Continued isolation of anaerobic gut fungi from marsupial hosts

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Introduction

- Anaerobic gut fungi (AGF) aid plant digestion as part of the herbivore microbiome.
- Prior studies suggest AGF occur in the marsupial gut, based on microscopic observation and sequence-based detection[1-4].
- In prior work we identified cultured strains of *Testudinimyces* and *Khoyollomyces* from koala and kangaroo feces, respectively (Fig. 1-2).
- We are currently attempting to identify a strain from wallaby feces (Fig. 4-5).

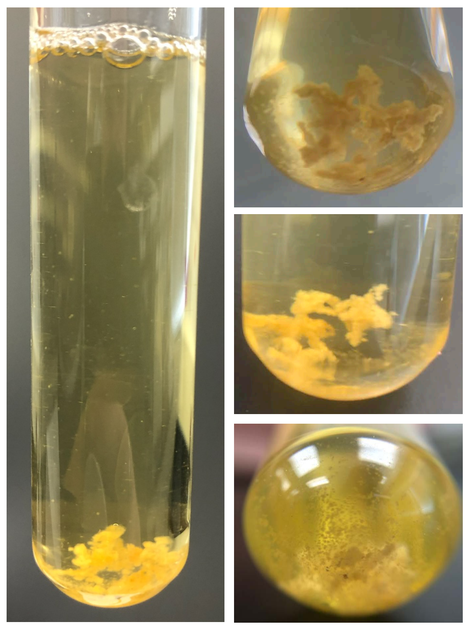


Figure 1: *Testudinimyces* strain from koala feces.

Methods

- We enriched fecal samples from marsupials under anaerobic conditions (Fig. 3)
- Samples were selected based on results from a separate culture-independent study of AGF communities and freshness of samples (Table 1).
- Feces were added to rumen fluid cellobiose media with antibiotics and switchgrass at 39°C (Table 2).
- Some enrichments also utilized cellulose or were incubated at 35°C.

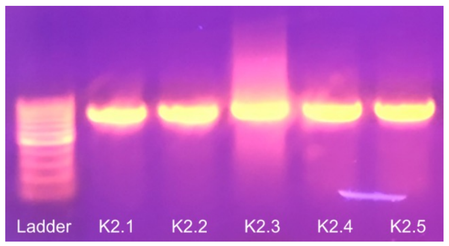
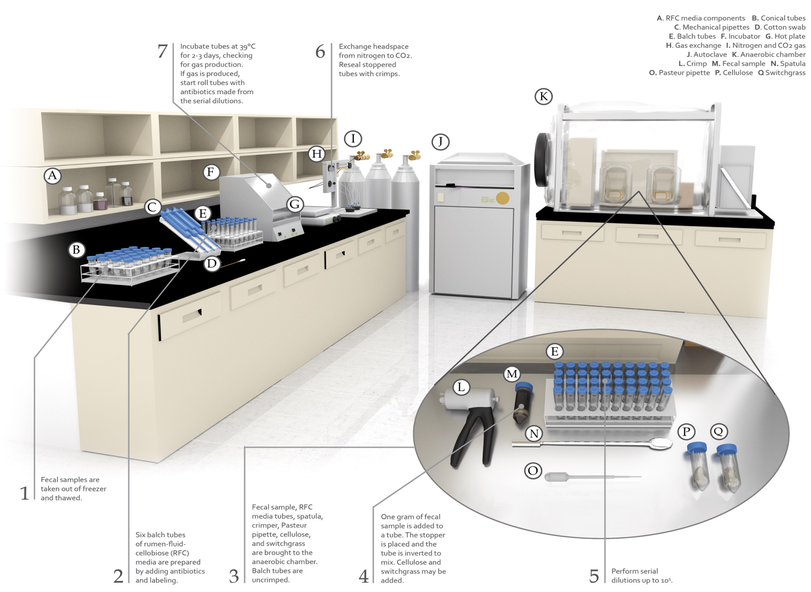


Figure 2: Agarose gel showing successful PCR products of five strains of *Khoyollomyces* from kangaroo feces.



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

Figure 3: Visual rendering of the enrichment process. Table 1. Select marsupial samples and number of sequences retrieved. Darker rows indicate those that have undergone enrichment attempts.

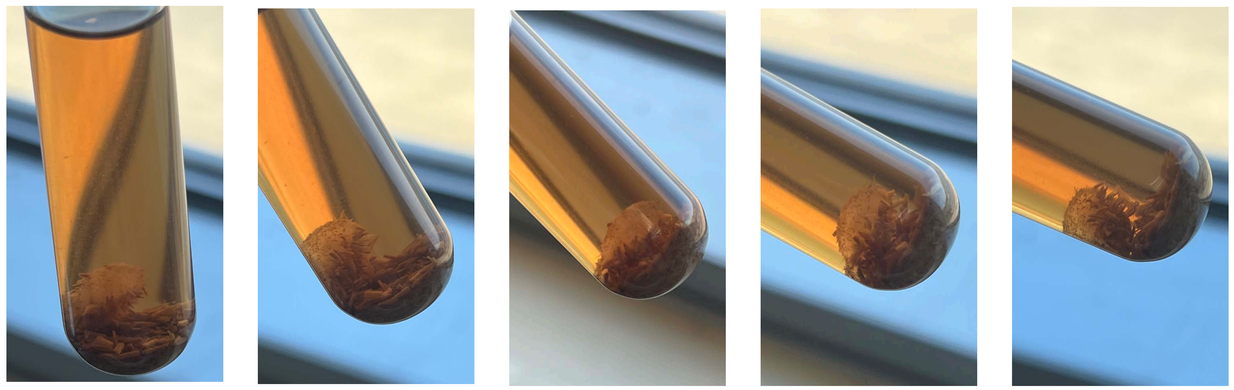


Figure 4: Visible biomass of wallaby enrichment in switchgrass.

Attempts	Sample Name	Species	+ Cell.	+ 35°C
1	Kangaroo-AUS9	eastern grey kangaroo		
1	Kangaroo-US-OK1	red kangaroo		
1	Kangaroo-US-OK4	red kangaroo		x
3	Kangaroo-US-OK7	red kangaroo	x	x
3	Kangaroo-US-OK8	red kangaroo		x
1	Koala-AUS30	koala		
2	Koala-AUS40	koala	x	
2	Koala-AUS80	koala		
1	Wallaby-US-OK16	wallaby		x
4	Wallaby-US-OK17	wallaby	x	x
2	Wallaby-US-OK3	wallaby		
1	Wallaby-US-OK5	wallaby		

Table 2: Isolation attempts from marsupials since October 2021. 22 total attempts with three successful enrichments. All enrichments were performed with switchgrass and at 39°C. Enrichments performed with cellulose (Cell.) and at 35°C are in the last columns.

Results

- While many marsupial enrichments produce bubbles and floating plant biomass after 24 hours, very few produce visible biomass.
- Of 22 attempts to enrich AGF from marsupial feces, only three have been successful (13.63%).
- *Testudinimyces* and *Khoyollomyces* were identified from koala and kangaroo feces, respectively.
- A recent wallaby enrichment produced visible biomass, but we have been unable to successfully isolate DNA or RNA.

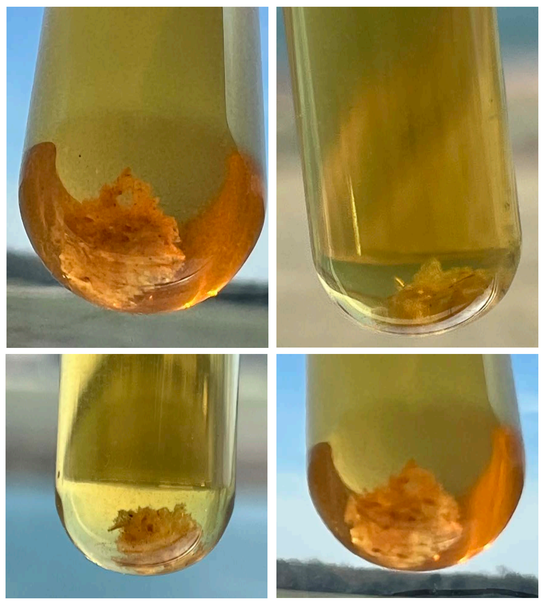


Figure 5: Visible biomass of wallaby enrichment.

Conclusions

- Attempts to culture AGF from marsupial feces have shown minor success. Although many enrichments appear to show growth early on, it typically fails to persist.
- The incongruence between culture-independent data indicating AGF presence and lack of successful culturing is likely due in part to the age and storage of the samples.
- The average age of our marsupial samples is 1.3 years. In this time, oxygen may have infiltrated and killed the extremely oxygen sensitive microbes.
- Future research will continue our attempts to isolate AGF from marsupials.

References

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