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Baeyer-Villiger Monooxygenases from *Yarrowia lipolytica* Catalyze Preferentially Sulfoxidations

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Highlights:

- - Nine new eukaryotic BVMOs (YMOA-I) from *Yarrowia lipolytica* identified
- - YMOA did not convert ketones, but DMSO and sulfides with high sulfone yields
- - A mutational study identified residues influencing sulfoxidation activity
- - YMOA together with four other YMOs might belong to a new class of BVMOs

Abstract:

Nine new putative Baeyer-Villiger monooxygenase encoding genes were identified in the eukaryote *Yarrowia lipolytica* and eight were subsequently cloned and expressed. Eight of these enzymes, *Yarrowia* monooxygenases A-H (YMOA-H), were used in biocatalysis reactions with ketones, sulfides and sulfoxides as substrates. YMOB converts ketones and sulfides, albeit with low activities. However, YMOA did not convert any of the tested ketone substrates, but showed activity towards sulfides and sulfoxides and also showed very high stereoselectivity. This enzyme produced high amounts of sulfones and even converted dimethylsulfoxide (DMSO). Therefore, the sulfoxidation activity of YMOA was investigated in a mutational study. Variants with increased and reduced sulfone yields were created, indicating relevant amino acid position for the control of sulfoxidation activity. This work expands the set of eukaryotic BVMOs and explores the *Yarrowia* monooxygenase A, which might belong to a new class of BVMOs as indicated by its unique activity and a phylogenetic analysis.

Keywords: Baeyer-Villiger monooxygenase; biocatalysis; protein engineering; sulfoxidation; *Yarrowia lipolytica*