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Towards culturing anaerobic gut fungi from an avian herbivore

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INTRODUCTION

- Anaerobic gut fungi (AGF, phylum Neocallimastigomycota) are an understudied group of microorganisms that reside in the digestive tracts of herbivorous mammals and aid in plant biomass degradation.
- The presence of AGF in non-mammalian herbivores is not yet well understood, and no AGF have been cultured from avian hosts.
- AGF are typically found in digestive tracts that promote fermentation, i.e., the cow rumen (Figure 1).
- Ostriches are hindgut fermenters with large caeca and a very long colon. This, in addition to their long ~40 h ingesta mean retention time, led us to the hypothesis that ostriches are likely a novel host for AGF and likely also house novel AGF taxa.

OBJECTIVES

This project aims to successfully culture anaerobic gut fungi from fecal samples of avian herbivores. This is important to understanding the diversity of AGF and their host variability.

METHODS

Culturing AGF follows a standard enrichment process (Figure 2) that can be amended in several ways (Table 1). Cow fecal samples were used as a methods control.

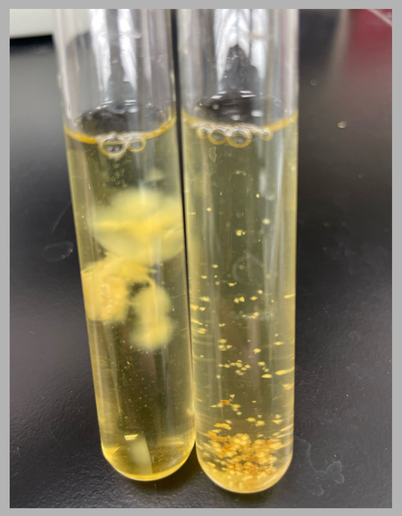


Figure 4. Cow isolates Orpinomyces joyonii (left) *and Anaeromyces mucronatus* (right).

RESULTS

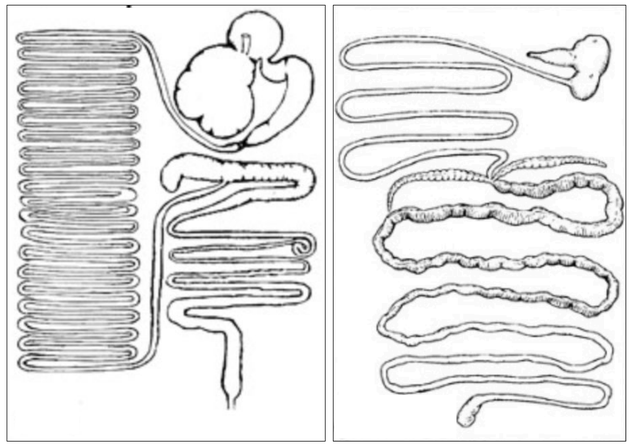


Figure 1. Comparison of ruminant (left) and ostrich (right) digestive tracts.

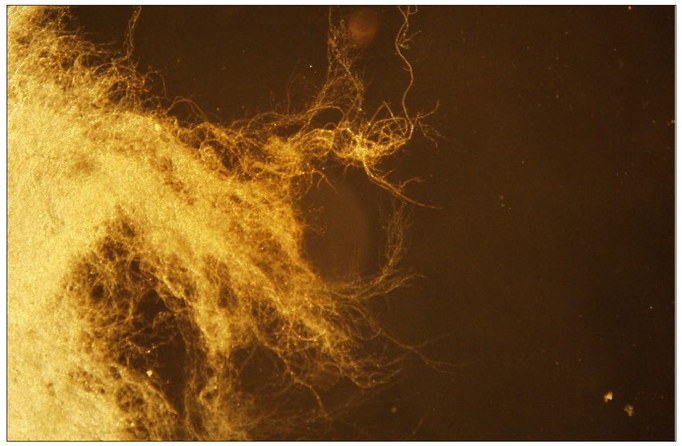


Figure 3. Microscopic view of Anaeromyces mucronatus.

Temperature (°C)	Substrates	Antibiotics	
		Standard Cocktail	Super Cocktail
30	Cellulose	Penicillin G	Penicillin G
39	Switchgrass	Streptomycin	Streptomycin
	Starch	Chloramphenicol	Chloramphenicol
	Filter paper		Kanamycin
			Norfloxacin

Table 1. Variables for enrichment procedure.

Sample	Sequence Numbers
Ostrich 587	247
Ostrich 838	1,159
Ostrich 599	8,563
Ostrich 600	17,928
Ostrich 598	63,881

Table 2. Sequences per sample.

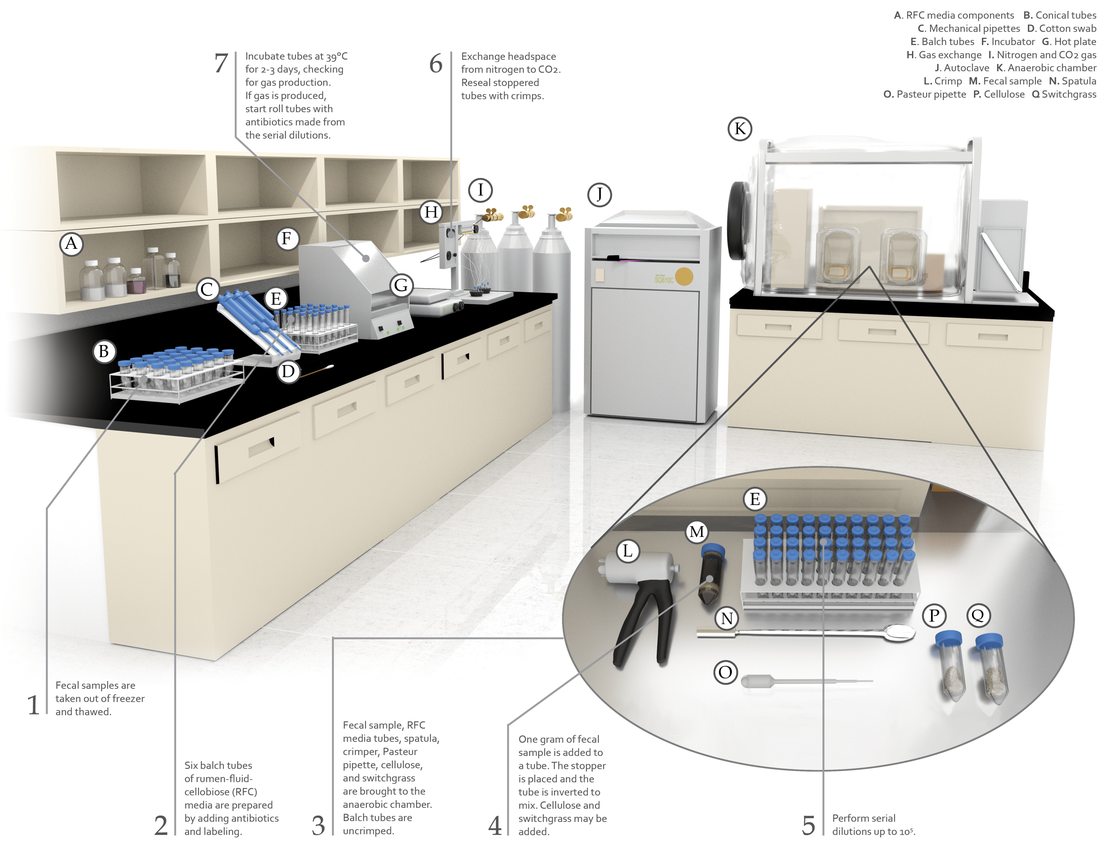
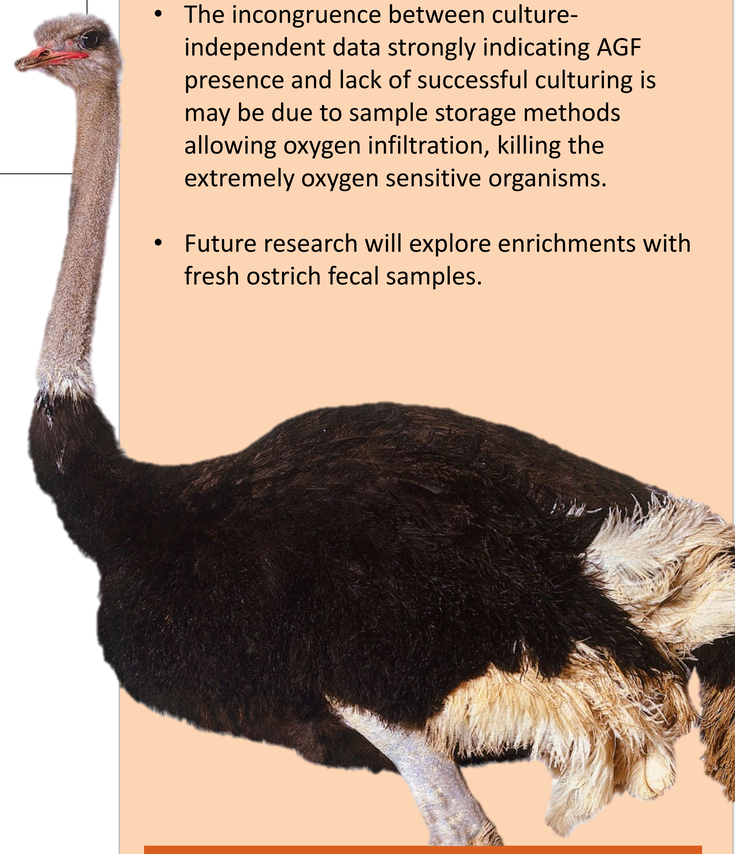


Figure 2. Visual procedure of the enrichment process

CONCLUSION

- Despite altering the enrichment process with variable temperature, substrates, and antibiotic cocktails, none of the ostrich samples yielded any growth.
- The control cow samples resulted in the isolation of seven isolates (Figure 1). These isolates were identified via the DNA extraction and PCR amplification of the D1/D2 LSU region of the 28S ribosomal subunit. Five of the isolates were identified as *Anaeromyces mucronatus*, and two were identified as *Orpinomyces joyonii* (Figure 3).
- Culture-independent surveys of AGF diversity using the D1/D2 LSU region reveal that AGF are present within ostrich samples (Table 2).
- The incongruence between culture-independent data strongly indicating AGF presence and lack of successful culturing is may be due to sample storage methods allowing oxygen infiltration, killing the extremely oxygen sensitive organisms.
- Future research will explore enrichments with fresh ostrich fecal samples.



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