

Metabolic pathway rerouting in *Paraburkholderia rhizoxinica* evolved long-overlooked derivatives of coenzyme F₄₂₀.

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Abstract

Coenzyme F₄₂₀ is a specialized redox cofactor with a negative redox potential. It supports biochemical processes like methanogenesis, degradation of xenobiotics or the biosynthesis of antibiotics. Although well-studied in methanogenic archaea and actinobacteria, not much is known about F₄₂₀ in Gram-negative bacteria. Genome sequencing revealed F₄₂₀ biosynthetic genes in the Gram-negative, endofungal bacterium *Paraburkholderia rhizoxinica*, a symbiont of phytopathogenic fungi. Fluorescence microscopy, high-resolution LC-MS, and structure elucidation by NMR demonstrated that the encoded pathway is active and yields unexpected derivatives of F₄₂₀ (3PG-F₄₂₀). Further analyses of a biogas-producing microbial community showed that these derivatives are more widespread in nature. Genetic and biochemical studies of their biosynthesis established that a specificity switch in the guanylyltransferase CofC re-

programmed the pathway to start from 3-phospho-d-glycerate, suggesting a rerouting event during the evolution of F₄₂₀ biosynthesis. Furthermore, the cofactor activity of 3PG-F₄₂₀ was validated, thus opening up perspectives for its use in biocatalysis. The 3PG-F₄₂₀ biosynthetic gene cluster is fully functional in Escherichia coli, enabling convenient production of the cofactor by fermentation.

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