RESEARCH

De Silva, G.L.S.N.; Vinzelj, J.; Miller, S.L.; Jemmett, A.M.; Elshahed, M.S.; Youssef, N.H. **Diversity and community structure of anaerobic gut fungi in camel feces.** *Fungal Biology* 2026, *130*, 101872, doi:10.1016/j.funbio.2026.101872.

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Abstract: Anaerobic gut fungi (AGF) are key members of the herbivorous gut microbiome. While AGF communities have been well-studied in foregut and hindgut fermenters, they remain poorly characterized in pseudoruminants such as camels. Here, we present a comprehensive culture-independent diversity survey of 142 fecal samples from all three extant camel species (*Camelus dromedarius, Camelus bactrianus*, and *Camelus ferus*). The AGF community in *Camelus* was highly diverse, with representatives of 42 AGF genus-level lineages identified. However, this diversity was unevenly distributed, with three AGF genera (*Neocallimastix, Caecomyces,* and *Orpinomyces*) accounting for 70.7% of sequences encountered, and only 12 AGF genus-level lineages exceeding 1% relative abundance in the entire dataset. While several of the AGF genus-level lineages identified as major components of the AGF community in camels are highly ubiquitous in all herbivores, others, including the genera *Oontomyces, Aestipascuomyces, Liebetanzomyces*, as well as the yet-uncultured genera NY09, NY03, and JV-2025d identified through culture-independent surveys are extremely rare in ruminants and hindgut fermenters, hinting at their preference and potential co-evolution with the Camelidae. Ordination approaches identified host species and biogeography as key determinants driving AGF community structure differences between various camel species. Comparative community structure analysis between AGF community in camels versus reference foregut and hindgut fermenters identified the relative enrichment of the genera *Oontomyces* and *Aestipascuomyces* in pseudoruminants datasets. Our results demonstrate a distinct AGF community composition in Camelidae, elucidate factors impacting AGF diversity and community structure variations in *Camelus*, and identify key distinct taxa differentially enriched in pseudoruminants compared to ruminants and hindgut fermenters. The ecological and evolutionary drivers of such patterns are discussed.

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Schulz, K.E.; Pfau, A.; Tarhan, T.; van Almsick, C.; Scholz, D.; Sikirić, A.R.; Neumann, A.; Ochsenreither, K. **Beyond carbon dioxide: decoupling headspace and buffering reveals a bicarbonate requirement for anaerobic gut fungi growth.** *Fungal Biology and Biotechnology* 2026, *13*, 18, doi:10.1186/s40694-026-00224-0.

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Abstract: **Background:** Anaerobic gut fungi (*Neocallimastigomycota*) are major lignocellulose degraders in herbivore gastrointestinal tracts, but reproducible axenic cultivation remains difficult because conventional serum-bottle systems intrinsically couple CO_2 headspace, bicarbonate buffering, dissolved inorganic carbon availability, and pH control. This study aimed to decouple these variables and determine whether bicarbonate is required beyond its buffering function. **Results:** Comparison of CO_2- and N_2-based headspaces in otherwise identical bicarbonate-buffered cultures showed consistently higher hydrogen production under N_2. Screening of chemically defined non-carbonate buffers demonstrated that stable external pH alone was insufficient to sustain activity, as bicarbonate-free cultures lost metabolic performance after serial transfer. Reintroduction of sodium hydrogen carbonate under N_2 restored activity in a strain-dependent manner, revealing low bicarbonate thresholds that supported reproducible growth-associated metabolism in filamentous taxa. To test whether this effect reflected a general carboxylate requirement, twenty structurally diverse carboxylic acids were screened using *Aestipascuomyces dupliciliberans*; none restored the bicarbonate-supported hydrogen production phenotype, although maleic acid increased soluble metabolite accumulation. A dual-buffer strategy combining Bis-Tris with minimal bicarbonate enabled independent pH control and identified maximal metabolic activity at pH 6.4. In addition, an OD_{750}-based biomass assay calibrated with activated carbon was established and validated in an ammonium sulfate perturbation experiment, providing a practical framework for biomass estimation and future analyses of nitrogen effects. **Conclusions:** Headspace composition and inorganic carbon availability are key determinants of anaerobic gut fungal cultivation. The results indicate that bicarbonate supports sustained anaerobic gut fungal activity beyond extracellular pH stabilization and cannot be functionally replaced by the tested organic

carboxylates. Decoupling gas phase, buffering chemistry, and pH control provides a more interpretable and reproducible cultivation framework for physiological studies and biotechnological application of anaerobic gut fungi.

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Kim, B.-C.R.; Blair, E.M.; Howard, J.P.; Gois, I.M.; Flick, R.; Lankiewicz, T.S.; Mondo, S.; Pangilinan, J.; Lipzen, A.; Guo, J.; et al. **Engineering anaerobic fungal-bacterial consortia for direct conversion of lignocellulosic biomass into medium-chain fatty acids.** *Trends in Biotechnology* 2026, doi:10.1016/j.tibtech.2026.09.013.

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Abstract: Lignocellulosic biomass is a renewable feedstock for sustainable fuels and chemicals, yet industrial conversion remains constrained by carbohydrate solubilization. Inspired by herbivore rumen microbiomes, we engineered an anaerobic fungal–bacterial consortium thatconverts native lignocellulose into medium-chain fatty acids (MCFAs) without pretreatment. Systematic screening identified the anaerobic fungus isolated here, *Neocallimastix* sp. FC1, together*with Megasphaerahexanoica,* as a top-performing consortium, achieving a lignocellulose-to-MCFA yield of 21.0% (carbon-to-carbon basis) through tight lactate cross-feeding without competition for soluble sugars. Fungal lactate production limited the growth of *M. hexanoica* in co-culture, and the bacterium reallocated protein from growth toward chain elongation, resulting in increased MCFA production. These findings identify fungal lactate production as the primary biological constraint, and balancing lactate production and consumption as a key engineering strategy for improving lignocellulose-to-MCFA conversion. Techno-economic analysis identified high cultivation medium costs as the primary economic constraint and established quantitative cost–yield targets for profitable MCFA production.