***RESEARCH***

Zhang, B.; Hrdy, I.; Tachezy, J.; Gao, Y.; Williams, S.M.; Fulcher, J.M.; Munoz, N.; Burnet, M.; Baker, S.E.; O'Malley, M.A. **The anaerobic fungus *Caecomyces churrovis* produces $H_2$ via a non-bifurcating NADH-dependent enzyme complex.** *mBio 0*, e01643-01626, doi: 10.1128/mbio.01643-26.

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**Abstract:** Hydrogenosomes are mitochondrion-derived organelles that produce ATP and $H_2$ to support energy metabolism in anaerobic eukaryotes. $H_2$ production allows reoxidation of reduced cofactors generated during fermentative metabolism; however, the metabolic mechanisms for $H_2$ production in anaerobic eukaryotes remain incompletely understood. In particular, it remains unclear whether anaerobic fungi (AF) hydrogenosomes use a ferredoxin-dependent pathway or a distinct mechanism to regenerate $NAD(P)^+$ and link electron transfer to $H_2$ formation. Here, by combining genomic search, proteomic analysis, and enzymology, we reveal the molecular mechanism for $H_2$ production in the AF *Caecomyces churrovis*. Our enzyme assays on the organelle fraction of *C. churrovis* revealed the activity of $H_2$:$NAD^+$ oxidoreductase but not pyruvate:ferredoxin oxidoreductase, which is usually linked to $H_2$ formation. We identified genes encoding [FeFe] hydrogenase (Hyd) and NADH dehydrogenase subunits E and F (NuoE and NuoF) in *C. churrovis* and confirmed their expression in the isolated hydrogenosomal fractions by proteomic analysis. Combining the individually purified enzymes, we found Hyd and NuoEF proteins formed $H_2$ directly from NADH independently of ferredoxin, functioning as a non-bifurcating NADH-dependent enzyme rather than an electron-bifurcating enzyme known from anaerobic prokaryotes. We identified homologs of hydrogenosomal NuoE, NuoF, and Hyd in many other AF, indicating this pathway is commonly shared among the AF. This work demonstrates the existence of a non-bifurcating NADH-dependent enzyme complex for $H_2$ production in eukaryotes. Moreover, this complex could potentially be exploited as a target for controlling AF $H_2$ production and altering fungal metabolism.

***RESEARCH***

Young, D.; Stüer-Patowsky, K.; Huang, L.; Elshahed, M.S.; Youssef, N.H.; Hanafy, R.; Cheng, Y.; Moon, C.D.; Soni, P.; Joshi, A.; et al. **Full ribosomal operon sequencing of anaerobic gut fungi (phylum Neocallimastigomycota): insights on its markers and phylogenetic resolution.** *IMA Fungus* 2026, *17*, doi:10.3897/imafungus.17.195921.

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**Abstract:** The phylogenetic affiliations of anaerobic gut fungi (*Neocallimastigomycota*) are typically evaluated using single-gene markers. However, this approach often fails to resolve relationships between closely related lineages. To address this issue and identify alternative markers, we created a curated database comprising the complete ribosomal operon sequences of 156 isolates, representing 20 of the 22 recognized genera and two new genus-level clades. Using long-read sequencing, we obtained ~9 kbp operon sequences and developed a robust analysis pipeline. Incorporating both coding genes and non-coding regions (excluding IGS1) improved phylogenetic resolution. This phylogenetic approach successfully resolved the *Cyllamyce* and *Caecomyces* clades (hard-to-distinguish genetically), as well as seven analysed *Piromyces* species. We also scanned the operon for markers that are suitable for short-read sequencing platforms, with the aim of enhancing biodiversity and phylogenetic studies. Notably, the ETS1 genetic region also enabled the distinction between these lineages, indicating its phylogenetic value within the ribosomal operon. The resulting database is a valuable resource for expanding and strengthening phylogenetic frameworks.