EXPLORING THE POTENTIAL OF ANCESTRAL PHENYLALANINE/TYROSINE AMMONIA-LYASES FOR THERAPEUTIC APPLICATIONS

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Phenylalanine/tyrosine ammonia-lyases (PAL/TAL) have been approved by the FDA for treatment of phenylketonuria and may also harbor potential for complimentary treatment of hereditary tyrosinemia type II. Herein, we explore ancestral sequence reconstruction as an enzyme engineering tool to increase stability and alter substrate specificity, which could enhance the therapeutic potential of these enzymes. We used MEGA and PAML to reconstruct putative ancestors of PAL/TAL from fungi and compared them to two modern enzymes that have a relatively low PAL/TAL activity ratio. The majority of ancestors could be functionally expressed in *E. coli* and showed activity towards both phenylalanine and tyrosine. All ancestral enzymes displayed increased thermostability compared to both modern enzymes, however, the increase in thermostability was accompanied by a loss in activity when going back in the phylogenetic tree. One reconstructed ancestral enzyme could be interesting for further development, as its catalytic turnover of tyrosine is slightly higher than one of the modern enzymes and it is significantly more thermostable than both modern enzymes. More detailed characterization of the ancestral variants with a focus on stability is currently ongoing. Our results indicate that ancestral sequence reconstruction programs are robust in terms of stability, whereas activity of ancestral variants seems to vary depending on the reconstruction method. We believe that this approach has great potential for enhancing the properties of therapeutic enzymes and biocatalysts for various applications.