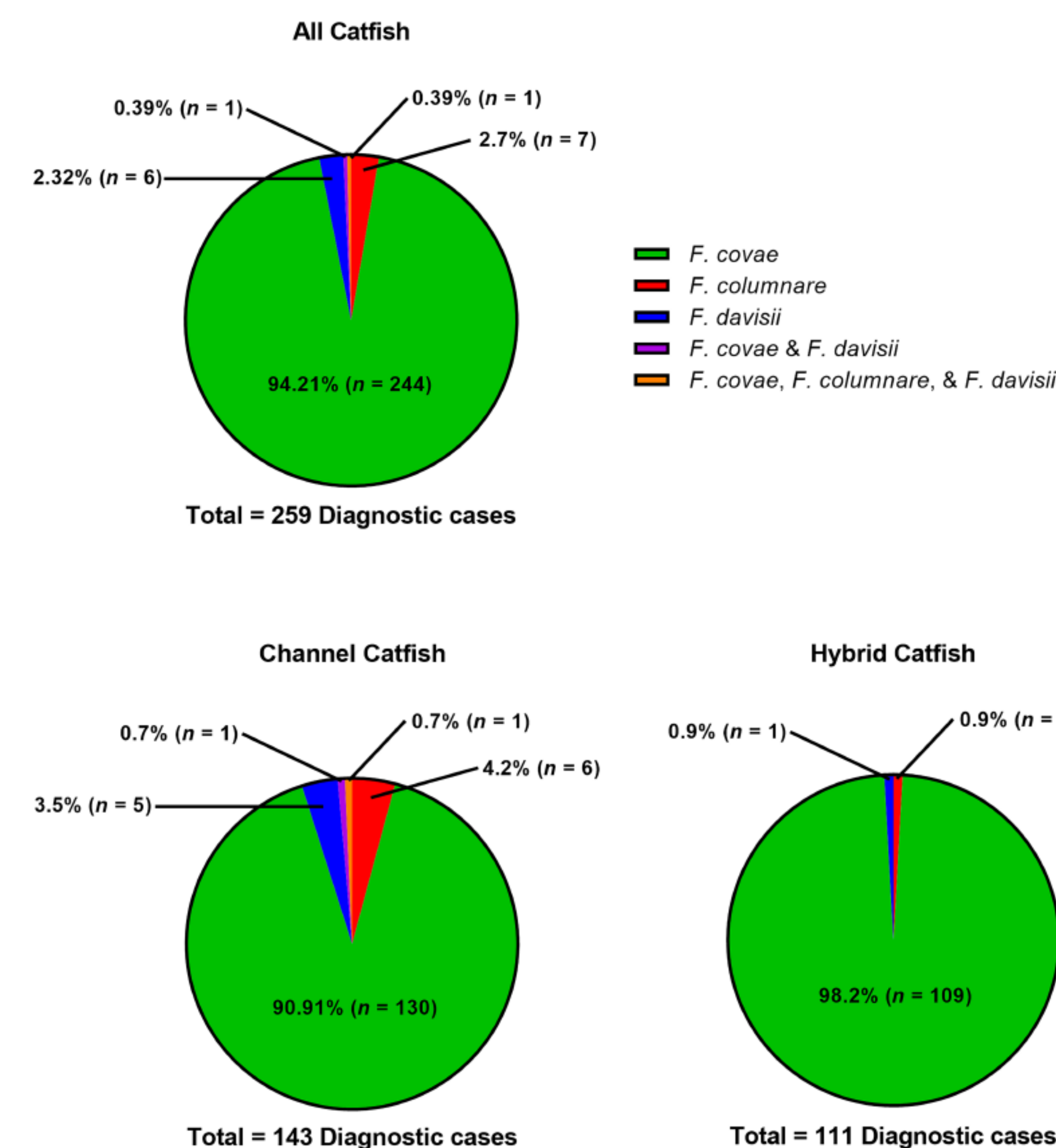


Figure 2. Percentage of examined diagnostic cases attributed to each species of columnaris-causing bacteria (*Flavobacterium* spp.) or coinfections in Mississippi and Alabama during 2015–2019²

Columnaris disease in Channel Catfish threatens fish health and the sustainability of aquaculture businesses, thereby impacting overall food security. Despite its importance, there is a lack of consistency in the culture media used for the optimal growth of *F. covae*, delaying in vitro and in vivo studies due to the bacterium's fastidious nature.

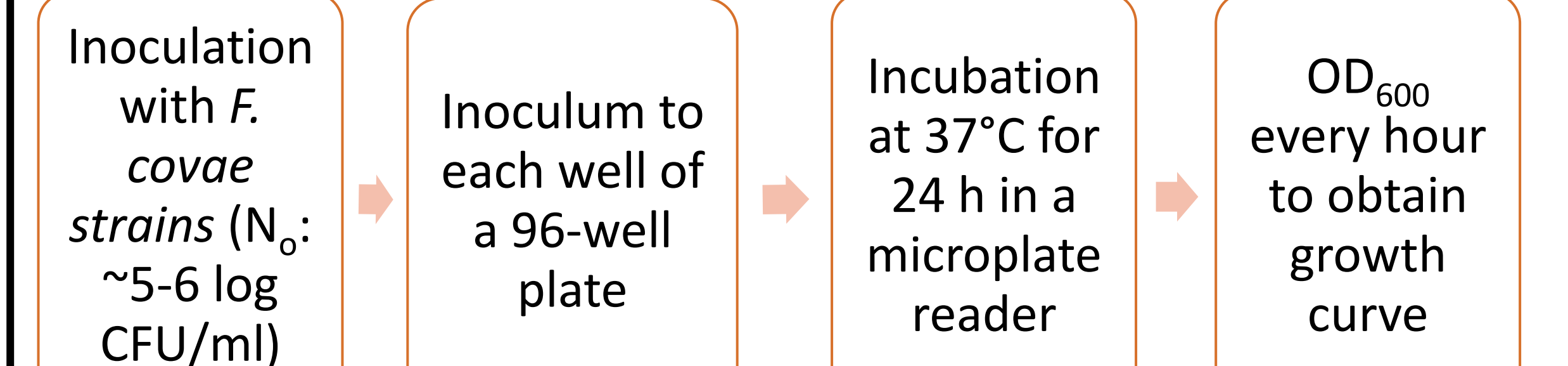
Purpose

The objective of this study was to evaluate and compare the efficacy of various culture media to support the growth of *F. covae* during initial resuscitation, preparation of a stationary-phase inoculum, and throughout the bacterial growth curve.

Methodology

Initial resuscitation at 28 °C for 24, 48, and 72 h using Nutrient Agar, Tryptic Soy Agar, Tryptic Soy Agar with Blood, Brain Heart Infusion Agar and Broth, Reasoner's 2A (R2A) Agar and Broth, and Shieh Broth

Subsequent inoculation to obtain a stationary-phase inoculum and generate growth curves in R2A Broth and Shieh Broth at 28 °C for 48 h with constant agitation.



Results

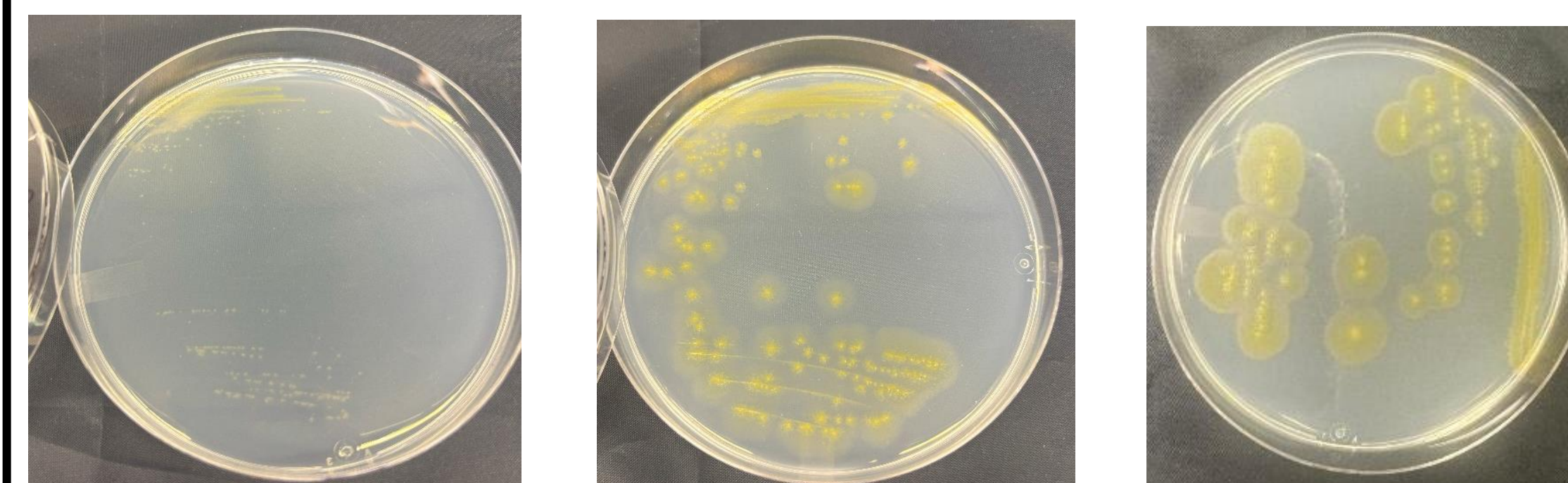


Figure 3. R2A Agar at 28 °C for 24 (left), 48 (middle), and 72 (right) h

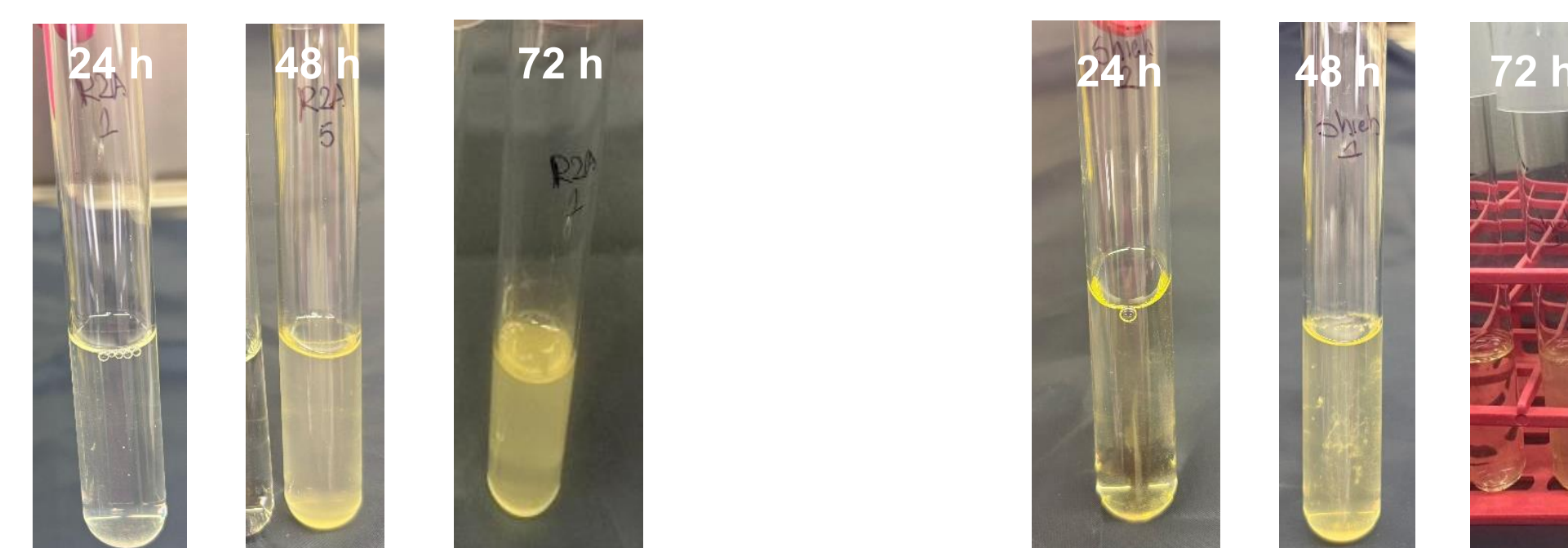
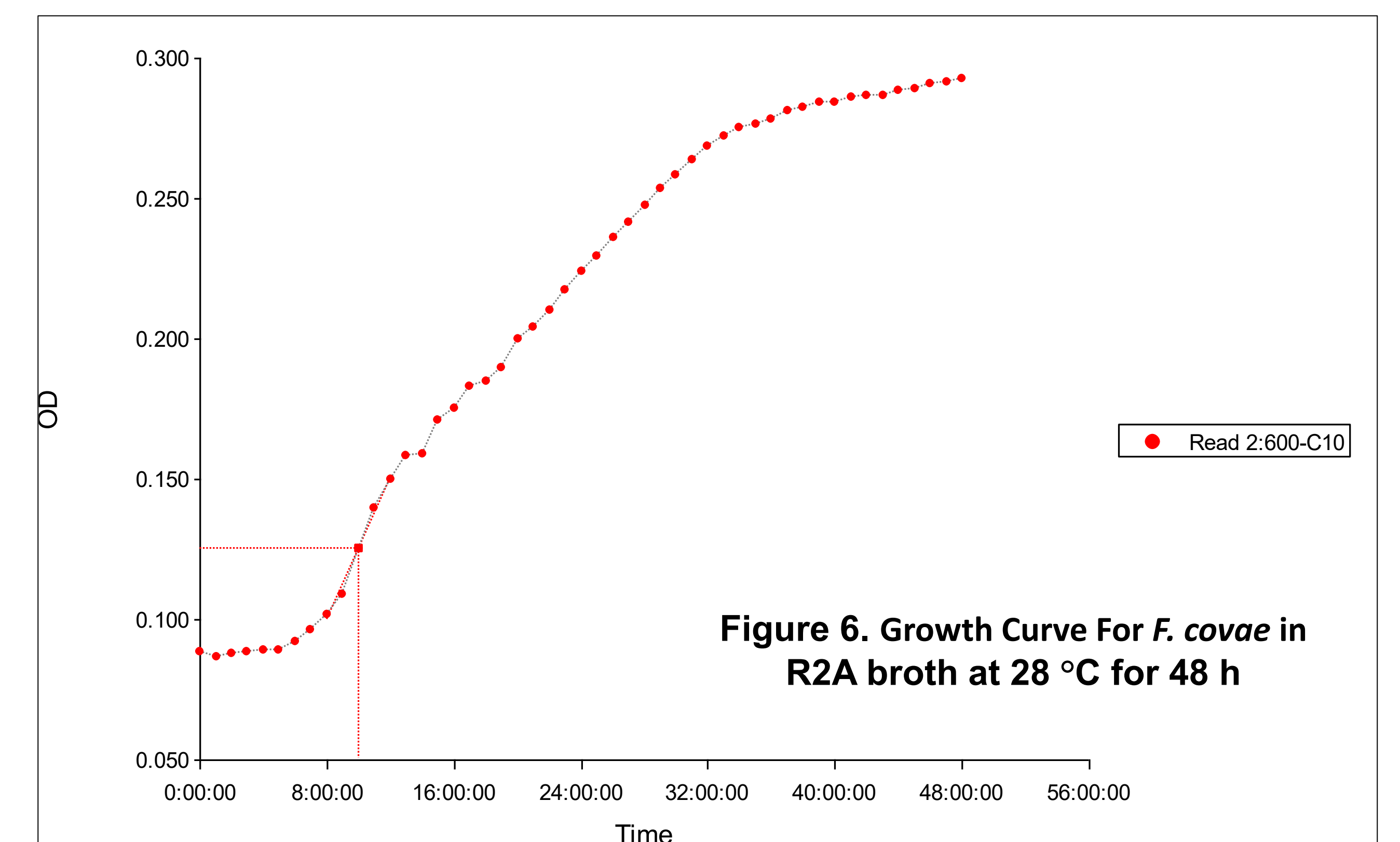


Figure 4 & 5. R2A (left) and Shieh (right) Broth at 28 °C for 24, 48, and 72 h



Conclusions

- The viability of the bacterial stock and freshness of the media significantly influenced outcomes. Sensitivity to pH and complex preparation requirements may affect reproducibility.
- Bacterial growth was consistently observed in R2A Broth across all replicates. Therefore, this media could be a reliable option for cultivation of *F. covae*, being a promising candidate for standardization in laboratory protocols.

References

- Schoch CL, et al. (2020). NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford), 2020:baaa062.
- LaFrentz, B. R., Khoo, L. H., Lawrence, M. L., Petrie-Hanson, L., Hanson, L. A., Baumgartner, W. A., & Griffin, M. J. (2024). *Flavobacterium covae* is the predominant species of columnaris-causing bacteria impacting the Channel Catfish industry in the southeastern United States. Journal of Aquatic Animal Health, 36(1), 3-15.