ENGINEERING A LIPASE FOR ORGANIC COSOLVENT RESISTANCE – HOW DO CURRENT DIRECTED EVOLUTION APPROACHES COMPETE WITH THE POTENTIAL THAT NATURE OFFERS?

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Key Words: directed evolution; gene saturation; mutational diversity; lipase; organic solvent resistance.

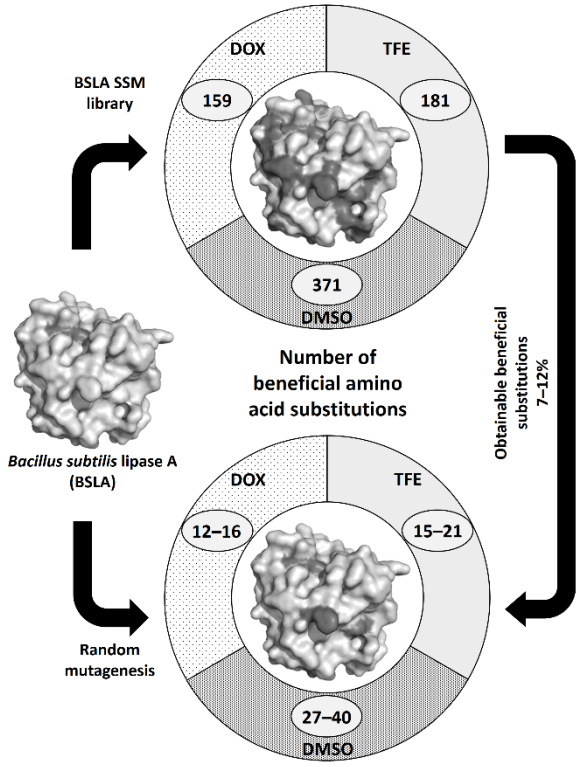

Figure 1 – Comparative analysis of random mutagenesis vs. total single amino acid substitution diversity of BSLA in the context of organic cosolvent resistance.

Our desire to design enzymes resistant to organic cosolvents is still challenged by our level of molecular understanding of this important issue. This is why currently directed evolution is utilized as the method of choice to discover promising enzyme variants. As directed evolution-based studies typically report only few beneficial amino acid exchanges, the deduction of general principles for the design of enzymes with increased resistance to water/organic solvent mixtures is challenging. Here, we present the comparative analysis of a *Bacillus subtilis* lipase A (BSLA) library, covering the full diversity of single amino acid exchanges at all 181 positions of BSLA (BSLA SSM library), and three random mutagenesis libraries (error-prone PCR with low and high mutagenesis frequencies, as well as a transversion-enriched Sequence Saturation Mutagenesis (SeSaM-Tv P/P) library). Screening of the BSLA SSM library for resistance to the water-miscible organic cosolvents 1,4-dioxane (DOX), 2,2,2 trifluoroethanol (TFE), and dimethyl sulfoxide (DMSO) revealed that 5 – 11% of all possible single substitutions promote organic cosolvent resistance. However, only 7 – 12% of these beneficial substitutions were identified in the three random mutagenesis libraries. To our knowledge, this is the first study quantifying the number of beneficial substitutions obtainable by random mutagenesis compared to the total number of beneficial single-substitutions (BSLA SSM library). Moreover, comprehensive analysis of the BSLA SSM library revealed that only few beneficial amino acid substitutions were common for all three organic cosolvents tested. These findings illustrate that – even when the total single-substitution diversity is available – our understanding of organic cosolvent resistance still remains incomplete. Hence, deducing general design principles based on relatively few amino acid exchanges, as it is common practice in directed evolution campaigns, seems counterintuitive. Furthermore, analysis of the BSLA SSM library conferred valuable insights into the role of surface-exposed charges for organic cosolvent resistance. Structural inspection of beneficial variants revealed that this is due to the attraction of water rather than to the formation of salt bridges.