

Supplementary Materials: Are Directed Evolution Approaches Efficient in Exploring Nature's Potential to Stabilize a Lipase in Organic Cosolvents?

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1. Auto-induction medium

The auto-induction medium consisted of 800 mL media component (12 g casein hydrolysate, 24 g yeast extract, 5 g glycerol), 90 mL KPi buffer (pH 7.0, 1 M), 10 mL glucose (50 g/L) and 100 mL α -lactose monohydrate (20 g/L).

2. Definition of residual activity

The residual activity (R) was calculated using the following equation:

$$\text{Residual activity [\%]} = \frac{\text{slope (BSLA variant or WT - EV)}_{\text{organic solvent}}}{\text{slope (BSLA variant or WT - EV)}_{\text{buffer}}}$$

3. Supplementary tables and figures

Table S1. Number of beneficial amino acid positions ^{a)} in BSLA generated by SSM, epPCR-low, epPCR-high, SeSaM and theoretical combinations.

Diversity Generation Method	DOX	(%)	TFE	(%)	DMSO	(%)
SSM	75	(100)	74	(100)	107	(100)
epPCR-low	11	(15)	14	(19)	24	(22)
epPCR-high	13	(17)	19	(26)	29	(27)
SeSaM	15	(20)	13	(18)	34	(32)
epPCR-low + SeSaM	20	(27)	21	(28)	43	(40)
epPCR-high + SeSaM	23	(31)	24	(32)	45	(42)
epPCR-low + epPCR-high	15	(20)	22	(30)	33	(31)
epPCR-low + epPCR-high + SeSaM	24	(32)	26	(35)	47	(44)

^{a)} In contrast to beneficial substitutions the term beneficial positions refers to positions with at least one beneficial amino acid substitution (*i.e.*, a position with ≥ 2 different beneficial amino acid substitutions is counted as one beneficial position).

Table S2. Location of amino acid positions within BSLA with at least one beneficial substitution improving organic cosolvent resistance to DOX, TFE and DMSO.

Organic Cosolvent	Diversity Generation Method	Location of Beneficial Positions in BSLA			
		Exposed	(%)	Buried	(%)
DOX	BSLA WT	128	(71)	53	(29)
	SSM	61	(81)	14	(19)
	epPCR-low	9	(82)	2	(18)
	epPCR-high	12	(92)	1	(8)
	SeSaM	13	(87)	2	(13)
TFE	SSM	58	(78)	16	(22)
	epPCR-low	12	(86)	2	(14)
	epPCR-high	18	(95)	1	(5)
	SeSaM	12	(92)	1	(8)
DMSO	SSM	79	(74)	28	(26)
	epPCR-low	17	(71)	7	(29)
	epPCR-high	22	(76)	7	(24)
	SeSaM	26	(76)	8	(24)

Table S3. Number, category and location of common beneficial amino acid substitutions and positions for resistance to more than one organic solvent.

Organic Cosolvent	Substitutions	Positions ^{a)}	Amino Acid Category	Location of Amino Acid Substitutions in BSLA			
				Exposed	(%)	Buried	(%)
DMSO & DOX	81	48	Total	76	(94)	5	(6)
			Aromatic ^{b)}	9		0	
			Hydrophobic	11		3	
			Special	6		2	
			Polar	15		0	
			Charged	35		0	
DMSO & TFE	47	30	Total	44	(94)	3	(6)
			Aromatic	5		0	
			Hydrophobic	7		1	
			Special	6		1	
			Polar	14		1	
			Charged	12		0	
DOX & TFE	49	31	Total	45	(92)	4	(8)
			Aromatic	8		1	
			Hydrophobic	7		1	
			Special	4		1	
			Polar	9		0	
			Charged	17		1	
DMSO & DOX & TFE	21	16	Total	21	(100)	0	(0)
			Aromatic	2		0	
			Hydrophobic	4		0	
			Special	2		0	
			Polar	6		0	
			Charged	7		0	

^{a)} Beneficial positions are defined as positions with at least one beneficial substitution (*i.e.*, a position with ≥ 2 different beneficial amino acid substitutions was counted as one beneficial position).

^{b)} Amino acids were categorized as defined in the Material and Methods section.

Table S4. Relative permittivity of DOX, TFE and DMSO.

Relative Permittivity	DOX	TFE	DMSO	Water
ϵ [-] (at 25°C)	2	27	47	78

ϵ : Relative permittivity as described in the literature. [1-3]

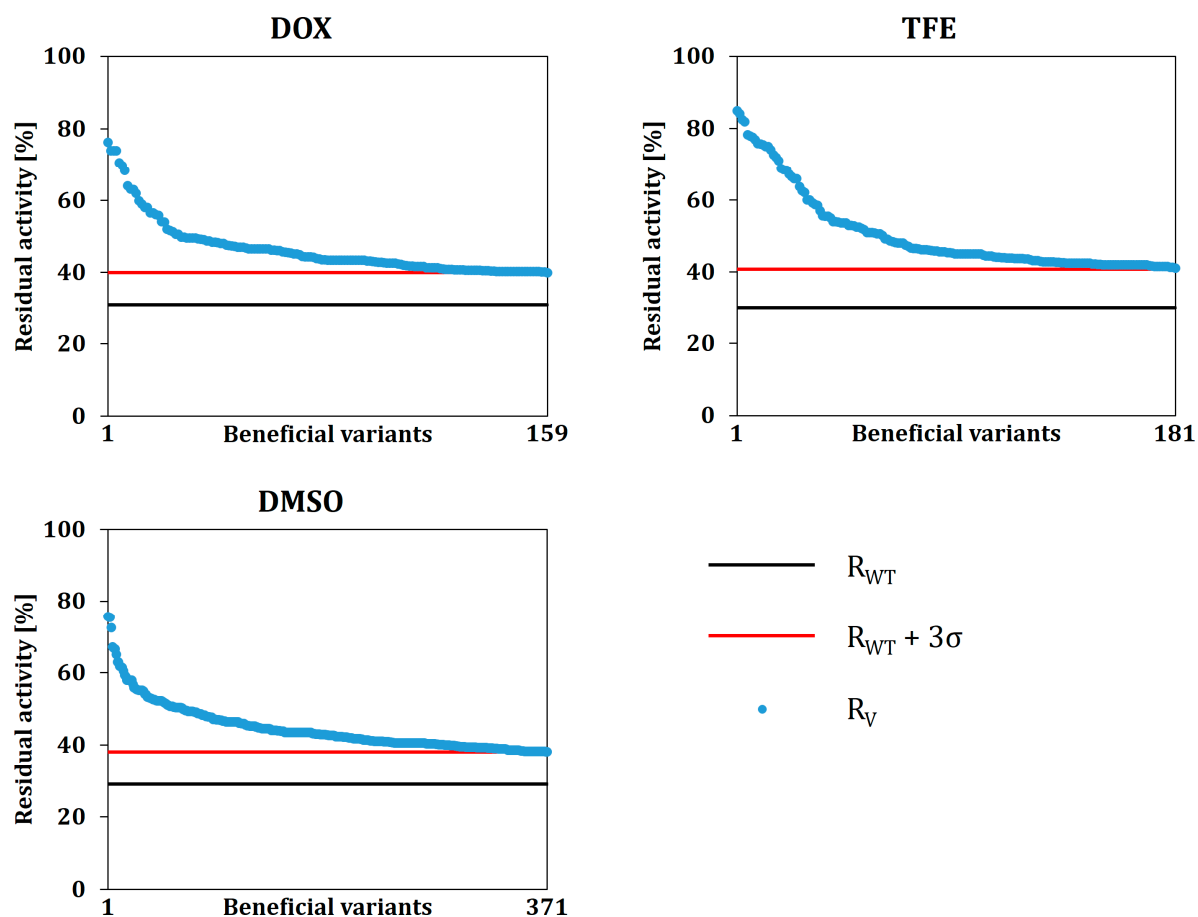


Figure S1. Distribution of residual activity of beneficial BSLA variants for three organic cosolvents. The figure shows the residual activity of BSLA^{WT} (R_{WT} ; black line) as reported in Table 1, the threshold of the residual activity for beneficial variants ($R_{WT} + 3\sigma$; red line; σ reported in Table 1) as well as the residual activity of BSLA variants (R_V ; blue dots; only beneficial variants are shown) for the three organic cosolvents DOX, TFE and DMSO. Beneficial variants (for DOX: 159; for TFE: 181; for DMSO: 371) were plotted sorted according to their residual activity.

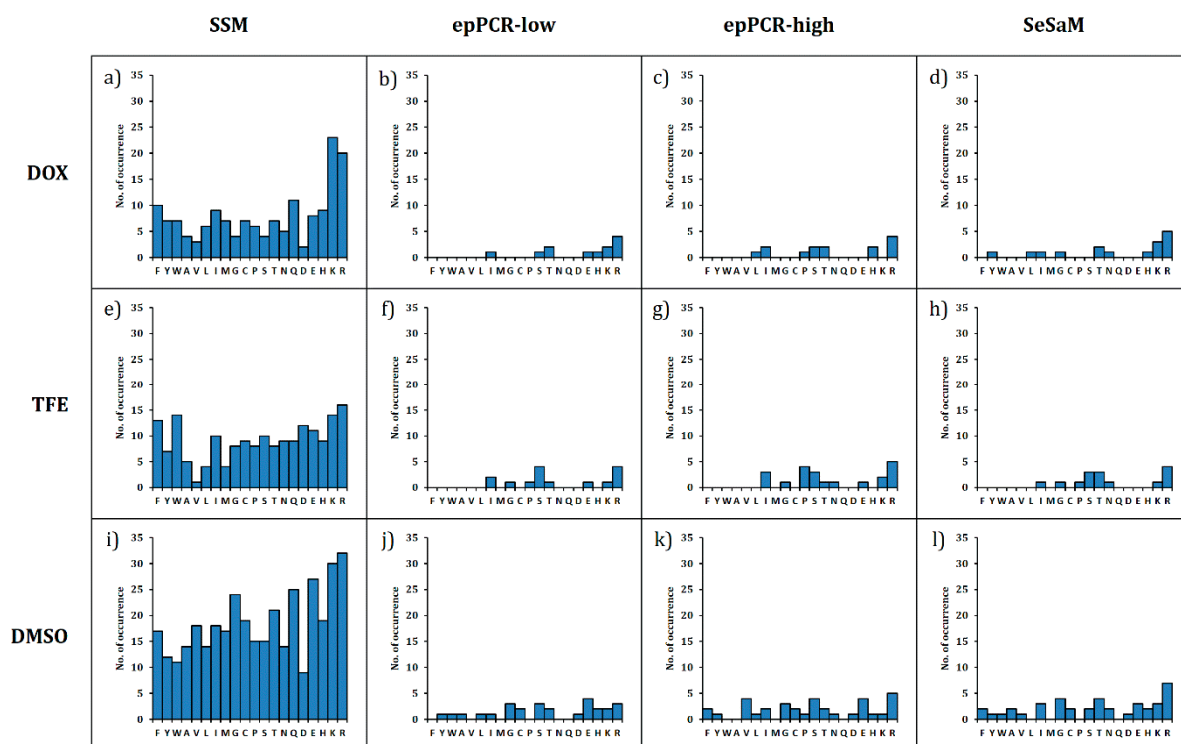


Figure S2. Number of beneficial amino acid substitutions. Beneficial substitutions found in the BSLA SSM library and the three random mutagenesis libraries (epPCR-low, epPCR-high, SeSaM). The order of amino acids reflects their category as defined in the Material and Methods section. From left to right: aromatic, hydrophobic, special, polar, and charged.

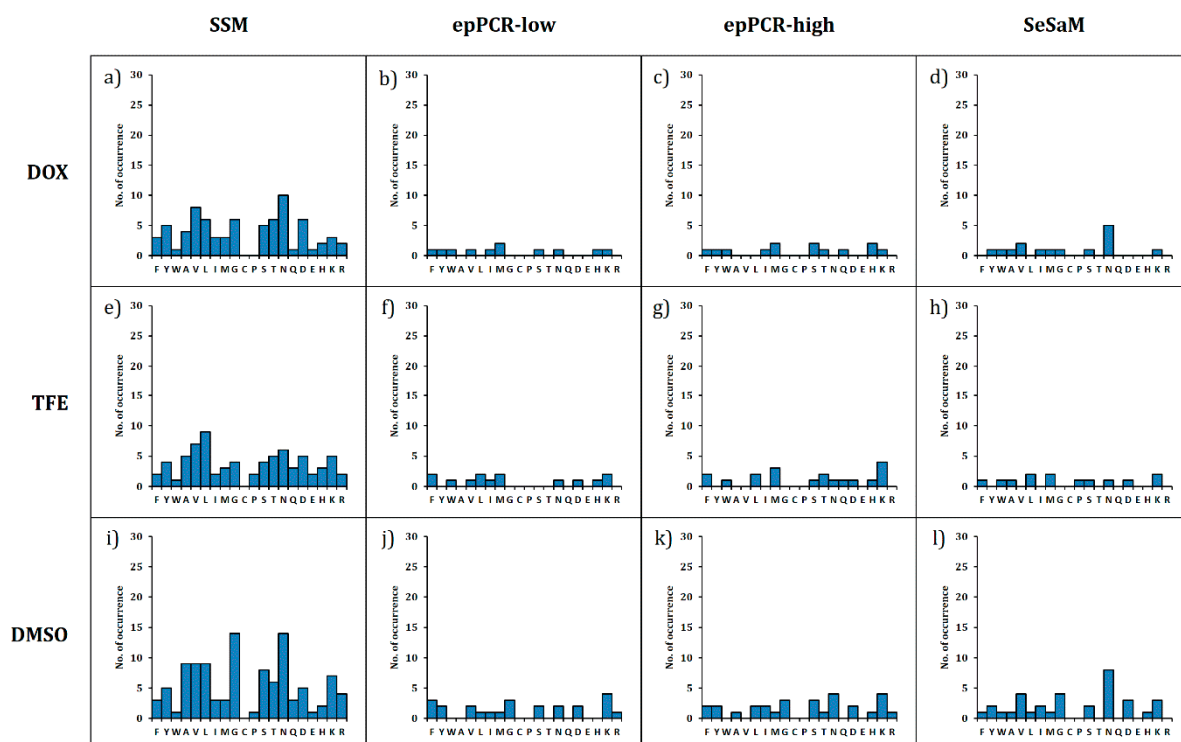


Figure S1. Numbers of replaced amino acids. These amino acids were replaced by more favorable amino acids (not shown) from the BSLA SSM library and the three random mutagenesis libraries. From left to right: aromatic, hydrophobic, special, polar, and charged amino acids.

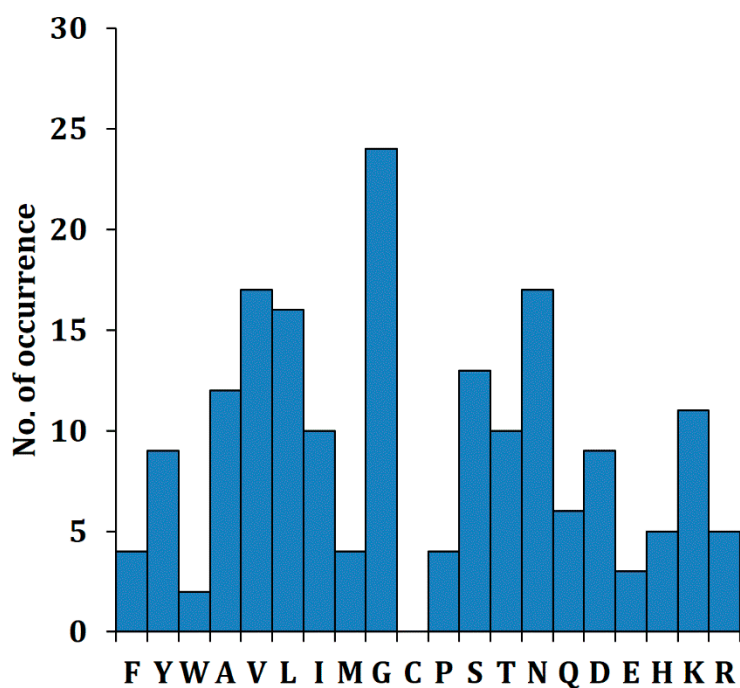


Figure S2. Amino acid distribution of BSLA^{WT}. Numbers of the 20 canonical amino acids found in the BSLA sequence. The order of amino acids reflects their category. From left to right: aromatic, hydrophobic, special, polar, and charged.

References

1. MacGregor, W.S. The chemical and physical properties of DMSO. *Ann. N. Y. Acad. Sci.* **1967**, *141*, 3–12.
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3. Uematsu, M.; Frank, E. Static dielectric constant of water and steam. *J. Phys. Chem. Ref. Data* **1980**, *9*, 1291–1306.