Culturing novel anaerobic gut fungi from marsupials

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Introduction

- Anaerobic gut fungi (AGF) are an essential part of the microbiome in herbivores that aid in the digestion of plant biomass.
- Prior studies have suggested AGF occurrence in the marsupial gut, based on microscopic observation and sequence-based detection[1-4].
- Culturing AGF from marsupials will allow for deeper perspectives on the diversity and biology of anaerobic gut fungi and enable their applications in veterinary medicine, biofuels, and biomedical engineering.

Methods

- We enriched fecal samples from three types of marsupials, kangaroos, koalas, and wallabies obtained from Australian natural reserves and the Oklahoma City Zoo. Cow fecal samples were used as a positive control for the enrichment process
- Multiple strategies were employed to enrich for AGF (Figure 2A, Table 1).
- Fecal samples selected for enrichment were chosen based on results from a separate study that examining the occurrence and relative abundance of AGF in marsupial fecal samples. Samples with highest perceived AGF abundance (based on number of sequences obtained) were selected. (Table 2).

Substrates	Antibiotics	
	Standard Cocktail	Super Cocktail
Cellulose Switchgrass Starch	Penicillin G Streptomycin Chloramphenicol	Penicillin G Streptomycin Chloramphenicol Kanamycin Norfloxacin

Table 1. Substrates and antibiotic cocktails used.

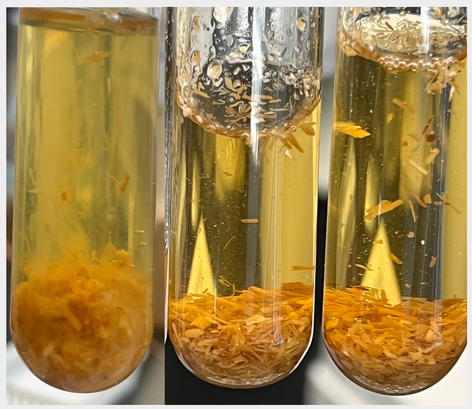


Figure 1. Switchgrass enrichments from wallaby (left) and koala (middle, right) fecal samples.

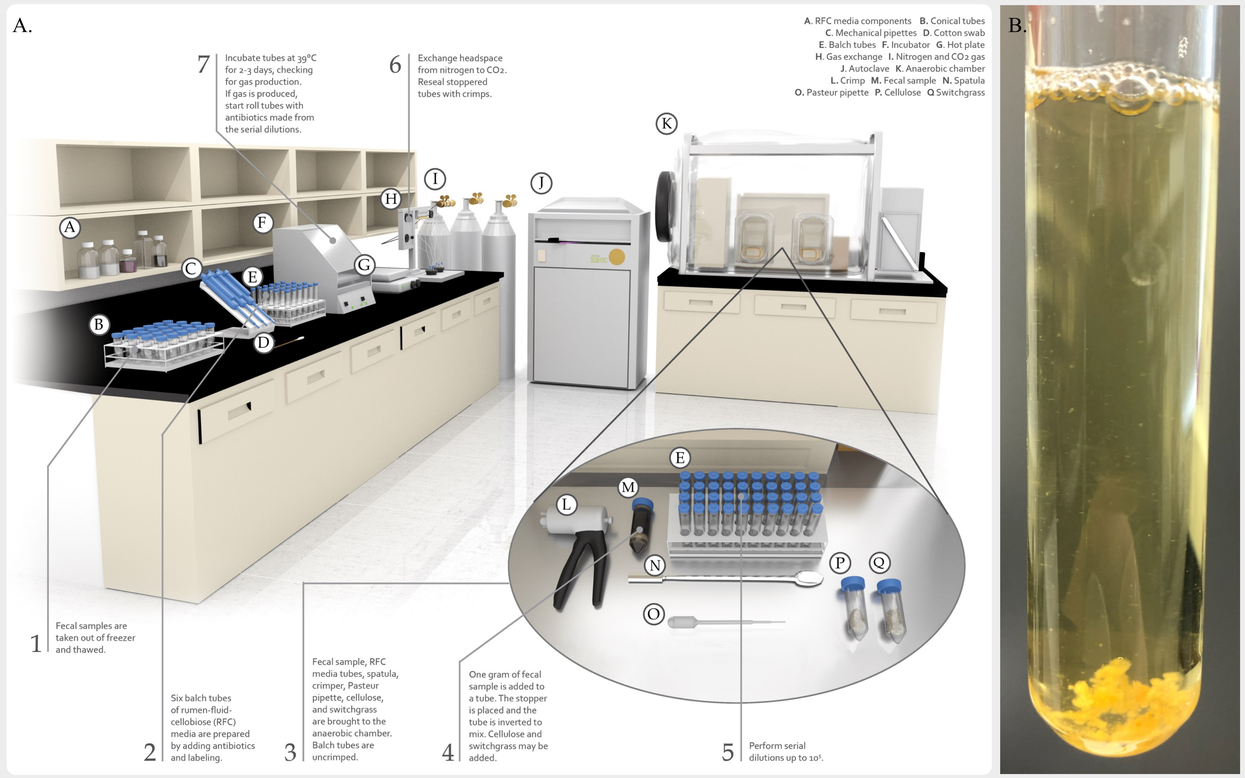


Figure 2. A. Visual rendering of the enrichment process. B. Visible fungal biomass in the successful koala enrichment.

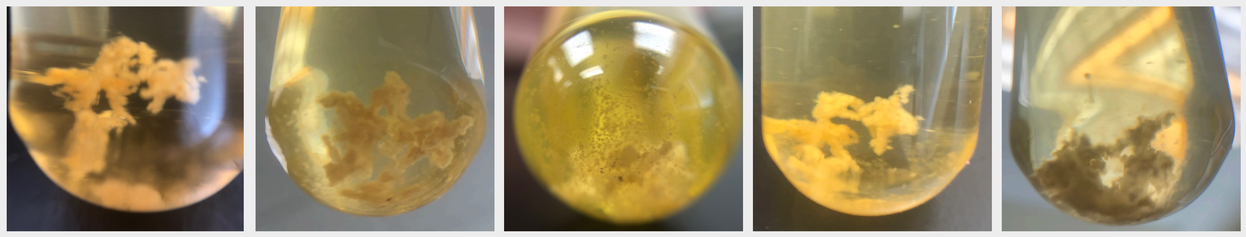
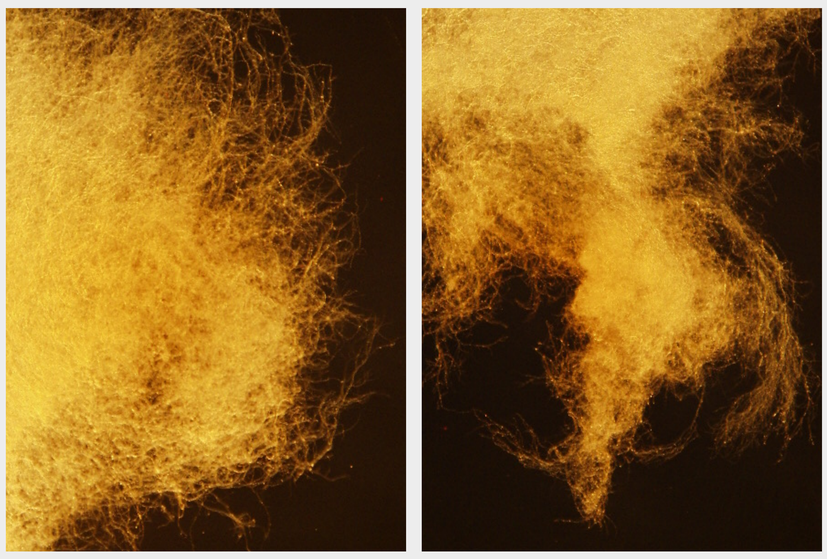


Figure 3. Images of the visible fungal biomass in the successful koala enrichment.

Sample	Illumina Sequences
Koala_43	4938
Kangaroo_83	5192
Koala_33	6538
Kangaroo_82	6609
Kangaroo_G30	6741
Koala_146	7291
Koala_39	8465
Koala_74	12046
Kangaroo_55	25552
Kangaroo_1035	-
Kangaroo_1036	-
Wallaby_G31	-

Table 2. Select marsupial samples and number of sequences retrieved. Darker rows indicate those that have undergone enrichment attempts. Figure 4. Microscopy images of *Orpinomyces joyonii* isolate.



Results

- While some enrichments produced bubbles and floating plant biomass (Figure 1) after 24 hours, only one koala enrichment produced visible fungal biomass (Figure 2B and 3).
- Further research will attempt to isolate and identify the fungus.
- The control cow samples resulted in the isolation of four isolates. Three of the isolates were identified as *Anaeromyces mucronatus*, and the fourth was identified as *Orpinomyces joyonii*.

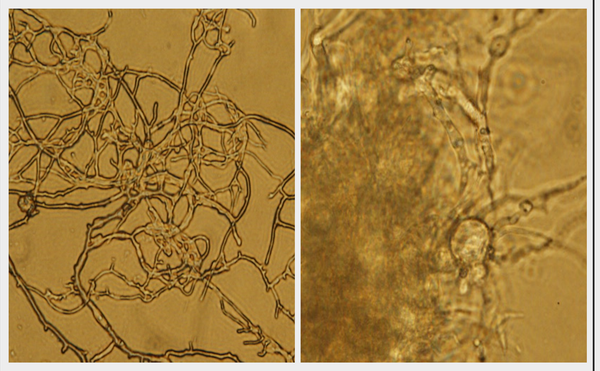


Figure 5. Microscopy images of *Anaeromyces mucronatus*.

Conclusions

- Preliminary attempts to culture AGF using marsupial samples have shown minor success. Although many enrichments appeared to show growth in the first days, on all but one occasion it has failed to persist.
- The incongruence between culture-independent data strongly indicating AGF presence and lack of successful culturing is likely due to age and storage of the fecal samples. The average age of the marsupial samples is about 1.3 years. In this time, oxygen may have infiltrated the tubes used for storage and killed the extremely oxygen sensitive microbes.
- Future research will utilize fresh fecal samples with limited oxygen exposure. This may better enable the survival of AGF within the sample and provide viable cells to culture.

References

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