

RESEARCH

Butkovich, L.V.; Swift, C.L.; Clendinen, C.S.; Olson, H.M.; Purvine, S.O.; Vining, O.B.; O'Malley, M.A. **Untargeted GC-MS Metabolic Profiling of Anaerobic Gut Fungi Reveals Putative Terpenoids and Strain-Specific Metabolites.** *Metabolites* 2025, 15, 578, doi:10.3390/metabo15090578.

Full text access:

<https://www.mdpi.com/2218-1989/15/9/578>

Abstract: **Background/Objectives:** Anaerobic gut fungi (Neocallimastigomycota) are biotechnologically relevant, lignocellulose-degrading microbes with under-explored biosynthetic potential for secondary metabolites. Untargeted metabolomic profiling with gas chromatography–mass spectrometry (GC-MS) was applied to two gut fungal strains, *Anaeromyces robustus* and *Caecomyces churrovis*, to establish a foundational metabolomic dataset to identify metabolites and provide insights into gut fungal metabolic capabilities. **Methods:** Gut fungi were cultured anaerobically in rumen-fluid-based media with a soluble substrate (cellobiose), and metabolites were extracted using the Metabolite, Protein, and Lipid Extraction (MPLEx) method, enabling metabolomic and proteomic analysis from the same cell samples. Samples were derivatized and analyzed via GC-MS, followed by compound identification by spectral matching to reference databases, molecular networking, and statistical analyses. **Results:** Distinct metabolites were identified between *A. robustus* and *C. churrovis*, including 2,3-dihydroxyisovaleric acid produced by *A. robustus* and maltotriitol, maltotriose, and melibiose produced by *C. churrovis*. *C. churrovis* may polymerize maltotriose to form an extracellular polysaccharide, like pullulan. GC-MS profiling potentially captured sufficiently volatile products of proteomically detected, putative non-ribosomal peptide synthetases and polyketide synthases of *A. robustus* and *C. churrovis*. The triterpene squalene and triterpenoid tetrahymanol were putatively identified in *A. robustus* and *C. churrovis*. Their conserved, predicted biosynthetic genes—squalene synthase and squalene tetrahymanol cyclase—were identified in *A. robustus*, *C. churrovis*, and other anaerobic gut fungal genera. **Conclusions:** This study provides a foundational, untargeted metabolomic dataset to unmask gut fungal metabolic pathways and biosynthetic potential and to prioritize future efforts for compound isolation and identification.

Kovács, E.; Szűcs, C.; Juhász-Erdélyi, A.; Bagi, Z.; Kovács, K.L. **Anaerobic fungi - effective warriors in lignocellulosic biomass degradation and fermentation.** *FEMS Microbiology Ecology* 2025, 10.1093/femsec/fiaf108, doi:10.1093/femsec/fiaf108.

Full text access:

<https://academic.oup.com/femsec/advance-article/doi/10.1093/femsec/fiaf108/8300365>

Abstract: The significant advancements in understanding the roles of anaerobic fungi within microbial ecology have opened numerous avenues for biotechnological exploitation, particularly in enhancing the productivity of livestock. The efficient, unique, and complex enzyme systems of anaerobic fungi play a determining role in the metabolic conversion of lignocellulosic plant matter into animal products such as milk and meat by mammalian herbivores. Mitigation of methane emissions through microbial or dietary strategies in ruminants is a major environmental climate change issue. In turn, controlled management of the inter-kingdom syntrophic interactions among the eukaryotic anaerobic fungi, prokaryotic bacteria and archaea can lead to the production of valuable biofuels, (biomethane, biohydrogen, bioethanol), and organic acids. These products can also serve as building blocks in numerous processes to generate high value chemicals in circular bioeconomy.