# RESEARCH

Blair, E.M.; Navaratna, T.A.; Ahern, C.B.; Ragunathan, R.; Brown, J.L.; Mondo, S.J.; Lipzen, A.; Hanafy, R.A.; LaButti, K.; Talag, J.; et al. Genomic and transcriptomic characterization of Carbohydrate-Active Enzymes in the anaerobic fungus *Neocallimastix cameroonii* var. constans. *G3* Genes|Genomes|Genetics 2025, doi:10.1093/g3journal/jkaf137.

### Full text access:

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Abstract: Anaerobic gut fungi effectively degrade lignocellulose in the guts of large herbivores, but there remains a limited number of isolated, publicly available, and sequenced strains that impede our understanding of the role of anaerobic fungi within microbial communities. We isolated and characterized a new fungal isolate, Neocallimastix cameroonii var. constans, providing a transcriptomic and genomic understanding of its ability to degrade diverse carbohydrates. This anaerobic fungal strain was stably cultivated for multiple years *in vitro* among members of an initial enrichment microbial community derived from goat feces, and it demonstrated the ability to pair with other microbial members, namely archaeal methanogens to produce methane from lignocellulose. Genomic analysis revealed a higher number of predicted carbohydrate-active enzymes encoded in the N. cameroonii var. constans genome compared to most other sequenced anaerobic fungi. The carbohydrate-active enzyme profile for this isolate contained 660 glycoside hydrolases, 160 carbohydrate esterases, 194 glycosyltransferases, and 85 polysaccharide lyases. Differential gene expression analysis showed the upregulation of thousands of genes (including predicted carbohydrate-active enzymes) when N. cameroonii var. constans was grown on lignocellulose (reed canary grass) compared to less complex substrates, such as cellulose (filter paper), cellobiose, and glucose. AlphaFold was used to predict functions of transcriptionally active yet poorly annotated genes, revealing feruloyl esterases that likely play an important role in lignocellulose degradation by anaerobic fungi. The combination of this strain's genomic and transcriptomic characterization, omics-informed structural prediction, and robustness in microbial co-culture make it a wellsuited platform to conduct future investigations into bioprocessing and enzyme discovery.

Wang, J.; Liu, J.; Guo, T.; Zheng, C.; Wang, F.; Liu, T.; Yuan, C.; Lu, Z. Multi-omics insights into key microorganisms and metabolites in Tibetan sheep's high-altitude adaptation. *Frontiers in Microbiology* 2025, *Volume* 16 - 2025, doi:10.3389/fmicb.2025.1616555.

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Abstract: Tibetan sheep gastrointestinal microbial communities and metabolites showed adaptive differences with altitude, but we do not know which flora or metabolites may play an important role in acclimatization to the altitude environment. Therefore, we systematically analyzed the microbial structure and metabolites in the rumen and feces of Tibetan sheep at two altitudes (4,424 m and 2,364 m) using amplicon sequencing and untargeted metabolomics. The results showed that the bacterial communities differed greatly between the two groups, with high altitude Tibetan sheep having a higher forage fermentation capacity, and the abundance of some bacteria and fungi that were conducive to the decomposition of cellulose in rumen fluid increased significantly (especially Bacteroidota, Neocallimastigomycota, and Ascomycota), and the short chain fatty acids and NH<sub>3</sub>-N produced by metabolism also increased. There was also a significant increase in the abundance of Naganishia, which is prone to survive in extreme environments. In addition, the metabolite profiles in the rumen and feces of Tibetan sheep at two altitudes were also significantly different, and further correlation analysis showed that the differential bacteria in the rumen were mainly related to the products related to amino acid metabolism and lipid metabolism, and the differential bacteria in the feces were mainly correlated with some metabolites related to antibacterial, anti-inflammatory, anti-tumor and other disease treatment components. Collectively, these changes in microbiota and metabolites may have facilitated the adaptation of Tibetan sheep to the harsh plateau environment, contributing to their better survival and reproduction. This study provides a basis for research on the mechanisms of adaptation of Tibetan sheep to the plateau environment.

Chaverri, P.; Escudero-Leyva, E.; Mora-Rojas, D.; Calvo-Obando, A.; González, M.; Escalante-Campos, E.; Mesén-Porras, E.; Wicki-Emmenegger, D.; Rojas-Gätjens, D.; Avey-Arroyo, J.; et al. Differential microbial composition and fiber degradation in two sloth species (*Bradypus variegatus* and *Choloepus hoffmanni*). *Current Microbiology* 2025, *82*, 327, doi:10.1007/s00284-025-04303-0.

# Full text access:

# https://doi.org/10.1007/s00284-025-04303-0

Abstract: Sloths have the slowest digestion among mammals, requiring 5-20 times longer to digest food than other herbivores, which suggests differences in their gut in plant-fiber-degrading microbiota. particularly microorganisms. Bradypus variegatus has a lower metabolic rate and moves less than Choloepus hoffmanni. However, no comprehensive studies have compared the microbiota (e.g., fungi) of these species. We hypothesized that differences in digestion and metabolism between the two species would be reflected in their microbiota composition and functionality, which we characterized using metagenomics, metabarcoding, and cellulose degradation. Results revealed significant differences in microbiota composition and functionality. Both species are dominated by bacteria; fungi comprised only 0.06–0.5% of metagenomic reads. Neocallimastigomycota, an anaerobic fungus involved in fiber breakdown in other herbivores, was found in low abundance, especially in *B. variegatus*. Bacterial communities showed subtle differences: C. hoffmanni was dominated by Bacillota and Bacteroidota, while *B. variegatus* showed higher Actinomycetota. Expected herbivore bacterial taxa (e.g., Fibrobacter and Prevotella) were scarce. Functional analysis showed a low abundance of carbohydrate-active enzymes essential for polysaccharide breakdown. Cellulose degradation assays confirmed that sloths digest only  $\sim 3-30\%$  of ingested plant material. This research sheds light on the potential multidirectional links between the gut microbiota, metabolism, and digestion.

Manlapig, J.J.D.; Crisostomo, J.C.A.; Kondo, M.; Ban-Tokuda, T.; Matsui, H. Assessment of rice bran with acrylate or pyruvate in modulating ruminal fermentation and methane production *in vitro*. *Journal of the Science of Food and Agriculture*, doi:10.1002/jsfa.14437.

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Abstract: (BACKGROUND) Livestock production contributes largely to climate change and methane (CH<sub>4</sub>) from enteric fermentation is the primary greenhouse gas emitted from ruminants. Thus, developing effective strategies to reduce the impact of ruminant production on the environment is crucial, and the combination of CH<sub>4</sub> mitigating agents may be a viable strategy to attain this. The present study aimed to evaluate the effect of rice bran alone (control+rice bran, RB) or in combination with acrylate (control+rice bran+acrylate, AA) or pyruvate (control+rice bran+pyruvate, PA) on rumen fermentation parameters, CH<sub>4</sub> production, and microbial populations *in vitro*. (RESULTS) Gas production was highest in control+rice bran+pyruvate (PA) treatments and lowest (P < 0.01) in control+rice bran+acrylate (AA), whereas dry matter digestibility (DMD) was significantly lower (P < 0.05) in AA compared to control (CON) and PA. CH<sub>4</sub> and CH<sub>4</sub>/digested dry matter decreased (P < 0.05) by more than 66% and 63%, respectively, in AA cultures, whereas total organic acid production increased (P < 0.01) compared to CON. Lactate levels were significantly higher (P < 0.05), while acetate, propionate and butyrate were notably lower (P < 0.05) in AA cultures than CON. Microbial analysis showed a significant decrease in methanogen populations in AA than CON (P < 0.01). Compared to CON, anaerobic fungi and Megasphaera elsdenii populations declined across all treatments (P < 0.01), whereas Selenomonas ruminantium populations decreased in RB and AA, and *Prevotella ruminicola* populations were higher in RB and PA (P < 0.05). (CONCLUSION) These findings suggest that incorporating RB with AA could be a viable CH4 inhibitor; however, because of its negative effect on gas production and DMD, further investigations should be done to determine its optimum inclusion level.