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Vinzelj, J.; Nash, K.; Jones, A.L.; Young, R.T.; Meili, C.H.; Pratt, C.J.; Wang, Y.; Elshahed, M.S.; Youssef, N.H. Anaerobic gut fungal community in ostriches (*Struthio camelus*). *ISME Communications* 2025, 5, doi:10.1093/ismeco/ycaf144.

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Abstract: Anaerobic gut fungi (AGF; Neocallimastigomycota) are crucial for the degradation of plant biomass in herbivores. While extensively studied in mammals, information regarding their occurrence, diversity, and community structure in nonmammalian hosts remains sparse. Here, we report on the AGF community in fecal samples of 13 domesticated ostriches. The ostrich (Struthio camelus) is an herbivorous, flightless, hindgut-fermenting member of the class Aves (birds). Illumina-based metabarcoding targeting the D2 region of the large ribosomal subunit (28S rRNA) revealed a uniform AGF community with low alpha diversity in the fecal samples. The community was mostly comprised of sequences potentially representing two novel species in the genus *Piromyces*, and a novel genus in the *Neocallimastigomycota*. Sequences affiliated with these novel taxa were absent or extremely rare in datasets derived from mammalian and tortoise samples, indicating a strong pattern of AGF-host association. One *Piromyces* strain (strain Ost1) was successfully isolated. Transcriptomics-enabled molecular dating analysis suggested a divergence time of \approx 30Mya, a time frame in line with current estimates for ostrich evolution. Comparative gene content analysis between strain Ost1 and other Piromyces species from mammalian sources revealed a high degree of similarity. Our findings expand the range of AGF animal hosts to include members of the birds (class Aves), highlight a unique AGF community in the ostrich alimentary tract, and document the occurrence of a strong pattern of fungal-host association in ostriches, similar to previously observed patterns in AGF canonical mammalian hosts.

Schulz, K.E.; Scholz, D.; Sikirić, A.R.; Rambow, D.; Neumann, A.; Ochsenreither, K. Anaerobic gut fungi as biocatalysts: metabolic and physiological analysis of anaerobic gut fungi under diverse cultivation conditions. *Frontiers in Microbiology* 2025, *Volume 16 - 2025*, doi:10.3389/fmicb.2025.1662047.

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https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2025.1662047/full

Abstract: [Background] Anaerobic gut fungi, known for their diverse carbohydrate-active enzymes and hydrogen production, have promising potential for the valorization of lignocellulosic materials. Despite being classified nearly 50 years ago and re-categorized into the phylum Neocallimastigomycota in 2007, their growth conditions and metabolism remain largely underexplored. This study investigates the metabolic responses of Aestipascuomyces dupliciliberans, Caecomyces churrovis, Khyollomyces ramosus, Orpinomyces joyonii, Pecoramyces ruminantium, and Neocallimastix cameroonii under various conditions, including different growth temperatures, wheat straw particle sizes, alternative carbon sources, and cultivation methods. [Results] Strain-specific differences were observed in temperature tolerance and metabolite production. Optimal growth occurred at 39 °C, while hydrogen production peaked at 41 °C in N. cameroonii, P. ruminantium, and C. churrovis. Larger wheat straw particles (2–3 mm) partially enhanced hydrogen yields, and soluble carbon sources such as glucose and cellobiose were efficiently metabolized, whereas xylose led to stress responses and low hydrogen output, particularly in K. ramosus and O. joyonii. High sugar concentrations triggered overflow metabolism, with increased lactate and formate production in A. dupliciliberans and N. cameroonii, while K. ramosus, lacking lactate dehydrogenase, accumulated formate and succinate. Fed-batch cultivation did not improve yields, likely due to substrate overfeeding and end-product inhibition. Biowaste substrates such as cucumber, carrot, and potato peels were effectively degraded and supported fungal growth. Notably, a novel morphological growth form was observed in O. joyonii under starvation conditions, suggesting a stress-induced developmental transition. [Conclusion] This study provides valuable insights into the growth and physiology of anaerobic gut fungi and complements existing genomic data. The robustness of the process with respect to temperature, carbon source and substrate properties was evaluated, improving the understanding of anaerobic gut fungi cultivation and handling.