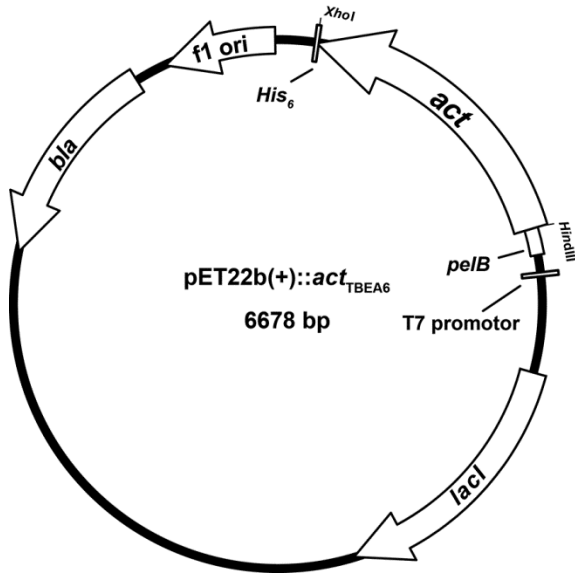
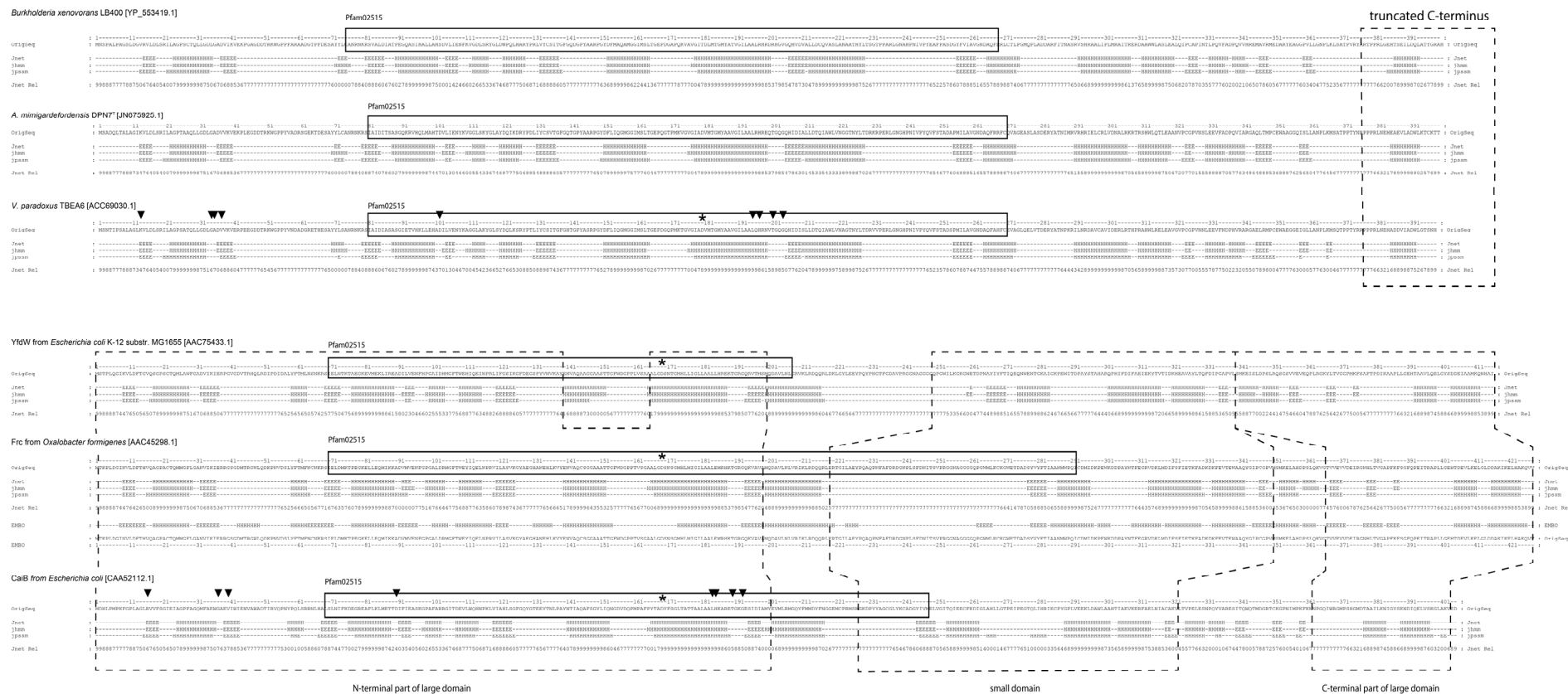


Supplemental Material



Supplemental Fig. S1

Physical map of pET22b(+)::act_{TBEA6}. *Act*, succinyl-CoA:3-sulfino-propionate CoA-transferase from *V. paradoxus* strain TBEA6; *bla*, β-lactamase; *pelB*, pelB signal sequence for potential periplasmic localization; f1 ori, f1 origin of replication.



Supplemental Fig. S2

Secondary structure prediction of ACTBEA6-orthologues. Amino acid sequences were subjected to secondary structure prediction using the Jpred3 server (44). Due to their available solved crystal structures, formyl-CoA:oxalate CoA-transferase from *E. coli* (YfdW) (27, 28), its orthologue Frc from *O. formigenes* (20, 26) and crotonobetainyl-CoA:carnitine CoA transferase from *E. coli* (CaiB) (29, 30) as members of the CoA-transferase III family were included for comparison. For them, the N-terminal and C-terminal part of the large domain as well as the small domain are indicated

(dashed outline). For each sequence amino acids annotated as Pfam02515 (CoA-transferase family III) are contoured with a solid line. The truncated C-terminus found in Act_{TBEA6} is highlighted by an additional box. Jnet, final secondary structure prediction for the amino acid query sequence; H, α -helix; E, extended β -sheet; jhmm, Jnet hmm (hidden Markov model) profile prediction; jpssm; Jnet PSIBLAST pssm (position specific scoringmatrix) profile prediction. JnetRel, Jnet reliability of prediction accuracy. The latter ranges from 0 to 9 and higher values are better.

Supplemental Fig. S3

Organism	Accession	Gene	Start	End	Score	
<i>Chloroflexus aurantiacus</i>	J-10-f1	(YP_001633818.1)	1	58		
<i>Burkholderia xenovorans</i>	LB400	(YP_559695.1)	1	58		
<i>A. mimigardefordensis</i>	DPN7	(JN675925.1)	1	58		
<i>V. paradoxus</i>	TBEA6	(ACC69030.2)	1	58		
<i>V. paradoxus</i>	EPS	(YP_004152464.1)	1	58		
<i>V. paradoxus</i>	S110	(YP_002942048.1)	1	58		
<i>V. paradoxus</i>	B4	(contig47_RVVP05211)	1	58		
<i>Verminephrobacter eiseniae</i>	EF01-2	(YP_996507.1)	1	58		
<i>Pseudomonas putida</i>	KT2240	(NE_742328.1)	1	58		
<i>Burkholderia xenovorans</i>	LB400	(YP_553419.1)	1	58		
<i>Ralstonia eutropha</i>	H16	(YP_727217.1)	1	58		
<i>Chloroflexus aurantiacus</i>	J-10-f1	(BY35475.1)	1	58		
<i>E. coli</i>	K-12 substr.	MG1655	(AAC75433.1)	1	58	
<i>Oxalobacter formigenes</i>	(AAC45298.1)	1	58			
<i>Escherichia coli</i>	(CA52112.1)	1	58			
<i>Chlostridium scindens</i>	(AAC45415.1)	1	58			
<i>Chloroflexus aurantiacus</i>	J-10-f1	(YP_001633818.1)	52	131		
<i>Burkholderia xenovorans</i>	LB400	(YP_559695.1)	52	131		
<i>A. mimigardefordensis</i>	DPN7	(JN675925.1)	56	131		
<i>V. paradoxus</i>	TBEA6	(ACC69030.2)	56	131		
<i>V. paradoxus</i>	EPS	(YP_004152464.1)	54	131		
<i>V. paradoxus</i>	S110	(YP_002942048.1)	56	131		
<i>V. paradoxus</i>	B4	(contig47_RVVP05211)	55	131		
<i>Verminephrobacter eiseniae</i>	EF01-2	(YP_996507.1)	91	131		
<i>Pseudomonas putida</i>	KT2240	(NE_742328.1)	51	131		
<i>Burkholderia xenovorans</i>	LB400	(YP_553419.1)	58	131		
<i>Ralstonia eutropha</i>	H16	(YP_727217.1)	51	131		
<i>Chloroflexus aurantiacus</i>	J-10-f1	(BY35475.1)	57	131		
<i>E. coli</i>	K-12 substr.	MG1655	(AAC75433.1)	50	131	
<i>Oxalobacter formigenes</i>	(AAC45298.1)	57	131			
<i>Escherichia coli</i>	(CA52112.1)	57	131			
<i>Chlostridium scindens</i>	(AAC45415.1)	58	131			
<i>Chloroflexus aurantiacus</i>	J-10-f1	(YP_001633818.1)	131	217		
<i>Burkholderia xenovorans</i>	LB400	(YP_559695.1)	139	217		
<i>A. mimigardefordensis</i>	DPN7	(JN675925.1)	144	217		
<i>V. paradoxus</i>	TBEA6	(ACC69030.1)	144	217		
<i>V. paradoxus</i>	EPS	(YP_004152464.1)	142	217		
<i>V. paradoxus</i>	S110	(YP_002942048.1)	144	217		
<i>V. paradoxus</i>	B4	(contig47_RVVP05211)	143	217		
<i>Verminephrobacter eiseniae</i>	EF01-2	(YP_996507.1)	179	217		
<i>Pseudomonas putida</i>	KT2240	(NE_742328.1)	139	217		
<i>Burkholderia xenovorans</i>	LB400	(YP_553419.1)	146	217		
<i>Ralstonia eutropha</i>	H16	(YP_727217.1)	139	217		
<i>Chloroflexus aurantiacus</i>	J-10-f1	(BY35475.1)	138	217		
<i>E. coli</i>	K-12 substr.	MG1655	(AAC75433.1)	133	217	
<i>Oxalobacter formigenes</i>	(AAC45298.1)	133	217			
<i>Escherichia coli</i>	(CA52112.1)	134	217			
<i>Chlostridium scindens</i>	(AAC45415.1)	134	217			
<i>Chloroflexus aurantiacus</i>	J-10-f1	(YP_001633818.1)	215	272		
<i>Burkholderia xenovorans</i>	LB400	(YP_559695.1)	229	272		
<i>A. mimigardefordensis</i>	DPN7	(JN675925.1)	230	272		
<i>V. paradoxus</i>	TBEA6	(ACC69030.2)	230	272		
<i>V. paradoxus</i>	EPS	(YP_004152464.1)	232	272		
<i>V. paradoxus</i>	S110	(YP_002942048.1)	234	272		
<i>V. paradoxus</i>	B4	(contig47_RVVP05211)	233	272		
<i>Verminephrobacter eiseniae</i>	EF01-2	(YP_996507.1)	269	272		
<i>Pseudomonas putida</i>	KT2240	(NE_742328.1)	229	272		
<i>Burkholderia xenovorans</i>	LB400	(YP_553419.1)	232	272		
<i>Ralstonia eutropha</i>	H16	(YP_727217.1)	229	272		
<i>Chloroflexus aurantiacus</i>	J-10-f1	(BY35475.1)	224	272		
<i>E. coli</i>	K-12 substr.	MG1655	(AAC75433.1)	219	272	
<i>Oxalobacter formigenes</i>	(AAC45298.1)	219	272			
<i>Escherichia coli</i>	(CA52112.1)	218	272			
<i>Chlostridium scindens</i>	(AAC45415.1)	218	272			
<i>Chloroflexus aurantiacus</i>	J-10-f1	(YP_001633818.1)	273	360		
<i>Burkholderia xenovorans</i>	LB400	(YP_559695.1)	282	360		
<i>A. mimigardefordensis</i>	DPN7	(JN675925.1)	283	360		
<i>V. paradoxus</i>	TBEA6	(ACC69030.2)	283	360		
<i>V. paradoxus</i>	EPS	(YP_004152464.1)	285	360		
<i>V. paradoxus</i>	S110	(YP_002942048.1)	287	360		
<i>V. paradoxus</i>	B4	(contig47_RVVP05211)	286	360		
<i>Verminephrobacter eiseniae</i>	EF01-2	(YP_996507.1)	322	360		
<i>Pseudomonas putida</i>	KT2240	(NE_742328.1)	282	360		
<i>Burkholderia xenovorans</i>	LB400	(YP_553419.1)	282	360		
<i>Ralstonia eutropha</i>	H16	(YP_727217.1)	282	360		
<i>Chloroflexus aurantiacus</i>	J-10-f1	(BY35475.1)	278	360		
<i>E. coli</i>	K-12 substr.	MG1655	(AAC75433.1)	294	360	
<i>Oxalobacter formigenes</i>	(AAC45298.1)	294	360			
<i>Escherichia coli</i>	(CA52112.1)	306	360			
<i>Chlostridium scindens</i>	(AAC45415.1)	275	360			
<i>Chloroflexus aurantiacus</i>	J-10-f1	(YP_001633818.1)	361	409		
<i>Burkholderia xenovorans</i>	LB400	(YP_559695.1)	365	409		
<i>A. mimigardefordensis</i>	DPN7	(JN675925.1)	368	409		
<i>V. paradoxus</i>	TBEA6	(ACC69030.2)	368	409		
<i>V. paradoxus</i>	EPS	(YP_004152464.1)	371	409		
<i>V. paradoxus</i>	S110	(YP_002942048.1)	373	409		
<i>V. paradoxus</i>	B4	(contig47_RVVP05211)	372	409		
<i>Verminephrobacter eiseniae</i>	EF01-2	(YP_996507.1)	408	409		
<i>Pseudomonas putida</i>	KT2240	(NE_742328.1)	365	409		
<i>Burkholderia xenovorans</i>	LB400	(YP_553419.1)	370	409		
<i>Ralstonia eutropha</i>	H16	(YP_727217.1)	370	409		
<i>Chloroflexus aurantiacus</i>	J-10-f1	(BY35475.1)	361	409		
<i>E. coli</i>	K-12 substr.	MG1655	(AAC75433.1)	361	409	
<i>Oxalobacter formigenes</i>	(AAC45298.1)	361	409			
<i>Escherichia coli</i>	(CA52112.1)	361	409			
<i>Chlostridium scindens</i>	(AAC45415.1)	360	409			

Supplemental Fig. S3

Multiple sequence alignment of Act_{TBEA6} and orthologues from various related species.

Identical amino acids are shaded in dark-grey. Similar amino acids are shaded in light-grey.

Accession numbers are given in parentheses. Amino acid residues considered to be highly conserved throughout class III CoA-transferases (26) are indicated with a hash. Residues corresponding to Asp169, which represents the putative active site key residue in formyl-CoA-transferase (20), are highlighted with a frame. The glycine-rich loop of formyl-CoA transferases is highlighted as well.

Supplemental Table S1.

oligonucleotide	sequence (5'-3') ^a	Source or reference
Primers for PCR:		
<i>act_HindIII_For</i>	<u>AAGCTT</u> ATGAGCAACACCATTCTAGC	MWG Biotech AG, Ebersberg, Germany
<i>act_XhoI_Rev_oS</i>	CTC <u>GAGGTGGTTG</u> AGGTTCCGAGC	MWG Biotech AG, Ebersberg, Germany
<i>XbaI_upAct</i>	AATCTAGAGGATCGCAGTCTCTCGCGC	MWG Biotech AG, Ebersberg, Germany
<i>NdeI_upAct</i>	AAA <u>CATATG</u> CAATCATCTCCAAAAGGCC	MWG Biotech AG, Ebersberg, Germany
<i>NdeI_downAct</i>	AAAACATATGTAGAAGGAATATCACCATG	MWG Biotech AG, Ebersberg, Germany
<i>XbaI_downAct</i>	AAATCTAGACATCGACTTGTGGACCTTC	MWG Biotech AG, Ebersberg, Germany
Primers for sequencing:		
M13 <i>Forward</i>	GTAAAACGACGGCCAG	MWG Biotech AG, Ebersberg, Germany
M13 <i>Reverse</i>	CAGGAAACAGCTATGAC	MWG Biotech AG, Ebersberg, Germany
<i>act_oS_451_For</i>	CCTGGCTACGACTTCCTG	MWG Biotech AG, Ebersberg, Germany
<i>act_oS_451_Rev</i>	CAGGAAGTCGTAGCCAGG	MWG Biotech AG, Ebersberg, Germany
<i>act_oS_876_For</i>	CTTCGGATTGTGGCGTAGC	MWG Biotech AG, Ebersberg, Germany
<i>act_oS_876_Rev</i>	GCTACGCCACAAATCCGAAG	MWG Biotech AG, Ebersberg, Germany
Primers for screening of <i>V. paradoxus</i> TBEA6 Δact_{TBEA6} mutant		
<i>up_act_proof</i>	CGAAGTCGGGCGGCCGATAGC	MWG Biotech AG, Ebersberg, Germany
<i>down_act_proof</i>	GCGCTGGGCGTTGTAGGCGG	MWG Biotech AG, Ebersberg, Germany
<i>act_int_fwd</i>	GCAGATGTTGTCAAAGTCGAGCG	MWG Biotech AG, Ebersberg, Germany
<i>act_int_rev</i>	CGAGAATCATCGGCGAGTCG	MWG Biotech AG, Ebersberg, Germany
Primers for genomewalking		
ActWalk1	CGTGACAAATCCAGCACTTTGAGGC	MWG Biotech AG, Ebersberg, Germany
ActSeq1	GAATGGTGTGCTCATCAATCATC	MWG Biotech AG, Ebersberg, Germany
ActWalk2	CTTTCGTGTTTCAAGCCAAAAAGCG	MWG Biotech AG, Ebersberg, Germany
ActSeq2	GAAATTGGCCGAAGAGCAATTC	MWG Biotech AG, Ebersberg, Germany
ActSeq3rev	GAATTGCTCTTCGGCCAATTC	MWG Biotech AG, Ebersberg, Germany
ActSeq4rev	CAAAAGCCAAAGCTACCGCG	MWG Biotech AG, Ebersberg, Germany
ActSeq5rev	CGTTATCCCTGTCTATACGCGGC	MWG Biotech AG, Ebersberg, Germany
Act_Walk3	GGTTTGCGGCTCATTGAAATCCC	MWG Biotech AG, Ebersberg, Germany
ActSeq6	GAATTTATCCCCTATAGGCAGCCG	MWG Biotech AG, Ebersberg, Germany
ActWalk5	GGAGAAGTACAGCTACGCCACGCC	MWG Biotech AG, Ebersberg, Germany
Act_Seq7	GATCGGGCACGATGCAACACC	MWG Biotech AG, Ebersberg, Germany