

***VpStyA1* and *VpStyA2B* of *Variovorax paradoxus* EPS: rather an aryl alkyl sulfoxidase than a styrene epoxidizing monooxygenase**

Dirk Tischler ^{1,2*}, Ringo Schwabe ¹, Lucas Siegel ¹, Kristin Joffroy ¹, Stefan R. Kaschabek ¹, Anika Scholtissek ¹ and Thomas Heine ¹

¹ Affiliation 1; Institute of Biosciences, Environmental Microbiology, TU Bergakademie Freiberg, Leipziger Str. 29, 09599 Freiberg, Germany; dirk-tischler@email.de, ringoschwabe007@gmail.com, lucasbenedikt@aol.com, kristin.friebel@student.tu-freiberg.de, stefan.kaschabek@ioez.tu-freiberg.de, anika.scholtissek@gmail.com, heinet@tu-freiberg.de

² Affiliation 2; Microbial Biotechnology, Ruhr University Bochum, Universitätsstr. 150, 44780 Bochum, Germany

* Correspondence: dirk.tischler@rub.de; Tel.: +49-234-32-22656

SUPPLEMENTARY MATERIAL

Table S1. Strains, plasmids and primers used in this study.

Strain, plasmid, or primer	Relevant characteristic(s)	Source / Reference
<i>E. coli</i> DH5 α	F ⁻ ϕ 80d <i>lacZ</i> M15 (<i>lacZYA-argF</i>)U169 <i>endA1 recA1 hsdR17</i> (rK ⁻ , mK ⁺) <i>supE44</i> λ ⁻ <i>thi-1 gyrA96 relA1</i>	Gibco-BRL
<i>E. coli</i> BL21(DE3) (pLysS)	<i>hsdS gal</i> (λ clts857 <i>ind1 Sam7 nin5 lacUV5-T7</i> gene 1), pLysS (Cm ^R)	Stratagene
pEX_A_VpstyA1	<i>VpstyA1</i> of <i>V. paradoxus</i> EPS (~1.25 kb NdeI/KpnI-fragment) cloned in pEX vector with additional multiple cloning site, (Amp ^r)	This study Eurofins MWG
pEX_A_VpstyA2B	<i>VpstyA2B</i> of <i>V. paradoxus</i> EPS (~1.75 kb NdeI/KpnI-fragment) cloned in pEX vector with additional multiple cloning site, (Amp ^r)	This study Eurofins MWG
pET16bP	pET16b (Novagen) with additional multi-cloning site, allows expression of recombinant proteins with N-terminal 10x His-tag	Wehmeier (pers. comm)
pSVpstyA1_P01	<i>VpstyA1</i> of <i>V. paradoxus</i> EPS (~1.25 kb NdeI/KpnI-fragment) cloned in pET16bp	This study
pSVpstyA2B_P01	<i>VpstyA2B</i> of <i>V. paradoxus</i> EPS (~1.75 kb NdeI/KpnI-fragment) cloned in pET16bp	This study
pSVpAAAAA_P01	<i>VpstyA2_408-AAAAA_B</i> gene in pET16bP (plasmid generated by site-directed mutagenesis using a fw_AAAAA/pET16-check-rev PCR-fragment as megaprimer)	This study
pSVpHHHHH_P01	<i>VpstyA2_408-HHHHH_B</i> gene in pET16bP (plasmid generated by site-directed mutagenesis using a fw_HHHHH/pET16-check-rev PCR-fragment as megaprimer)	This study
pSVpWYHHH_P01	<i>VpstyA2_408-WYHHH_B</i> gene in pET16bP (plasmid generated by site-directed mutagenesis using a fw_WYHHH/pET16-check-rev PCR-fragment as megaprimer)	This study
pSVpGQWCSQY_P01	<i>VpstyA2_408-GQWCSQY_B</i> gene in pET16bP (plasmid generated by site-directed mutagenesis using a fw_GQWCSQY /pET16-check-rev PCR-fragment as megaprimer)	This study
pSVpWYHHHHH_P01	<i>VpstyA2_408-WYHHHHH_B</i> gene in pET16bP (plasmid generated by site-directed mutagenesis using a fw_WYHHHHH/pET16-check-rev PCR-fragment as megaprimer)	This study
pET16-check-fw	CATCACAGCAGCGGCCATATCGAAG	[34]
pET16-check-rev	CAGCTTCCTTTTCGGGCTTTGTTAG	[34]
fw_AAAAA	TTCCTGGAAGCACGTGCGGCCGCGCCGCGGTTGACCGCTTTGATC	This study
fw_HHHHH	TTCCTGGAAGCACGTATCACCATCACCATGTTGACCGCTTTGATC	This study
fw_WYHHH	TTCCTGGAAGCACGTTGGTATCACCACCACGTTGACCGCTTTGATC	This study
fw_GQWCSQY	TTCCTGGAAGCACGTGGCCAGTGGTGCAGCCAGTATGTTGACCGCTTTGATC	This study
fw_WYHHHHH	TTCCTGGAAGCACGTTGGTATCACCACCACCACGTTGACCGCTTTGATC	This study
fw_TIVVV	TTCCTGGAAGCACGTACCATAGTGGTGGTGGTTGACCGCTTTGATC	This study

Primer sequence direction is 5'→3'

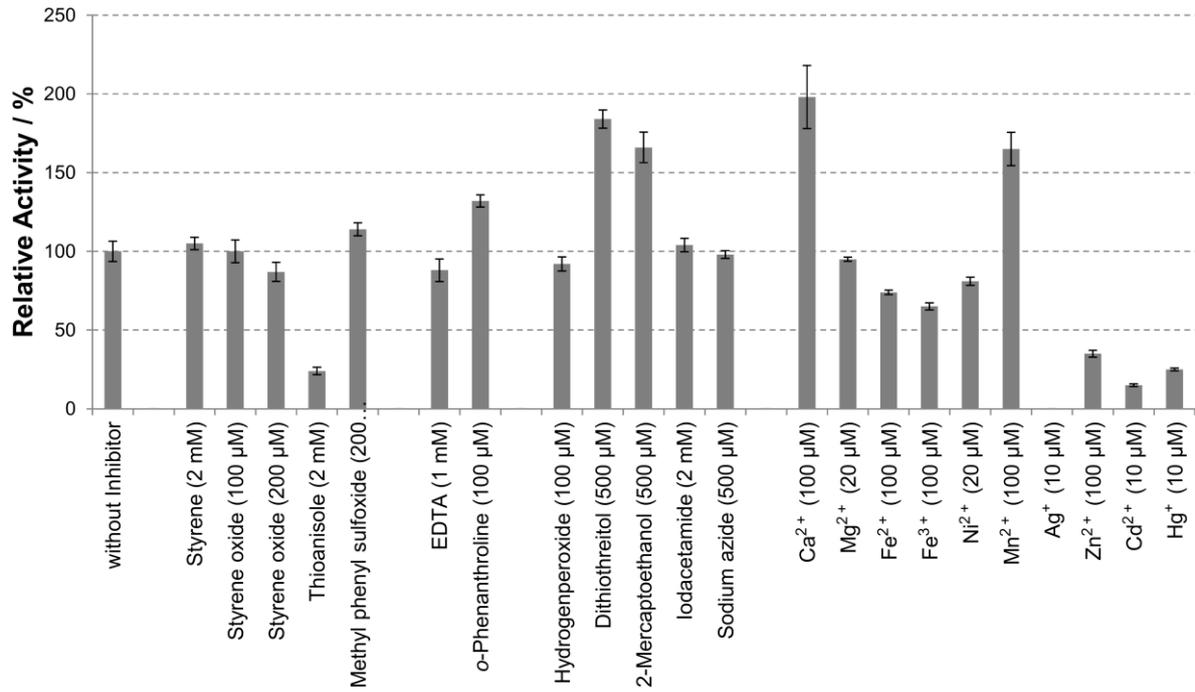


Figure S1. Sensitivity of VpStyA2B towards putative inhibitors determined by applying the NADH:FAD oxidoreductase assay.