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Wunderlich, G.; Bull, M.; McGilchrist, N.; Zhao, C.; Ross, T.; Rose, M.; Chapman, B. **The horse gut bacteriome and anaerobic mycobiome are influenced by seasonal forages and small intestinal starch digestibility**. *Journal of Applied Microbiology* 2025, *136*, doi:10.1093/jambio/lxaf203.

Full text access:
https://academic.oup.com/jambio/article/136/9/lxaf203/8233671

Abstract: **[Aims]** The equine gut microbiome is a complex and dynamic ecosystem, housing a diverse consortium of bacteria and anaerobic fungi (AF) capable of breaking down complex plant matter and converting it into vital energy sources for their host. The aim of this study was to broaden our current understanding of bacterial and AF diversity in the equine hindgut and how it differs between cohorts and responds to dietary shifts. **[Methods and results]** Faecal samples were collected from 48 horses and the bacteriome and anaerobic mycobiome analysed using long-read amplicon sequencing. Samples were collected from racehorses (RH) on high-concentrate diets and wild-foraging (WF) horses grazing on seasonally variable pastures. Distinct microbial profiles were observed, with bacterial diversity and composition strongly influenced by season. Reduced hindgut starch availability in RH promoted fibrolytic taxa such as *Roseburia* and reduced amylolytic *Lactobacillus*. AF communities, though relatively stable across dietary and seasonal changes, differed between the horse cohorts. *Piromyces* and *Khoyollomyces* were found to be more strongly associated with WF horses and *Caecomyces* with RH. **[Conclusion]** These findings emphasize the dynamic interplay between diet and microbial composition. Future multi-omics research will enhance our understanding of microbial functionality and inform strategies to optimize equine gut health.

Jin, S.; Farrand, I.R.; Chen, Y.; Gin, J.W.; Zhang, B.; Kirschke, E.; Petzold, C.J.; Adams, P.D.; O'Malley, M.A. **A large-scale screening campaign of putative carbohydrate-active enzymes reveals a novel xylanase from anaerobic gut fungi**. *mBio* 2025, e01007-01025, doi:10.1128/mbio.01007-25.

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https://journals.asm.org/doi/10.1128/mbio.01007-25

Abstract: The genomes of anaerobic gut fungi (AGF) encode a diverse array of carbohydrate-active enzymes (CAZymes), yet exceedingly few of these enzymes have been experimentally validated or expressed in heterologous systems. Here, we developed a predictive bioinformatic pipeline to annotate novel putative CAZymes from anaerobic fungi and validate their activity through large-scale heterologous expression in *Escherichia coli*. A total of 173 fungal proteins from *Piromyces finnis* associated with biomass degradation were synthesized and expressed in *E. coli*, and 9.8% were soluble with expression levels exceeding 5% of the total proteome using high-throughput proteomic screening. Among these 17 heterologously expressed proteins, analysis with AlphaFold and FoldSeek predicted 13 multi-functional proteins containing catalytic domains fused with repetitive fungal dockerins, and half of the substrate predictions were experimentally validated. One promising enzyme, celsome_012, exhibited robust and specific activity against beechwood xylan at 37°C and pH 6.4, with titers that were also fivefold higher than those of other recombinant proteins screened here. Both Michaelis-Menten kinetics and the linearized Lineweaver-Burk equation yielded consistent values for K_m, and its activation energy was estimated at 51.9 kJ/mol based on the Arrhenius model. This work supports the industrial translation of anaerobic fungal CAZymes due to their robust lignocellulolytic activity and provides a framework for prioritizing AGF proteins for efficient *E. coli* heterologous expression.

Yazdic, F.C.; Akyol, I.; Ozkose, E. **Production and partial characterization of bifunctional cellulase genes of anaerobic gut fungus *Orpinomyces* sp. GMLF19**. *Applied Biochemistry and Microbiology* 2025, 10.1134/S0003683824605389, doi:10.1134/S0003683824605389.

Full text access:
https://link.springer.com/article/10.1134/S0003683824605389

Abstract: Fiber-degrading properties of anaerobic gut fungus, *Orpinomyces* sp. GMLF19, were investigated when it was grown on various carbon sources, namely carboxymethylcellulose, barley, oat, corncob and wheat straw. Fungi were developed under anaerobic conditions, and carboxymethylcellulase (CMCase), lichenase, and xylanase activities were determined by using DNS method. Meanwhile, the decreasing order of the enzyme activity of *Orpinomyces* sp. GMLF19 was observed to be lichenase > CMCase > xylanase. Relative activities of these enzymes in the isolate GMLF19 were found to be the highest when the culture was grown for 5 days. Cellulase gene domain was reproduced from chromosomal DNA and transferred into *Escherichia coli* with the aid of pCT plasmid vector and CMCase, lichenase and xylanase activities were determined using DNS method. The gene showed relatively higher CMCase activity and moderate lichenase activity while no xylanase activity was observed suggesting that the gene is bifunctional. The cell-associated activity of overexpressed Cel19 enzyme (*E. coli* cells containing this transferred gene were designated as C19) was relatively higher than extracellular activity. It was also recorded that this gene has 2 dockerin domains and 1 catalytic domain with the length of 1.585 bp, and it coded 50.59 kDa size of the protein which belongs to glycosyl hydrolase family 6.

Lankiewicz, T.S.; Amer, B.; Baidoo, E.E.K.; Leggieri, P.A.; O'Malley, M.A. **The anaerobic fungus *Neocallimastix californiae* shifts metabolism and produces melanin in response to lignin-derived aromatic compounds**. *Biotechnology for Biofuels and Bioproducts* 2025, *18*, 96, doi:10.1186/s13068-025-02696-5.

Full text access:

https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-025-02696-5

Abstract: **[Background]** Biological deconstruction of lignocellulose for sustainable chemical production offers an opportunity to harness evolutionarily specialized enzymes and organisms for industrial bioprocessing. While hydrolysis of cellulose and hemicellulose by CAZymes yields fermentable sugars, ligninolysis releases a heterogeneous mix of aromatic compounds that likely play a crucial role in shaping microbial communities and microbial metabolism. Here, we interrogated the metabolomic and transcriptomic response of a lignocellulolytic anaerobic fungus, *Neocallimastix californiae*, to a heterogeneous mixture of aromatic compounds derived from lignin. **[Results]** Through exposing the fungus to both a concentration it might experience in its native environment and an elevated concentration of alkaline lignin, we observe that *N. californiae* transforms vanillin and that supplying alkaline lignin at 0.125 g/L, alongside cellulose, enhances the growth and polysaccharide-degrading activity of *N. californiae*. Altogether, our results further suggest that vanillin consumption, increased polymer-degrading activity, increased metabolic activity, and transcriptomic remodeling of amino acid synthesis genes all coincide with increased melanin production by fungal cells. These observations challenge previous notions that aromatics from lignocellulose only inhibit the growth and polymer deconstruction capabilities of the biomass-degrading anaerobic fungi (Neocallimastigomycetes). **[Conclusions]** This study demonstrates that anaerobic fungi have a complex relationship with aromatic chemicals derived from lignin and hemicellulose and shift their metabolism in response to the addition of lignocellulose-derived aromatics to their growth medium. Further, as no known pathways for the biochemical transformation of aromatics were detected in these organisms despite observed transcriptome remodeling in the presence of aromatics, we suggest they might encode novel biochemical routes for scavenging amino acid building blocks from aromatic monomers derived from hemicellulose side chains and lignin.