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[P005] Poster

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[P03-311] Adaptive laboratory evolution enables autotrophic growth of *Eubacterium limosum* under defined medium conditions

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Acetogens convert C1 substrates into acetyl-CoA via the Wood-Ljungdahl pathway. However, most acetogens require complex media containing undefined components, such as yeast extract, to support their autotrophic growth. The presence of these undefined components has obscured a precise understanding of their autotrophic metabolism. In this study, to reduce dependence on yeast extract for autotrophic growth, we performed adaptive laboratory evolution of *Eubacterium limosum* ECO2 under syngas conditions by reducing the yeast extract concentration. The resulting evolved strain, designated ECO_X, demonstrated the capability to grow in a yeast extract-free chemically defined medium, achieving complete CO consumption with a growth rate of $0.101 \pm 0.001 \text{ h}^{-1}$. In addition, we found that ECO_X produced less biofilm than ECO2, resulting in a 2-fold increase in transformation efficiency. To elucidate the genetic basis of these phenotypic changes, we identified a causal mutation in the ABC transporter gene via whole-genome resequencing and validated that it enables autotrophic growth in the defined medium. Furthermore, transcriptomic analysis revealed a significant upregulation of the tryptophan biosynthetic pathway in ECO_X, indicating that tryptophan biosynthesis is essential for autotrophic growth in defined conditions. Collectively, these findings provide a foundation for developing chemically defined media and enhance our understanding of acetogen physiology. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through Technology Development Program for CO₂ Mitigation and Conversion to Value-Added Products Using Indigenous Organism, funded by Korea Ministry of Climate, Energy and Environment (MCEE) (RS-2026-25505528).