Supplementary Materials

Insights into Protein and Amino Acid Metabolism of *Thermoanaerobacter mathranii*

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Supplemental Table S1. API ZYM assay results for *Thermoanaerobacter mathranii* as compared to *T. pseudethanolicus*.

Enzyme Activity	*T. mathranii*	*T. pseudethanolicus*[1]
Alkaline phosphatase	-	+
Esterase (C4)	+	+
Lipase esterase (C8)	-	-
Lipase (C14)	-	-
Leu arylamidase	-	-
Val arylamidase	-	-
Cys arylamidase	-	-
Trypsin	+	-
α-Chymotrypsin	-	-
Acid phosphatase	+	+
Naphthol-AS-BI-phosophohydrolase	+	+
α -Galactosidase	-	-
β-Galactosidase	-	+
β-Glucuronidase	-	-
α-Glucosidase	-	-
β-Glucosidase	-	+
N-Acetyl-β-glucosaminidase	-	-
α-Mannosidase	-	-
α-Fucosidase	-	-

[1] Data from (Scully, 2019).

Supplemental Table S2. Comparison of end products formed from branched-chain amino acid fermentation by *Caldanaerobacter* and *Thermoanaerobacter* strains. M = co-culture with hydrogenotrophic methanogen, NA—Not applicable.

Strain	Substrate	RCOOH (mM)	ROH (mM)	ROH/RCOOH ratio	Reference
T. mathranii (DSM 11426)	Isoleucine + S_2O_3	8.2	3.1	0.38	This study
	Leucine + S_2O_3	8.6	2.8	0.33	
	Valine + S_2O_3	10.9	1.7	0.16	
T. pseudethanolicus (DSM 2355)	Isoleucine + S_2O_3	9.8	0.7	0.07	(Scully & Orlygsson, 2020)
	Leucine + S_2O_3	10.3	1.2	0.12	
	Valine + S_2O_3	11.2	1.1	0.10	
T. ethanolicus (DSM 2246)	Isoleucine + S_2O_3	6.8	0.6	0.09	(Scully et al., 2015)
	Leucine + S_2O_3	5.1	0.6	0.12	
	Valine + S_2O_3	6.2	0.3	0.05	
T. wiegelii (DSM 10319)	Isoleucine + S_2O_3	13.8	3.0	0.22	(Scully et al., 2015)
	Leucine + S_2O_3	12.0	2.5	0.21	
	Valine + S_2O_3	14.0	1.8	0.13	
T. thermohydrosulfuricus (DSM 567)	Isoleucine + S_2O_3	15.5	1.5	0.10	(Scully et al., 2015)
	Leucine + S_2O_3	10.7	1.8	0.17	
	Valine + S_2O_3	15.0	1.5	0.10	
Thermoanaerobacter strain AK15	Isoleucine + S_2O_3	14.5	1.9	0.13	(Scully et al., 2015)
	Leucine + S_2O_3	13.9	2.1	0.15	
	Valine + S_2O_3	14.0	0.9	0.06	
Thermoanaerobacter strain AK85	Isoleucine + S_2O_3	13.1	2.0	0.15	(Scully & Orlygsson, 2019)
	Leucine + S_2O_3	10.6	1.4	0.13	
	Valine + S_2O_3	12.5	2.1	0.17	
Thermoanaerobacter strain AK68	Isoleucine + S_2O_3	12.8	4.0	0.31	(Scully et al., 2015)
	Leucine + S_2O_3	13.0	2.5	0.15	
	Valine + S_2O_3	12.2	2.3	0.19	
Thermoanaerobacter strain AK90	Isoleucine + S_2O_3	15.9	4.4	0.28	(Scully & Orlygsson, 2015)
	Isoleucine + M	15.6	0.2	0.01	
	Leucine + S_2O_3	9.7	3.5	0.36	
	Leucine + M	11.7	0.4	0.03	
	Valine + S_2O_3	19.2	1.8	0.09	
	Valine + M	12.3	<0.1	NA	
Thermoanaerobacter strain AK152	Isoleucine + S_2O_3	12.7	3.2	0.25	(Scully et al., 2015)

	Leucine + S_2O_3	8.8	2.3	0.26	
	Valine + S_2O_3	13.8	1.6	0.12	
Thermoanaerobacter brockii (DSM 1457)	Isoleucine + S_2O_3	14.4	1.2	0.08	(Scully & Orlygsson, 2014)
	Isoleucine + M	15.4	<0.1	NA	
	Leucine + S_2O_3	13.6	1.5	0.11	
	Leucine + M	15.9	<0.1	NA	
	Valine + S_2O_3	11.0	1.3	0.12	
	Valine + M	14.8	<0.1	NA	
C. subterraneus subsp. *yonseiensis* (DSM 13777)	Isoleucine + S_2O_3	17.4	4.0	0.23	(Scully & Orlygsson, 2014)
	Isoleucine + M	16.9	<0.1	NA	
	Leucine + S_2O_3	16.5	3.5	0.21	
	Leucine + M	17.0	<0.1	NA	
	Valine + S_2O_3	17.4	2.1	0.12	
	Valine + M	17.9	<0.1	NA	
C. subterraneus subsp. *subterraneus* (DSM 13054)	Isoleucine + S_2O_3	14.0	3.4	0.24	(Scully et al., 2015)
	Leucine + S_2O_3	14.1	5.7	0.40	
	Valine + S_2O_3	15.6	3.5	0.22	
Caldanaerobacter strain AK112	Isoleucine + S_2O_3	12.5	3.5	0.28	(Scully et al., 2015)
	Leucine + S_2O_3	11.7	4.0	0.34	
	Valine + S_2O_3	12.9	1.4	0.11	
Caldanaerobacter strain AK113	Isoleucine + S_2O_3	10.0	2.6	0.26	(Scully et al., 2015)
	Leucine + S_2O_3	12.0	5.3	0.44	
	Valine + S_2O_3	10.9	1.2	0.11	
Caldanaerobacter strain AK131	Isoleucine + S_2O_3	14.2	3.9	0.27	(Scully et al., 2015)
	Leucine + S_2O_3	11.1	6.5	0.58	
	Valine + S_2O_3	11.5	1.2	0.10	

Supplemental Table S3. *Thermoanaerobacter manthranii* genes. Table 2 Genes detected within the genome potentially involved in the pathways of Figure 4 with enzymes involved in RCOOH reduction and ethanol fermentation also listed. NA indicates that the gene was not found in the annotation. * Multiple WP Genome Locations are for subunits.

Gene	Pathways	Number of Copies	Length (aa)	Genome Location
Bifunctional aldehyde alcohol dehydrogenase	BCAA fermentation RCOOH reduction Ethanol fermentation	1	872	>WP_013150937.1
Alcohol dehydrogenase	BCAA fermentation RCOOH reduction Ethanol fermentation	4	389 400 403 83	>WP_013149746.1> WP_013150423.1 >WP_013150922.1> WP_041591635.1
Branched-chain-amino-acid transaminase	BCAA fermentation	1	291	>WP_013150144.1
Branched-chain amino acid ABC transporter permease	BCAA fermentation	2	294 322	>WP_041591693.1> WP_013149986.1
Oxaloacetate decarboxylase subunit alpha	BCAA fermentation	1	465	>WP_013150302.1
Butyrate kinase	RCOOH reduction	2	357 354	>WP_198002304.1 >WP_198002305.1
Butyryl phosphotransferase	RCOOH reduction	1	298	>WP_013150780.1
Pyruvate:ferredoxin oxidoreductase	Pyruvate fermentation	2	1175 1171	>WP_013149847.1 >WP_013150870.1
Acetate kinase	Pyruvate fermentation	1	401	>WP_013150373.1
Acetyl phosphotransferase	Pyruvate fermentation	1	330	>WP_012995339.1
Trypsin-like peptidase	Protease	2	456 367	>WP_013150958.1> WP_013151009.1
Thiosulfate reductase	Thiosulfate utilization	Na	NA	Na
Ars sulfite reductase *	Thiosulfate utilization	1	347 263 336	>WP_013149784.1 >WP_013149785.1 >WP_013149786.1
YeeE/YedE thiosulfate transporter family protein	Thiosulfate utilization	2	177 217	>WP_012994254.1> WP_013149588.1