Investigation and characterization of Thermostable Baeyer Villiger Monooxygenase Variant

The Baeyer-Villiger oxidation is a ubiquitous reaction in organic chemistry that was discovered at the end of the 19th century.[1] It is particularly useful for the synthesis of lactones from cyclic ketones which makes this reaction industrially relevant for synthesis of nylon.[2] Unfortunately, despite its history and industrial relevance Baeyer-Villiger oxidations still rely on hazardous peroxyacid or peroxide oxidants, the latter of which requires organo- or metallo- catalysts to facilitate the oxidation.[2] Peroxyacids tend to be shock-sensitive and result in carboxylic acid salts as the waste by-product of Baeyer-Villiger oxidations. Moreover, the requirement for a catalyst for oxidation via peroxides means that a complex series of organic reactions is usually required before the desired oxidation can take place.[2] All of these factors make upscaling of Baeyer-Villiger reactions to industrially relevant scales dangerous and environmentally damaging. In short, traditional Baeyer-Villiger oxidations are precarious in a world that is working towards greener energy and sustainable processes. As a potential alternative to chemical synthesis, Baeyer-Villiger monooxygenases (BVMOs) use oxygen as an oxidant and have the capacity to carry out Baeyer-Villiger reactions at milder conditions with safe, environmentally benign reagents. One of the major bottlenecks that has impeded the widespread implementation of BVMOs as industrial biocatalysts is the poor stability of these enzymes. While a handful of BVMOs with moderate melting temperatures (T_m) have been reported, these BVMOs have relatively narrow substrate scopes and limited industrial utility. Herein, we report our efforts to identify novel thermostable BVMOs using sequence similarity networks to mine the genomes of thermophilic microorganisms. This presentation will illustrate our efforts to characterize one such BVMO that was identified in the genome of the thermophilic *Chloroflexi bacterium* G233 (previously known as *Thermoflexus hugenholtzii*). This enzyme, which we have dubbed *ssn*BVMO, is active on a range of substrates over a wide range of conditions. Perhaps most importantly, we will demonstrate that our variant biocatalyst uncovered by our genome mining efforts exhibits the highest T_m reported to date for a naturally occuring BVMO.[3] In addition to our efforts to characterize this novel biocatalyst structurally and kinetically, we have developed a directed evolution methodology for potentially improving the substrate scope of *ssn*BVMO further. Taken together, the results presented here will serve to illustrate the potential utility of *ssn*BVMO as a potent biocatalyst that might serve as a useful starting point for subsequent enzyme engineering efforts.

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(2) Fürst, M. J. L. J.; Gran-Scheuch, A.; Aalbers, F. S.; Fraaije, M. W. Baeyer–Villiger Monooxygenases: Tunable Oxidative Biocatalysts. *ACS Catal.* **2019**, *9* (12), 11207–11241. https://doi.org/10.1021/acscatal.9b03396.

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