Using Phylogenetic and Genomic Characterization to Discover an Enzyme That Produces Selenoneine: A Natural Product Promoting Lifespan and Healthy Aging

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Abstract

Selenoneine, a selenium-containing analog of ergothioneine, is a potent antioxidant with potential therapeutic relevance to healthy aging and mitigating chronic disease. However, its biosynthesis is not well understood beyond the inefficient, multi-enzyme SEN pathway. This study aimed to identify naturally-occurring homologs of EanB—an anaerobic sulfurtransferase—that may serve as a simpler, alternative route to selenoneine. Using the EanB protein from *Chlorobium limicola* as a query, we performed a BLASTp search and filtered the results for organisms native to selenium-rich environments. Phylogenetic and gene neighborhood analyses of 36 candidates led to the prioritization of four promising homologs from *Methanolobus* sp., *Acetohalobium arabaticum*, *Cedecea lapagei*, and *Citrobacter enshiensis*, based on phylogenetic divergence and co-localized selenium-related genes like *selD*. To validate this strategy, the candidate from *Cedecea lapagei* (EanB_Ced) was purified and tested for activity. In an anaerobic, EGTase coupled assay, EanB_Ced catalyzed a rapid, concentration-dependent reaction consistent with the synthesis of selenoneine from hercynine and a selenium source. The estimated catalytic rate was comparable to the original sulfur-incorporating EanB, suggesting this is an evolved, specialized function. These results provide strong experimental evidence for a novel, anaerobic pathway for selenoneine biosynthesis, validating our genomic approach to enzyme discovery