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Identification of the evolutionary origins of the biosynthesis of the antioxidant ergothioneine

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Ergothioneine is a histidine-derived beta-amino acid found in high amounts in mushrooms and fermented foods, which functions as an anti-inflammatory antioxidant intracellularly[(1)]. Biosynthesis of ergothioneine occurs only in specific bacteria and fungi, therefore humans acquire ergothioneine exclusively from the diet. Interestingly, recent data shows that gut pathogens such as Helicobacter pylori compete with host cells for ergothioneine[(2)].

One of three indispensable enzymes responsible for ergothioneine biosynthesis, EgtB, is a metal-dependent sulfoxide synthase[(3)]. In spite of significant interest in the biological roles, bioengineering and the evolution of ergothioneine biosynthetic enzymes, little attention has been given to the specific origins of the key sulfoxide synthase domain involved. Therefore, the aim of this study was to identify the evolutionary origin of sulfoxide synthase domains of EgtB and its homologues in cyanobacteria.

Amino acid sequences of EgtB and its homologoues, FGE (formylglycine-generating enzyme family protein) and OvoA (ovothiol biosynthetic enzyme), from cyanobacteria were identified from NCBI Basic Local Alignment Search Tool (BLAST), using Gloeobacter violaceus (NCBI WP_011141636.1) reference sequence as the query sequence. Default protein-protein BLAST (BLASTp) was used against the non-redundant protein sequence database from cyanobacteria. The returned amino acid sequences (n = 85) were aligned using Multiple Alignment using Fast Fourier Transform (MAFFT) with default parameters. The redundancy (n = 603), gap-inducing sequences (n = 21) and YfmG-containing sequences (n = 273) were removed before alignment. The phylogenetic tree was built using Maximum-Likelihood Phylogenies (PhyML) with approximate likelihood- ratio test with nonparametric Shimodaira–Hasegawa correction (alRT SH-like) and the default Le and Gascuel (LG) model for branch support. The tree was analysed and edited using Treegraph2.

As hypothesised, the FGE domains were found at the root of our phylogenetic tree, as they have related biochemical activity but are not sulfoxide synthetases. Whereas, interestingly, the OvoA domains clearly fall within the EgtB clade, suggesting ergothioneine biosynthesis is the more ancient function of this domain. In addition, the data show two branches of EgtB domain containing sequences, which may be explained by differences in the three dimensional structure of functional form (e.g. EgtB type 1 are monomers while type II are tetrameric[(3)]) or by whether they use cysteine vs gamma-glutamyl cysteine as substrate, which we are currently investigating through residue analysis.

In summary, this phylogenetic analysis identifies the evolutionary origin of sulfoxide synthase domains critical to microbial ergothioneine biosynthesis.

References

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