

Supplementary Material

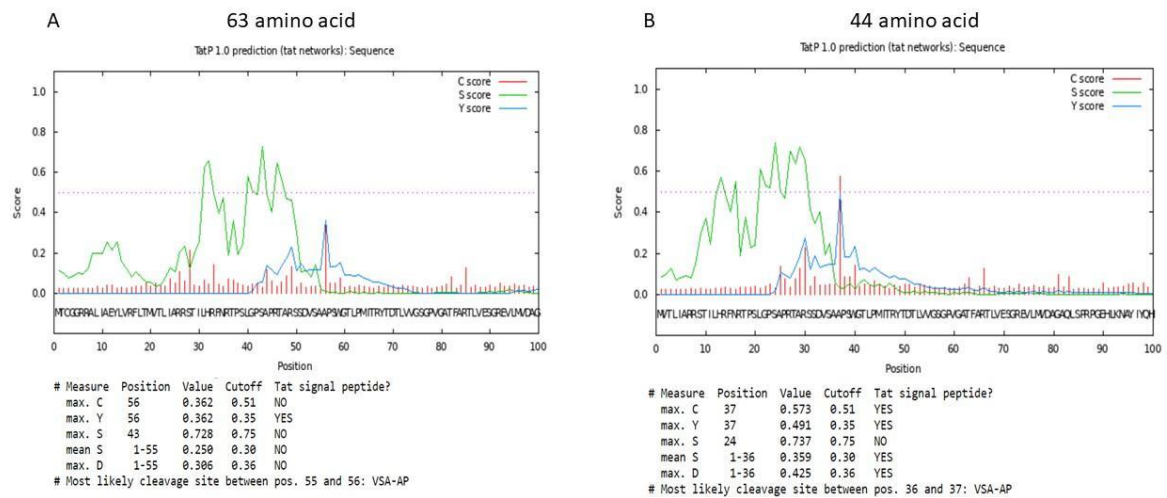


Figure S1: Signal peptide prediction using TatP-1.0 for the additional upstream 63 amino acids (A) and 44 amino acids (B)

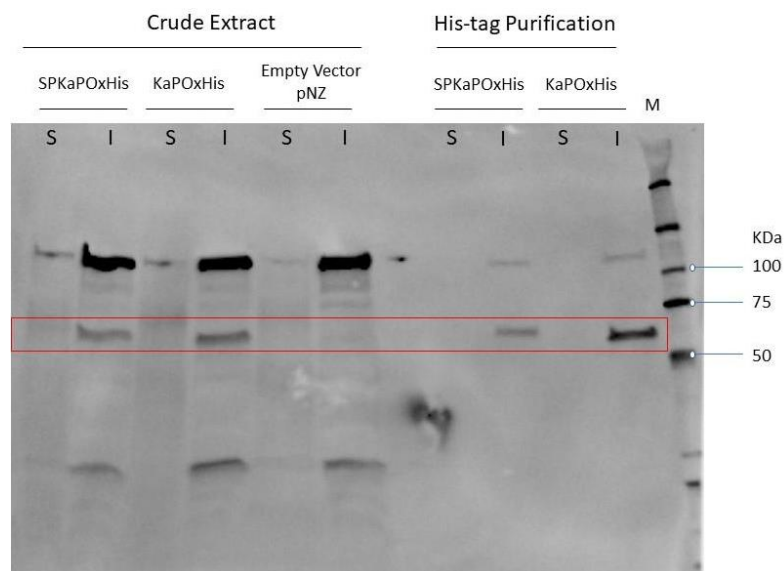
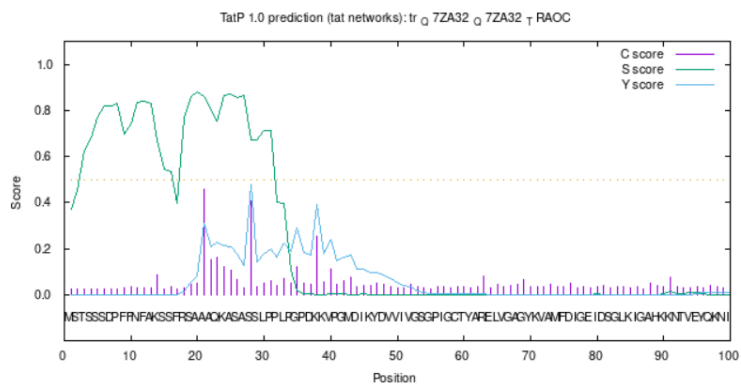


Figure S2: Western blot of samples from *B. subtilis* expression system harbouring SPKaPOxHis, KaPOxHis, and the empty pNZ vector in un-purified form (crude extract) and purified form (His-tag purification). Samples from supernatant are indicated as S, samples from the cell extract are indicated as I, the western blot marker is marked as M. Expected molecular weight of targeted protein was 60 KDa (shown in red box).



data

```
>tr_Q7ZA32_Q7ZA32_TRA length = 100
# Measure Position Value Cutoff Tat signal peptide?
max. C 21 0.456 0.51 NO
max. Y 28 0.480 0.35 YES
max. S 20 0.883 0.75 YES
mean S 1-27 0.735 0.30 YES
max. D 1-27 0.607 0.36 YES
# Most likely cleavage site between pos. 27 and 28: ASA-SS
# Potential Tat signal peptide but No Tat motif was found
# Used regex: RR.[FGAVML][LITMVF]
//
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Figure S3: Signal peptide prediction using TatP-1.0 for Pyranose Oxidase from *Trametes ochracea* (POx Q7ZA32)

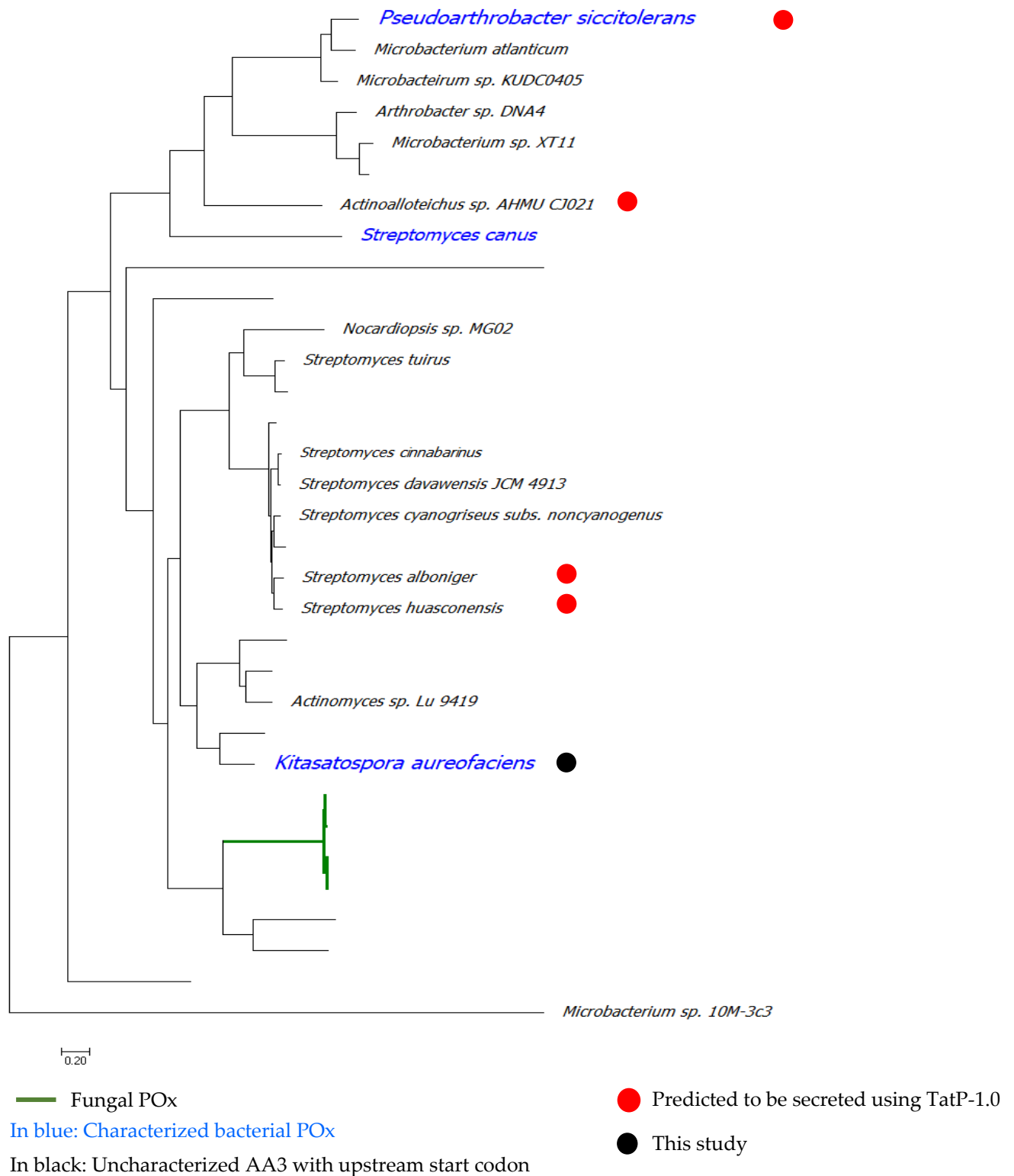


Figure S4. Phylogenetic tree constructed from extant bacterial POx-like sequences with *KaPOx* as query. Multiple alignment was conducted using Mafft (<https://mafft.cbrc.jp/>) with iterative refinement method for < 200 sequence with global homology. Tree was constructed using NGPhylogeny (<https://ngphylogeny.fr/>) with TrimAl as alignment curation and PhyML for tree inference.

Table S1. Oligonucleotides used in this study

Name	Sequence (5' - 3')	Note
pNZ_SPKaPOx_F	AACTGCAGAAATGGTTACATTGATAGCCCGTAGATCAAC	SPKaPOX to pNZ8901
pNZ_KaPOx_F	AACTGCAGAAATGATTACTCGCTATACGGAC	KaPOX to pNZ8901
KaPOxHis_R	CTCTCTAGATCAGTGATGATGATGATGGCTTCGGACAGCGAGCACCTC	(SP)KaPOX to pNZ8901 / pUC19-P _{vsi}
Pvsi_F	TTTTTTAAGCTTGGGGATGACCACCGCGGGAG	P _{vsi} to pUC19
Pvsi_R	TTTTTCATCTGCAGCTCTCCTTCGATCGATG	P _{vsi} to pUC19
SPKaPOx_F	GCTGCAGATGGTTACATTGATAGCCCG	SPKaPOX to pUC19-P _{vsi}
KaPOx_F	GAGAGCTGCAGATGATTACTCGCTATACGGACACC	KaPOX to pUC19-P _{vsi}
mRFP_GA_F	CATCGATCGAAGGAGAGCTGCAGATGGCCTCCTCCGAGGACGTC	mRFP to pUC19-P _{vsi} without SP ^{Sec} with Gibson Assembly
mRFP_GA_R	AGCTCGGTACCCGGGGATCCGAGCTCTCTAGATTAGGCGCCGGTGGAGTGGCGGC	mRFP to pUC19-P _{vsi} without SP ^{Sec} with Gibson Assembly
SPKaPOx_GA_F	CGATCGAAGGAGAGCTGCAGATGGTTACATTGATAGCCCGTAG	fusion SPKaPOx-mRFP-His in pUC19-P _{vsi}
KaPOx_GA_F	GCTTCGGACAGCGAGCACCTC	fusion KaPOx-mRFP-His in pUC19-P _{vsi}
KaPOx_GA_R	CGGGGATCCGAGCTCTCTAGACCGCCGCTGCCGCCGCTTCGGACAGCGAGCACCTCG	fusion (SP) KaPOx-linker in pUC19-P _{vsi}
mRFP_F	ATGGCCTCCTCCGAGGACGTCATCAAG	
mRFP_lkk_GA_F	AGCGGCGGCAGCGGCGGTATGGCCTCCTCCGAGGACGTC	fusion (SP) KaPOx-mRFP-His in pUC19-P _{vsi}
mRFPHis_GA_R	GGGGATCCGAGCTCTCTAGATCAGTGATGATGATGATGATGGGCGCCGGTGGAGTGGCGCC	fusion (SP) KaPOx-mRFP-His in pUC19-P _{vsi}
eGFP_F	AGAGCTGCAGATGGTGAGCAAGGGCGAGGAG	eGFP to pUC-P _{vsi}
eGFP_R	GAGCTCTCTAGATCATTACTTGTACAGCTCGTCATGCCGAGAGTGATCCC	eGFP to pUC-P _{vsi}

eGFP_GA_F	<i>CGAGTTGGGGGACTTTGCCGGTGAGCAAGGGCG</i> <i>AGGAGCT</i>	SPeGFP to pUC-P _{vsi}
eGFP_GA_R	<i>GGGGATCCGAGCTCTCTAGATTACTTGTACAGCT</i> <i>CGTCCATGC</i>	SPeGFP to pUC-P _{vsi}
eGFP_A	<i>ATGTGTCAGCGGCGCCGAGTTGGGGGACTTTGC</i> <i>CGATGGTGAGCAAGGGCGAGGAG</i>	SPKaPOx to eGFP
eGFP_B	<i>TCACTTGGGCCCTCGGCGCCGCGAACGGCTCG</i> <i>GTCCAGTGATGTGTCAGCGGCGCCGAGT</i>	SPKaPOx to eGFP
eGFP_C	<i>TCAACAATTTTGCACAGGTTCAACCGCACTCCA</i> <i>TCACTTGGGCCCTCGGCGCCGCG</i>	SPKaPOx to eGFP
eGFP_D	<i>GAGAGCTGCAGATGGTTACATTGATAGCCCGT</i> <i>AGATCAACAATTTTGCACAGGTTTC</i>	SPKaPOx to eGFP

Bold: linker

Underline: Restriction enzyme

Italic: complement