Supplementary Information

For

Biocatalysed Synthesis of Chiral Amines: Continuous Colorimetric Assays for Mining Amine-Transaminases

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Content

1. General Informations

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. Chemical shifts are reported in ppm (δ) relative to TMS as internal standard. Enzyme purifications by immobilized metal affinity chromatography (IMAC) were performed using Ni-NTA agarose purchased from QIAGEN. Lysogeny Broth (LB) for cell culture was purchased from DIFCO. Before enzymes purifications, cells were disrupted using a Bandelin Sonopuls sonicator. All chemicals were purchased from various commercial sources and used without further purification. Isopropyl-β-D-thiogalactoside (IPTG) used for induction was purchased from Sigma Aldrich (Merck Sigma, StLouis, USA). Bradford reagent and bovine serum albumin (BSA) used as standard were purchased from Bio-Rad. Enzyme assays were run at 25 °C in disposable 1 mL cuvettes using a UV/Vis spectrometer (Agilent Cary™ 300) or at 30 °C in Greiner® 96-well microplates using a multimode microplate reader (Safire™ II, Tecan). All kinetic measurements were performed in 50 mM potassium phosphate (KP) buffer, pH 7.5, in a total volume of 1 mL (cuvette) or 200 µL (microplate). In the latter case, an optical path of 0.59 cm was determined. Variations of optical density (OD) were recorded at 412 nm and initial rates were calculated from slopes using ε = 14150 $M^{-1}.cm^{-1}$ for the thiolate anion formed from 5,5'-dithiobis-(2-nitro-benzoic acid) (DTNB). One Unit (U) corresponds to the quantity of enzyme allowing the conversion of 1 µmol of substrate per minute in the specified conditions.

2. Synthesis of hypotaurine

Hypotaurine (HPT) was prepared following a modified procedure described for the synthesis of cysteine sulfinic acid.[1] To a solution of cystamine hydrochloride (10 g, 44.4 mmol) in 95% formic acid (230 mL) was added 37% hydrochloric acid (9 mL). A solution of 10 M H_2O_2 (10.7 mL, 107 mmol) was then added dropwise while maintaining the solution temperature between 15 and 25°C. The mixture was then stirred at room temperature for 16 h before concentration under reduced pressure. The residue was dissolved in water (100 mL) and the solution concentrated again under reduced pressure. This operation was repeated twice until complete removal of formic acid. The residue was then dissolved in water (22 mL) and 30% NH_3 (22 mL) was added. The mixture was then stirred at room temperature for 4 h before concentration under reduced pressure. The residue was dissolved in water (100 mL) and the solution concentrated again under reduced pressure. This operation was repeated twice until complete removal of NH_3. The residue was then dissolved in water (10 mL) and pH was adjusted to 11 with 4 M NaOH. The solution was then poured on a column of Dowex® 50WX8 (200 mL, H^+ form). The column was eluted with water (3 L). Ninhydrin positive fractions were pooled and concentrated under reduced pressure to afford HPT as a slightly yellow solid (5.3 g, 82%). Mp = 186 °C. ^{1}H NMR (400 MHz, D_2O): δ 3.22 (1H, t, *J* = 6.5 Hz, 2H), 2,51 (2H, t, *J* = 6.5 Hz, 2H). ^{13}C NMR (100 MHz, D_2O): δ 55.4, 33.4. HRMS (ESI^+) m/z: calc. for $C_2H_8NO_2S$: 110.0276, found: 110.0279.

3. Cloning and production of histidine-tagged TA

3.1 Cloning of enzymes

Two previously described TA (Uniprot ID: Q9A3Q9[2] and A0A0H3K2P4[3]) and four enzymes identified in the present study (Uniprot ID: A9CV07, A0KEV7, I0JLI1 and Q48AP6**)** were cloned with a Histidine tag in N-terminal part in a pET22b(+) (Novagen) modified for ligation independent cloning as already described.[4] All primers and strains are listed in Table S1. All the strains along with their identifiers were purchased from DSMZ or ATCC collections. When DNA samples corresponding to the gene encoding the selected enzyme was not available, PCR was performed on the DNA of another strain from the same species. Oligonucleotides (Table S1) were from Merck-Sigma Aldrich. Enzymes were over-expressed in *E. coli* BL21-CodonPlus (DE3)-RIPL cells (Agilent) and cell lysates prepared as previously described.[5,6] After centrifugation, supernatants were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the NuPAGE system (Invitrogen). Protein concentration was determined by the Bradford method, with bovine serum albumin as the standard (Bio-Rad). Samples of transformed cells over expressing the different enzymes were stored at -80 °C in lysogeny broth (LB) medium (3 mL) containing ampicilline (100 $mg.L^{-1}$) and glycerol (10 % vol.).

Table S1. Set of transaminases cloned with a His-tag.

Uniprot KB Id.	***Organism***	***Strain used for PCR***	***Primer 5'***	***Primer 3'***
Q9A3Q9	*Caulobacter vibrioides*	DSMZ 4727	AAAGAAGGAGATAGGATCATG CATCATCACCATCACCATCCCG ATTTCGGCGCCAACGACCTCGA C	GTGTAATGGATAGTGATCTTAA TCGACCTGACCCAGCACCCTGC GGATCG
A0A0H3K2P4	*Synechococcus sp.*	ATCC 27144	AAAGAAGGAGATAGGATCATG CATCATCACCATCACCATGAAG ACAAATTGATGCTGATG	GTGTAATGGATAGTGATCTTAC TTCGCGAGTTCAGC
A9CV07	*Hoeflea phototrophica*	DSMZ 17068	AAAGAAGGAGATAGGATCATG CATCATCACCATCACCATAACA TGCCCATTCAATCCGGAATTC	GTGTAATGGATAGTGATCTTAG CCGAACACCCGCGTC
A0KEV7	*Aeromonas hydrophila*	DSMZ 30187	AAAGAAGGAGATAGGATCATG CATCATCACCATCACCATAAAC CGATCAGCGACATCAATACCCC	GTGTAATGGATAGTGATCTTAG TCCTGGGCCTGCAGGGCGTC
I0JLI1	*Halobacillus halophilus*	DSMZ 2266	AAAGAAGGAGATAGGATCATG CATCATCACCATCACCATACAA AAGCTGATGTGAAGAACGATC	GTGTAATGGATAGTGATCTTAC AACTGATGGAGTGCATCGAC
Q48AP6	*Colwellia psychrerythraea*	ATCC BAA-681	AAAGAAGGAGATAGGATCATG CATCATCACCATCACCATAACA ATAACCAAACAAAACATGGCAT C	GTGTAATGGATAGTGATCTTAC GCTATTTCGTTAAAGGTTTCGC C

3.2 Production and purification

A preculture was run by adding a 3 mL stored sample of transformed cells in 100 mL LB medium containing ampicillin (100 mg.L^{-1}). After stirring (200 rpm) at 37 °C for 24 h, 5 x 4 mL preculture samples were diluted in 5 x 200 mL of LB medium containing ampicillin (100 mg.L^{-1}). The cultures were stirred (200 rpm) at 37 °C until an OD at 600 nm of 0.7 was reached. After addition of 50 mM IPTG (2 mL), the culture medium was stirred (200 rpm) at 30 °C for 24 h. Cells were harvested by centrifugation (12,000 *g*, 15 min), resuspended in 50 mM KP buffer, pH 8 (30 mL) and the suspension was centrifuged again (12,000 *g*, 15 min). This washing step was repeated 3 times. Finally, the cell pellet (typically 3-4 g of wet-cells from 1 L culture) was stored at -20 °C until enzyme purification.

For IMAC purification, cell lysates were prepared from 3 g of wet cells suspended in 50 mM KP, 0.3 M KCl, 10 mM imidazole, pH 8 (20 mL). Cells were disrupted by sonication at 0 °C for 1 h (50% amplitude, 8 s on, 15 s off) and cell debris were removed by centrifugation at 4 °C (25,000 *g*, 20 min). A solution of 10 mM pyridoxal phosphate (PLP) was added to reach a final concentration of 0.1 mM. Clarified lysates were poured onto Ni-NTA agarose (10 mL) equilibrated with 50 mM KP, 0.3 M KCl, 10 mM imidazole, pH 8. The column was washed with the same buffer containing 20 mM imidazole (100 mL) before elution of the tagged enzyme with the same buffer containing 250 mM imidazole (100 mL). Fractions were tested for protein content by adding 20 µL aliquots to 200 µL of Bradford reagent. Protein containing fractions were pooled. TA solutions were then dialysed at 4 °C with 50 mM KP, 0.1 mM PLP, 3M $(NH_4)_2SO_4$, pH 7.5 (3 x 0.5 L, 3 x 6 h). The final enzyme suspensions were stored at 4°C. Protein concentrations were determined by the Bradford assay with BSA as standard. Before use, TA suspensions were centrifuged (14,000 *g*, 5 min) and the supernatant discarded.

4. Calibration curves with direct and coupled assays

All experiments were performed in triplicate, in 96-well microplates. For direct assay, initial rates were measured with 20 mM HPT, 2 mM Pyruvate, 0.05 mM PLP, 1 mM DTNB, 1% EtOH and 0.03-7 µg/well of purified Q9A3Q9 (3-722 nM). Results are reported in figure S1. For coupled assay, initial rates were measured using 20 mM Ala, 20 mM HPT, 2 mM 2-oxoglutaric acid, 0.1 mM PLP, 1 mM DTNB, 1% EtOH, 6.7

µg/well of A9CV07 (0.65 µM) and 0.6-47 µg/well of purified A0A0H3K2P4 (0.07-5.7 µM). Results are reported in figure 2 of the manuscript. In both cases, LOD was calculated from the linear part of the plot of activity versus quantity of TA using the formula LOD = 3.3*Sb/m where m and Sb are the slope and standard deviation of the ordinate intercept which were both calculated according to the least square method.

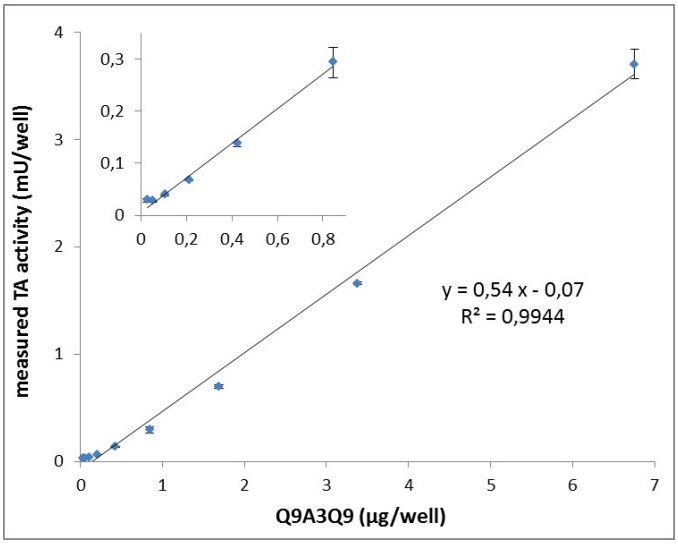

Figure S1. Calibration curve with the direct assay using Q9A3Q9 as model TA.

5. Construction of the amine-TA collection

A set of 60 TA from class III, experimentally characterized or reviewed in UniprotKB database (table S2), was used for pairwise sequence similarity with the BL2 option (BLAST allowing gaps) and a BLOSUM62 scoring matrix against the UniProtKB database. Adjusments of BLAST parameters between 40% and 80% identity on 80% length were applied. Then, redundancy between retrieved proteins was eliminated by removing all sequences with 100% of homology, leading to a final selection of 15 985 proteins. Then, protein sequences were clustered (80% of identity) as previously described,[5] and genes with 40-65% GC were chosen for facilitating cloning. In this way, a set of 889 proteins representative of these clusters for which genomic DNA was available in the Genoscope strain collection were selected, and the corresponding genes were cloned in an expression vector. Cloning of the genes , cell cultures, IPTG induction for protein production, and cell extraction were performed as reported previously.[3,7] Based on gel electrophoresis analysis, a set of 642 genes were successfully cloned. Clones were then transformed in expressing cells BL21-CodonPlus (DE3)-RIPL cells (Agilent). The overexpression (in the induced culture) and the solubility (in the clear lysate) of the proteins were checked on an E-PAGE 8% Protein Gels (SDS-PAGE,Invitrogen). A set of 549 proteins were overexpressed and induced successfully as shown by the protein gel analysis. Notably, around 65 % were found visible on SDS gel of the cell-free extracts. Protein concentrations were determined using the Bradford method. The samples were stored at -80 °C.

Table S2: Reference set of 60 experimentally characterised or reviewed amine-TA used for genes selection.

UniprotKB Id	*Organism*	Protein name / annotation	References
A6WVC6	*Ochrobactrum anthropi*	Aminotransferase class-III	[8,9]
C3K3T9	*Pseudomonas fluorescens*	Putative aminotransferase	[9–11]
B7IC89/A0A0D5YH21	*Acinetobacter baumannii*	ω-amino acid-pyruvate aminotransferase	[9]
C7KV78/C7JE89	*Acetobacter pasteurianus*	β-alanine-pyruvate transaminase	[9]
A4JTE9	*Burkholderia vietnamiensis*	Aminotransferase	[12]

Q12DH7	*Polaromonas sp.*	Glutamate-1-semialdehyde 2,1-aminomutase	[13,14]
A1B956	*Paracoccus denitrificans*	Aminotransferase	[11,15–17]
F2XBU9	*Vibrio fluvialis*	Pyruvate transaminase	[11,14,18–21]
Q7NWG4	*Chromobacterium violaceum*	Probable aminotransferase	[11,18,19,22–25]
Q9I700	*Pseudomonas aeruginosa*	β-alanine-pyruvate aminotransferase	[19,24,26]
Q7WWK8	*Achromobacter denitrificans*	ω-amino acid:pyruvate transaminase	[18,19,27]
Q9A3Q9	*Caulobacter vibrioides*	ω-amino acid-pyruvate aminotransferase	[2,19,28]
A3EYF7	*Mesorhizobium sp.*	β-transaminase	[19,29,30]
Q3J0Y0	*Rhodobacter sphaeroides*	Putative aminotransferase	[19]
A9CEZ4	*Agrobacterium fabrum*	Aminotransferase	[31]
A4WXQ0	*Rhodobacter sphaeroides*	Aminotransferase	[31]
Q98NJ9	*Mesorhizobium japonicum*	Aminotransferase	[31,32]
Q98L27	*Mesorhizobium japonicum*	Probable aminotransferase	[31,32]
Q98K53	*Mesorhizobium japonicum*	β-alanine-pyruvate transaminase	[31,32]
Q98AI4	*Mesorhizobium japonicum*	Probable aminotransferase	[31,32]
Q98AI1	*Mesorhizobium japonicum*	Aminotransferase	[31,32]
Q98A92	*Mesorhizobium japonicum*	Aminotransferase	[31,32]
Q987Q5	*Mesorhizobium japonicum*	Probable aminotransferase	[31,32]
Q987I6	*Mesorhizobium japonicum*	Family II aminotransferase	[31,32]
Q987B2	*Mesorhizobium japonicum*	Putative aminotransferase	[31,32]
Q98FQ6	*Mesorhizobium japonicum*	Aminotransferase	[32]
Q9AGD3	*Rhizobium leguminosarum*	4-aminobutyrate aminotransferase	[32,33]
A1B9Z3	*Paracoccus denitrificans*	(Hypo)taurine-pyruvate aminotransferase	[34]
Q976K0	*Sulfurisphaera tokodaii*	Acetylornithine/acetyl-lysine aminotransferase	[35]
H8WR05	*Variovorax paradoxus*	β-phenylalanine transaminase	[28,36]
Q9APM5	*Bilophila wadsworthia*	Taurine-pyruvate aminotransferase	[37]
P28269	*Pseudomonas putida*	ω-amino acid-pyruvate aminotransferase	[28,38–40]
U2H8Z1	*Sphingobacterium paucimobilis*	lysine aminotransferase	[41,42]
Q01767	*Streptomyces clavuligerus*	L-lysine-ε-aminotransferase	[43]
Q9EVJ7	*Flavobacterium lutescens*	L-lysine 6-aminotransferase	[44]
P12995	*Escherichia coli*	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	[45]
P9WQ77	*Mycobacterium tuberculosis*	Probable L-lysine-ε-aminotransferase	[46]
Q5SHH5	*Thermus thermophilus*	Acetylornithine/acetyl-lysine aminotransferase	[47]
P22256	*Escherichia coli*	4-aminobutyrate aminotransferase	[48,49]
L7ZI44/A0A0J9X1Q5	*Serratia sp.*	Aminotransferase PigE	[50]
Q5LMU1	*Ruegeria pomeroyi*	Aminotransferase, class III	[38,39]
P40732	*Salmonella typhimurium*	Acetylornithine/succinyldiaminopimelate aminotransferase	[47]
Q3IWE9	*Rhodobacter sphaeroides*	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	[38,39,51]
Q1GD43	*Ruegeria sp.*	Aminotransferase	[38,39]
Q9HV04	*Pseudomonas aeruginosa*	Probable class III aminotransferase	[16]
P18335	*Escherichia coli*	Acetylornithine/succinyldiaminopimelate	[52]

		aminotransferase	
Q07907	*Geobacillus stearothermophilus*	Acetylornithine aminotransferase	
P9WQ81	*Mycobacterium tuberculosis*	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	[53]
P53555	*Bacillus subtilis*	L-Lysine--8-amino-7-oxononanoate transaminase	[53]
Q4H4F5	*Bacillus circulans*	Neamine transaminase	[54]
P56744	*Acinetobacter baumannii*	Diaminobutyrate-2-oxoglutarate aminotransferase	[55]
O52250	*Halomonas elongata*	Diaminobutyrate--2-oxoglutarate transaminase	[56]
D2D3B2	*Sphingopyxis macrogoltabida*	Aminopentol aminotransferase	[57]
Q8NT73	*Corynebacterium glutamicum*	Glutamate-1-semialdehyde 2,1-aminomutase	[58]
B0VH76	*Cloacamonas acidaminovorans*	3-aminobutyryl-CoA aminotransferase	[59]
Q53U08	*Streptomyces fradiae*	Neamine transaminase	[54]
P42588	*Escherichia coli*	Putrescine aminotransferase	[60]
Q6L741	*Streptomyces kanamyceticus*	2'-deamino-2'-hydroxyneamine transaminase	[61]
P50457	*Escherichia coli*	4-aminobutyrate aminotransferase	[62]
P46395	*Corynebacterium glutamicum*	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	[58]

6. Substrate spectra of selected TA with hypotaurine as amine donor.

Substrate spectra of 4 purified TA (Uniprot ID: A9CV07, A0KEV7, IOJ1L1 and Q48AP6) was explored using hypotaurine as amine donor. All experiments were performed in triplicate in 96-well microplates. Initial rates were measured using the direct assay with 20 mM HPT, 2 mM acceptor, 0.1 mM PLP, 1 mM DTNB, 5% DMSO and 1-80 µg/well of purified TA adjusted to measure an activity within the calibration range. TA activity in the well was calculated using the following equation: Act (U/well) = ((dOD/dt) / (14150*0.59)*200). Results are reported in table 2 of the manuscript.

7. Thermostability study of A9CV07 from *Hoeflea phototrophica*

All experiments were performed in triplicate in 96-well microplates. A solution of 2.4 $mg.mL^{-1}$ of A9CV07 (0.2 mL) in 50 mM KP buffer (pH 7.5) was incubated for 100 h at 30 or 40 °C. At regular time intervals, a 10 µL aliquot was added to 90 µL buffer and 10 µL of the diluted solution was used for TA activity measurement using the direct assay with 20 mM HPT, 2 mM Pyr, 0.1 mM PLP, 1 mM DTNB. TA activity in the well was calculated using the following equation: Act (U/well) = ((dOD/dt) / (14150*0.59)*200). Results are reported in figure S2.

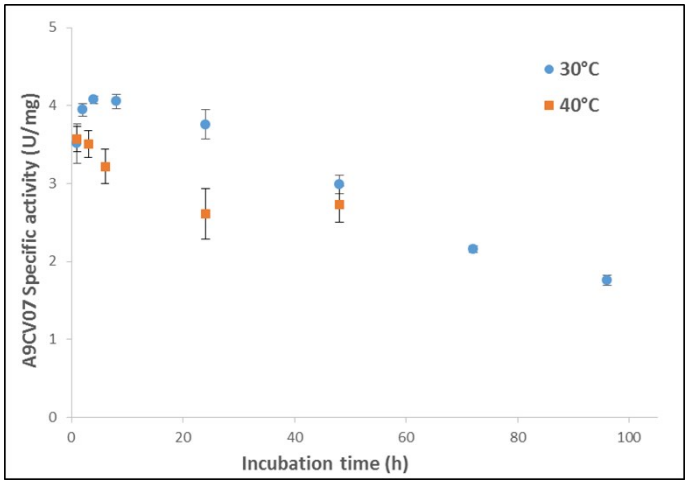

Figure S2. Thermostability of A9CV07.

8. Kinetic study of A9CV07

All measurements were done in triplicate in 96-well microplates at 30 °C using the direct assay with 1 mM DTNB, 0.1 mM PLP, 45 nM (2.35 µg.mL^{-1}) of purified A9CV07 and variable concentrations of HPT and Pyr. Apparent Km relative to Pyr (Km_{Pyr}) was determined using 20 mM HPT and 0.1-5 mM Pyr. Apparent Km relative to HPT (Km_{HPT}) was determined using 2 mM Pyr and 1-50 mM HPT. Kinetic parameters values and standard errors were calculated from the Hanes-Woolf plot ((S)/vi = 1/k_{cat}(E)*1/(S) + Km/k_{cat}(E)) according to the least-squares method and Gauss's error propagation law*:* Km_{HPT} = 22.0 ± 1.2 mM, Km_{Pyr} = 0.25 ± 0.03 mM, k_{cat} = 181 ± 7 min^{-1} (value determined with variable HPT concentration). Experimental plots are presented in figure S3.

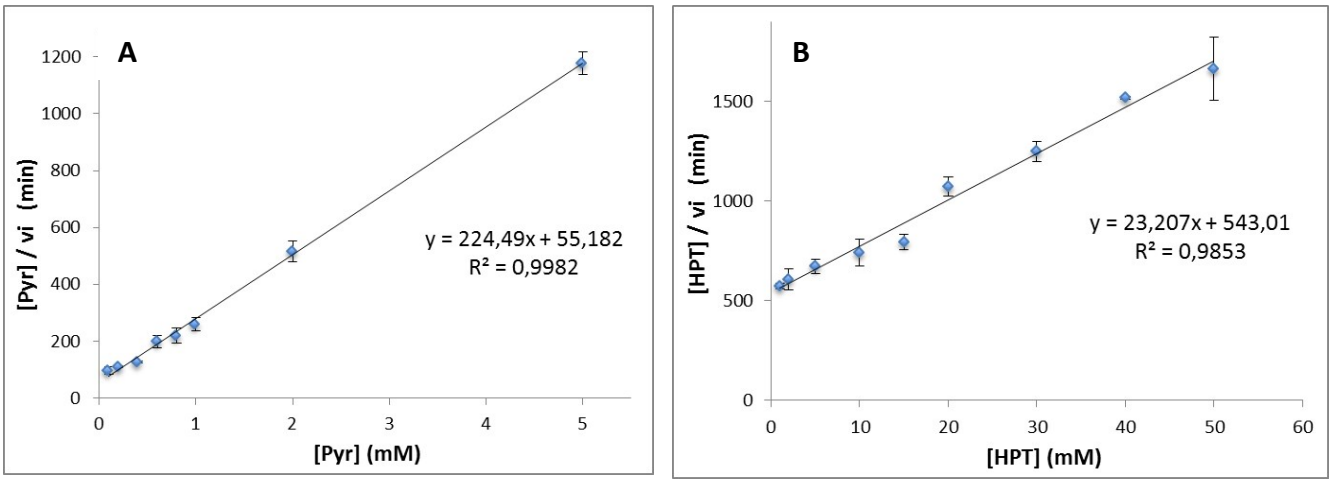

Figure S3. Hanes-Woolf plots of A9CV07 activity at variable substrate concentrations measured with the direct assay. A: variable [Pyr] and fixed 20 mM HPT; B: variable [HPT] and fixed 2 mM Pyr.

9. Comparison of the best hits sequences.

Figure S4 presents the identity matrix including the 20 best hits detected with each substrate, whereas figure S5 compiles the screening results including the 20 best hits with each couple of substrate, supplemented with hits displaying an activity above 0.2 mU/well. The Philogenetic tree was constructed using iTOL.

	D4YMU7	D4XGV2	F0SJ00	G2T7Q7	Q48AP6	Q47WV7	E1V5R2	D0KX56	B1KH99	A4G7S3	B2TF42	A0KEV7	E1SVL1	A0P1C1	A9CF07	Q2JYL2	L8QQZ4	A4IMV2	I0JLI1	A9CV07	B8J2Z6	Q13M75	A3SIW8	A9EB31	Q1MXW4	B9R1U5	B9K5I2	E1V9D2	A0NZD8	A4ABK4	C6XP14	B9JUL0	B9Z0Z6	A7HSN3	A3SLG7	A0KQL1	E1SVK1	A0P0G6	A3K8H0	B8G642	B9L0N2	J7GK03	A9DZF6	B2JR37	C4G8S3	C0BVS9	C6LGL6	A8RJD7	D9R961	A1HTU7	B5Y8A4
D4YMU7	100	24	24	22	22	20	21	20	22	21	22	22	23	22	22	23	20	18	19	18	22	25	19	19	22	21	19	20	23	21	19	21	18	20	21	21	19	23	24	23	22	26	29	29	22	22	24	22	24	28	26
D4XGV2	24	100	43	46	42	44	45	46	48	49	50	50	50	51	49	50	31	32	30	26	27	32	30	28	31	33	30	29	32	33	32	36	34	32	33	34	33	33	33	32	36	26	28	30	30	29	30	30	29	29	32
F0SJ00	24	43	100	51	46	47	49	50	51	49	51	51	53	54	51	50	35	34	31	29	28	32	33	32	33	31	31	31	33	35	33	32	33	36	34	37	36	32	34	35	37	27	27	30	27	29	28	30	30	33	32
G2T7Q7	22	46	51	100	63	65	67	54	54	51	55	58	55	55	52	56	31	30	30	27	26	34	29	27	31	31	30	29	33	35	31	36	31	33	35	35	33	33	34	34	34	26	28	28	27	27	28	28	27	31	30
Q48AP6	22	42	46	63	100	69	70	52	50	47	50	53	49	53	50	50	34	29	31	25	26	29	29	26	32	27	27	30	30	33	33	32	31	32	33	34	33	30	31	33	31	24	26	25	27	26	26	25	26	28	28
Q47WV7	20	44	47	65	69	100	78	54	54	50	51	54	52	54	52	51	32	29	28	25	26	30	29	29	30	27	27	29	33	32	32	33	31	32	33	34	34	31	34	33	32	25	26	26	27	25	26	25	26	29	31
E1V5R2	21	45	49	67	70	78	100	54	55	50	50	55	54	54	51	52	31	29	30	26	26	32	30	30	32	30	29	33	34	36	34	36	33	34	37	37	36	32	33	36	36	27	29	29	27	27	28	28	27	30	31
D0KX56	20	46	50	54	52	54	54	100	58	61	63	61	60	60	60	61	35	35	34	29	28	30	30	28	33	31	29	30	33	36	33	32	32	35	34	36	36	34	34	35	38	27	27	29	27	27	27	28	27	31	30
B1KH99	22	48	51	54	50	54	55	58	100	57	60	62	62	60	62	62	32	31	30	28	29	31	30	28	34	32	31	32	32	34	33	36	33	34	35	36	34	34	33	33	36	25	27	28	28	31	29	31	30	33	32
A4G7S3	21	49	49	51	47	50	50	61	57	100	61	63	62	61	58	61	33	31	31	27	28	32	32	29	31	31	33	30	35	37	32	35	34	36	33	38	39	33	32	33	37	27	28	30	26	29	28	26	28	35	32
B2TF42	22	50	51	55	50	51	50	63	60	61	100	66	62	65	64	61	34	33	31	26	28	30	30	28	31	32	30	31	33	34	30	34	32	34	32	36	37	31	34	32	36	24	27	28	26	29	27	27	27	31	32
A0KEV7	22	50	51	58	53	54	55	61	62	63	66	100	73	67	65	66	34	33	32	29	28	33	31	29	34	32	31	34	35	37	34	36	35	35	34	37	35	33	33	34	38	26	28	30	26	29	29	28	27	32	32
E1SVL1	23	50	53	55	49	52	54	60	62	62	62	73	100	65	64	68	33	33	32	29	29	34	30	29	35	32	31	33	37	38	33	36	34	36	34	36	35	33	35	37	37	26	28	29	27	29	28	28	28	32	32
A0P1C1	22	51	54	55	53	54	54	60	60	61	65	67	65	100	71	67	33	32	33	28	28	33	32	31	33	30	32	33	36	38	33	34	35	34	34	37	37	33	35	35	36	26	28	29	28	29	29	31	30	31	30
A9CF07	22	49	51	52	50	52	51	60	62	58	64	65	64	71	100	75	33	32	32	29	29	33	33	32	32	32	34	35	37	32	34	33	35	34	36	35	33	34	35	34	37	28	30	31	27	28	28	28	28	30	30
Q2JYL2	23	50	50	56	50	51	52	61	62	61	61	66	68	67	75	100	33	33	33	29	30	34	33	32	34	33	32	34	37	39	32	37	35	37	36	38	38	34	34	35	37	29	32	31	26	30	29	29	29	32	31
L8QQZ4	20	31	35	31	34	32	31	35	32	33	34	34	33	33	33	33	100	67	66	40	41	35	35	35	35	33	33	33	32	33	34	34	33	35	34	35	36	37	34	41	40	27	29	30	27	30	28	31	29	34	30
A4IMV2	18	32	34	30	29	29	29	35	31	31	33	33	33	32	32	33	67	100	68	39	40	33	37	34	36	34	33	36	34	33	36	35	32	36	36	35	36	37	34	40	39	26	30	28	27	31	29	31	31	34	32
I0JLI1	19	30	31	30	31	28	30	34	30	31	31	32	32	33	32	33	66	68	100	38	42	32	33	34	36	31	30	32	31	33	33	32	32	34	35	34	33	35	31	36	35	26	29	26	25	30	27	29	28	30	28
A9CV07	18	26	29	27	25	25	26	29	28	27	26	29	29	28	29	29	40	39	38	100	57	31	33	31	30	29	33	31	29	31	34	34	31	35	29	30	30	32	32	36	34	22	23	24	24	26	25	26	26	30	27
B8J2Z6	22	27	28	26	26	26	26	28	29	28	28	28	29	28	29	30	41	40	42	57	100	34	36	34	33	35	34	31	31	32	35	33	31	32	32	33	33	33	32	36	37	22	28	24	27	30	29	28	27	31	28
Q13M75	25	32	32	34	29	30	32	30	31	32	30	33	34	33	33	34	35	33	32	31	34	100	35	35	32	32	36	32	35	35	32	37	31	34	34	36	32	36	36	39	35	28	33	29	31	31	34	32	32	34	33
A3SIW8	19	30	33	29	29	29	30	30	30	32	30	31	30	32	33	33	35	37	33	33	36	35	100	67	39	42	44	36	37	41	38	41	38	40	38	41	41	34	32	38	35	24	26	27	26	29	30	28	27	29	26
A9EB31	19	28	32	27	26	29	30	28	28	29	28	29	29	31	32	32	35	34	34	31	34	35	67	100	40	40	43	35	36	39	40	40	37	39	38	43	38	33	33	37	33	25	28	27	25	27	28	27	26	27	26
Q1MXW4	22	31	33	31	32	30	32	33	34	31	31	34	35	33	33	34	35	36	36	30	33	32	39	40	100	42	44	39	37	38	39	40	37	40	39	39	38	36	32	37	34	22	28	25	23	26	26	28	25	29	26
B9R1U5	21	33	31	31	27	27	30	31	32	31	32	32	32	30	32	33	33	34	31	29	35	32	42	40	42	100	51	37	40	37	32	39	35	37	39	36	35	34	35	36	33	23	27	27	26	26	26	26	24	27	24
B9K5I2	19	30	31	30	27	27	29	29	31	33	30	31	31	32	32	32	33	33	30	33	34	36	44	43	44	51	100	35	38	38	35	38	38	40	38	37	37	39	35	40	36	25	28	28	26	29	32	31	30	25	23
E1V9D2	20	29	31	29	30	29	33	30	32	30	31	34	33	33	34	34	33	36	32	31	31	32	36	35	39	37	35	100	38	40	38	37	36	36	39	40	38	35	32	39	37	22	27	27	24	25	27	26	25	27	23
A0NZD8	23	32	33	33	30	33	34	33	32	35	33	35	37	36	35	37	32	34	31	29	31	35	37	36	37	40	38	38	100	38	35	38	36	38	42	42	41	35	35	38	36	26	29	34	24	27	28	26	26	28	28
A4ABK4	21	33	35	35	33	32	36	36	34	37	34	37	38	38	37	39	33	33	33	31	32	35	41	39	38	37	38	40	38	100	44	49	46	46	49	52	51	33	34	39	32	26	27	29	31	31	31	29	30	29	28
C6XP14	19	32	33	31	33	32	34	33	33	32	30	34	33	33	32	32	34	36	33	34	35	32	38	40	39	32	35	38	35	44	100	46	53	48	51	50	47	34	31	41	35	22	25	26	26	30	27	27	27	28	27
B9JUL0	21	36	32	36	32	33	36	32	36	35	34	36	36	34	34	37	34	35	32	34	33	37	41	40	40	39	38	37	38	49	46	100	52	49	53	54	54	37	35	43	33	23	27	26	27	28	29	27	26	31	26
B9Z0Z6	18	34	33	31	31	31	33	32	33	34	32	35	34	35	33	35	33	32	32	31	31	31	38	37	37	35	38	36	36	46	53	52	100	50	52	57	55	33	33	38	34	20	27	25	25	27	27	28	28	27	27
A7HSN3	20	32	36	33	32	32	34	35	34	36	34	35	36	34	35	37	35	36	34	35	32	34	40	39	40	37	40	36	38	46	48	49	50	100	54	56	54	35	33	42	35	23	28	27	28	29	28	30	29	28	26
A3SLG7	21	33	34	35	33	33	37	34	35	33	32	34	34	34	34	36	34	36	35	29	32	34	38	39	39	38	39	42	49	51	53	52	54	100	61	58	35	33	39	36	22	28	28	26	28	29	30	28	30	27	27
A0KQL1	21	34	37	35	34	34	37	36	36	38	36	37	36	37	36	38	35	35	34	30	33	36	41	43	39	36	37	40	42	52	50	54	57	56	61	100	72	35	36	43	37	24	28	28	29	30	28	29	29	29	28
E1SVK1	19	33	36	33	33	34	36	36	34	39	37	35	35	37	35	38	36	36	33	30	33	32	41	38	38	35	37	38	41	51	47	54	55	54	58	72	100	34	35	40	36	23	27	26	26	28	26	28	26	26	26
A0P0G6	23	33	32	33	30	31	32	34	34	33	31	33	33	33	33	34	37	37	35	32	33	36	34	33	36	34	39	35	35	33	34	37	33	35	35	35	34	100	49	40	36	28	30	29	28	32	30	29	30	31	31
A3K8H0	24	33	34	34	31	34	33	34	33	32	34	33	35	35	34	34	34	31	32	32	36	32	32	36	32	35	35	33	33	34	31	35	33	33	33	36	35	49	100	39	34	27	30	28	27	29	28	30	30	31	29
B8G642	23	32	35	34	33	33	36	35	33	33	32	34	37	35	35	35	41	40	36	36	36	39	38	37	37	36	40	39	38	39	41	43	38	42	39	43	40	40	39	100	44	25	32	30	28	30	28	29	29	34	29
B9L0N2	22	36	37	34	31	32	36	38	36	37	36	38	37	36	37	37	40	39	35	34	37	35	35	33	34	33	36	37	36	32	35	33	34	35	36	37	36	36	34	44	100	27	33	34	27	32	30	31	30	33	29
J7GK03	26	26	27	26	24	25	27	27	25	27	24	26	26	26	28	29	27	26	26	22	22	28	24	25	22	23	25	22	26	26	22	23	20	23	22	24	23	28	27	25	27	100	47	46	28	30	28	30	29	31	29
A9DZF6	29	28	27	28	26	26	29	27	27	28	27	28	28	28	30	32	29	30	29	23	28	33	26	28	28	27	28	27	29	27	25	27	27	28	28	28	27	30	30	32	33	47	100	58	29	30	31	33	33	34	30
B2JR37	29	30	30	28	25	26	29	29	28	30	28	30	29	29	31	31	30	28	26	24	24	29	27	27	25	27	28	27	34	29	26	26	25	27	28	28	26	29	28	30	34	46	58	100	32	33	34	36	36	36	32
C4G8S3	22	30	27	27	27	27	27	27	28	26	26	26	27	28	27	26	27	27	25	24	27	31	26	25	23	26	26	24	24	31	26	27	25	28	26	29	26	28	27	28	27	28	29	32	100	53	55	52	51	37	33
C0BVS9	22	29	29	27	26	25	27	27	31	29	29	29	29	29	28	30	30	31	30	26	30	31	29	27	26	26	29	25	27	31	30	28	27	29	28	30	28	32	29	30	32	30	30	33	53	100	59	53	58	39	35
C6LGL6	24	30	28	28	26	26	28	27	29	28	27	29	28	29	28	29	27	27	25	29	34	30	28	26	26	32	27	28	31	27	29	27	28	29	28	26	30	28	28	30	28	31	34	55	59	100	60	64	35	35
A8RJD7	22	30	30	28	25	25	28	28	31	26	27	28	28	31	28	29	31	31	29	26	28	32	28	27	28	26	31	26	26	29	27	27	28	30	30	29	28	29	30	29	30	29	31	30	53	53	60	100	72	37	35
D9R961	24	29	30	27	26	26	27	27	30	28	27	27	28	30	28	29	29	31	28	26	27	32	27	26	25	24	30	25	26	30	27	26	28	29	28	29	26	30	30	29	30	29	33	36	51	58	64	72	100	39	37
A1HTU7	28	29	33	31	28	29	30	31	33	35	31	32	32	31	30	32	34	34	30	30	31	34	29	27	29	27	25	27	28	29	28	31	27	28	30	29	26	31	31	34	33	31	34	36	37	39	35	37	39	100	53
B5Y8A4	26	32	32	30	28	31	31	30	32	32	32	32	32	30	30	31	30	32	28	27	28	33	26	26	26	24	23	23	28	28	27	26	27	26	27	28	26	31	29	29	29	29	30	32	33	35	35	35	37	53	100

Figure S4. Identity matrix of selected hits from tables 1 and 3.

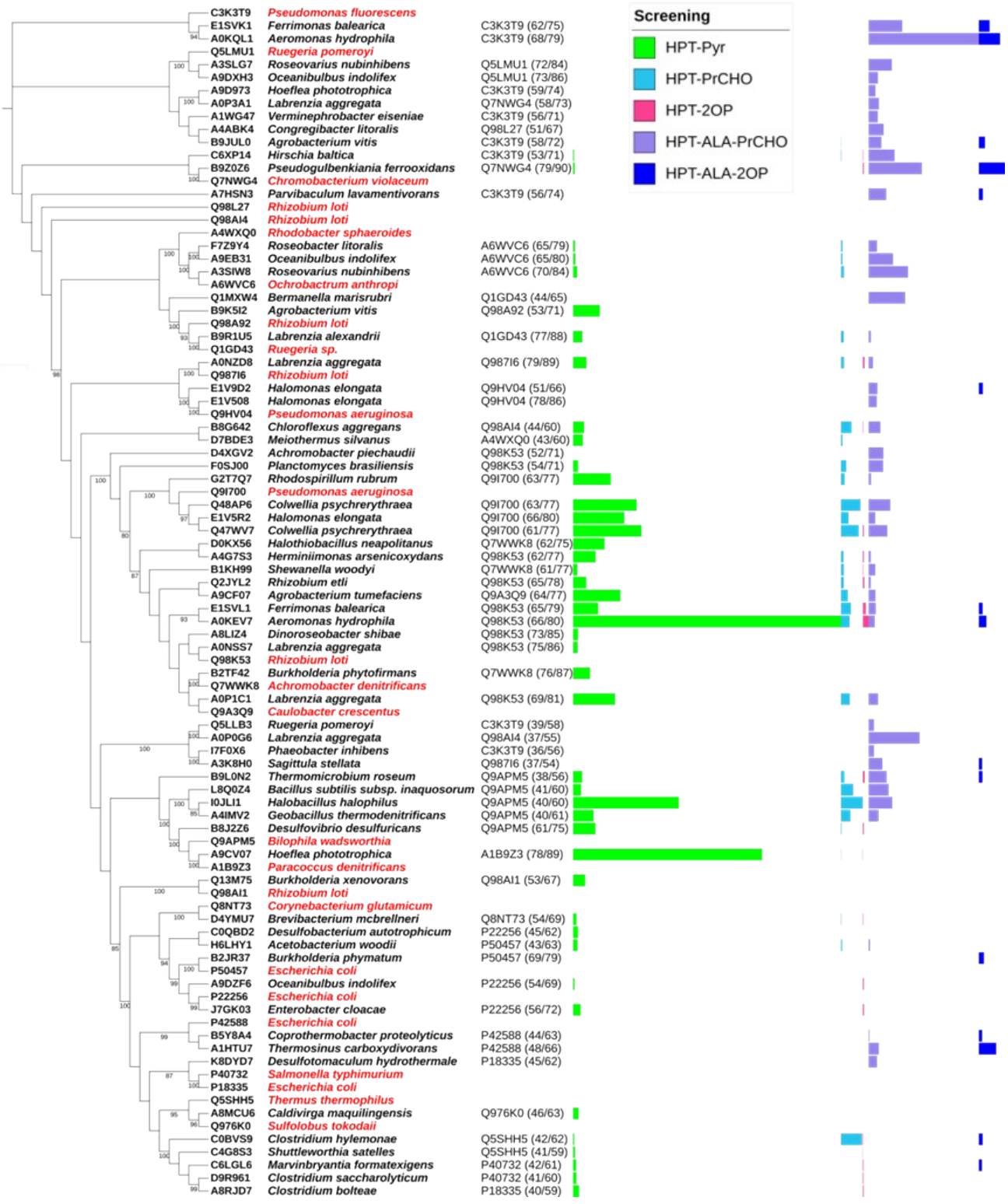

Figure S5. Compilation of selected screening results.

TA indicated in red correspond to homologous enzymes from the reference set. For each hit the closest enzyme from the reference set is indicated with identity/homology within brackets.

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