Supplemental material

Table S1. OrthoANI values[a].

Genome 1	Genome 2	OrthoANI (%)
B. thermosphacta DSM 20171	*B. thermosphacta* 7803	99.19
B. thermosphacta DSM 20171	*B. thermosphacta* 7804	99.09
B. thermosphacta DSM 20171	*B. thermosphacta* 7806	99.11
B. thermosphacta DSM 20171	*B. thermosphacta* 7807	99.00
B. thermosphacta DSM 20171	*B. thermosphacta* 7808	99.20
B. thermosphacta DSM 20171	*B. thermosphacta* 7809	98.99
B. thermosphacta DSM 20171	*B. thermosphacta* 7810	99.26
B. thermosphacta DSM 20171	*B. thermosphacta* 7811	99.23
B. thermosphacta DSM 20171	*B. thermosphacta* 7813	99.12
B. thermosphacta DSM 20171	*B. thermosphacta* 7816	99.54
B. thermosphacta DSM 20171	*B. thermosphacta* 7818	99.14
B. thermosphacta DSM 20171	*B. campestris* DSM 4712	75.05

[a] OrthoANI (Orthologous Average Nucleotide Identity) between *B. thermosphacta* DSM 20171 and the draft genomes (listed in column two) is shown in column three.

Table S2. DDH estimates[a].

	Formula 2[b]			
Reference genome	**DDH (%)**	**Model C.I. (%)**	**Prob. DDH >= 70% (%)**	**G+C difference[c]**
B. thermosphacta 7803	92.4	90.4 – 94.0	96.54	0.12
B. thermosphacta 7804	91.9	89.9 - 93.6	96.41	0.13
B. thermosphacta 7806	91.8	89.7 - 93.5	96.36	0.12
B. thermosphacta 7807	90.7	88.5 - 92.6	96.04	0.00
B. thermosphacta 7808	92.6	90.6 - 94.2	96.59	0.07
B. thermosphacta 7809	91.1	88.9 - 92.9	96.16	0.04
B. thermosphacta 7810	93.1	91.2 - 94.6	96.73	0.02
B. thermosphacta 7811	92.5	90.4 - 94.1	96.55	0.03
B. thermosphacta 7813	92.4	90.4 – 94.0	96.53	0.16
B. thermosphacta 7816	92.6	90.6 - 94.2	96.59	0.13
B. thermosphacta 7818	92.5	90.5 - 94.1	96.56	0.09
B. campestris DSM 4712	20.6	18.3 – 23.0	0.00	3.81

[a] DDH (digital DNA:DNA hybridisation) estimates determined for the comparison of the draft genome of *B. thermosphacta* DSM 20171 to the listed reference draft genomes in column one.

[b] DDH values, which correlate well with wet lab percentages are shown in the second column. In column three, model-based confidence intervals (Model C.I.) are listed followed by the probability that DDH values are >70% in column five.

[c] GC differences between the query (*B. thermosphacta* DSM 20171) and reference genomes, which cannot differ by more than one within a single species.

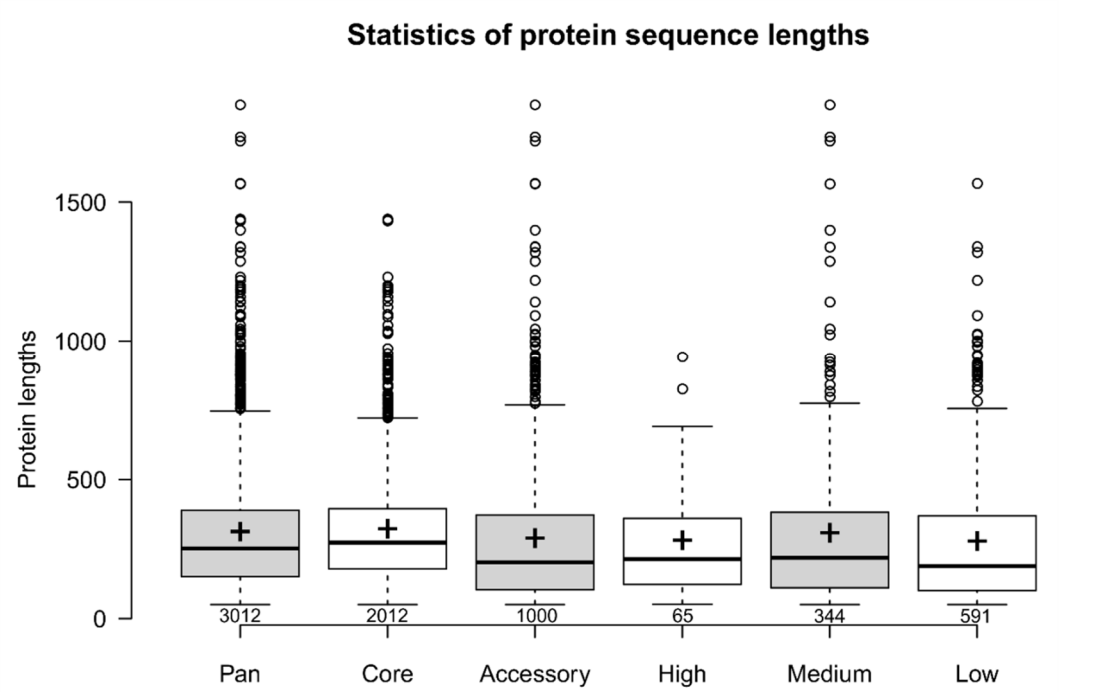

Figure S1. Statistics of protein sequence lengths. Box plots of protein lengths of all predicted coding regions in the pan-genome of the 12 *B. thermosphacta* strains are shown. Crosses represent sample means and whiskers extend 1.5 times the interquartile. Outliers are represented by circles. The number of protein sequences analyzed are listed below each plot.

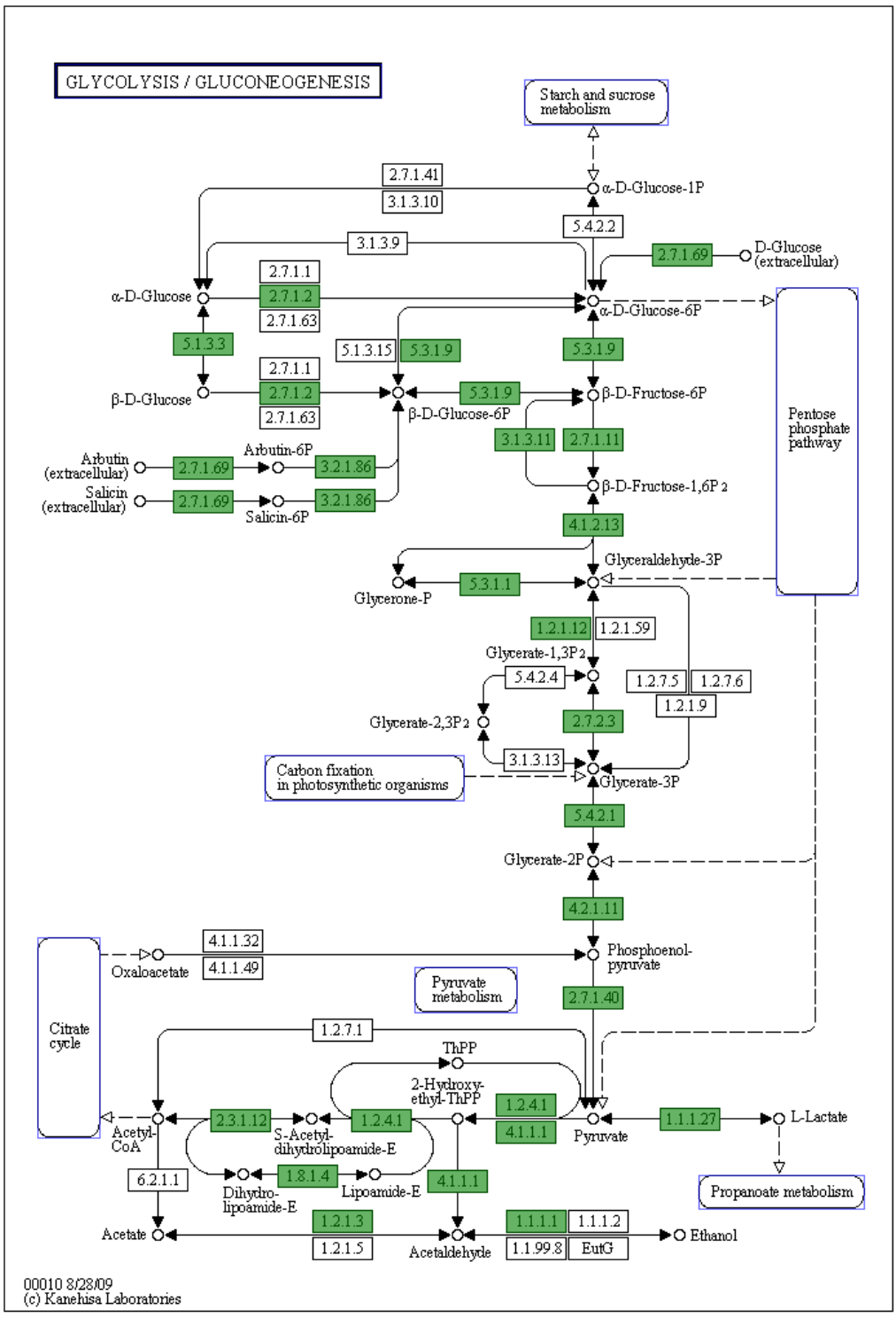

Figure S2. KEGG map of the glycolysis/gluconeogenesis pathways and subsequent pyruvate reactions in *Brochothrix*. Enzymes that are present in *Brochothrix* species are shown in green boxes, while enzymes that are absent are represented in uncoloured boxes, with the exception of a pyruvate decarboxylase

(EC 4.1.1.1) gene, which was not identified in the draft genome of *B. campestris* DSM 4712.

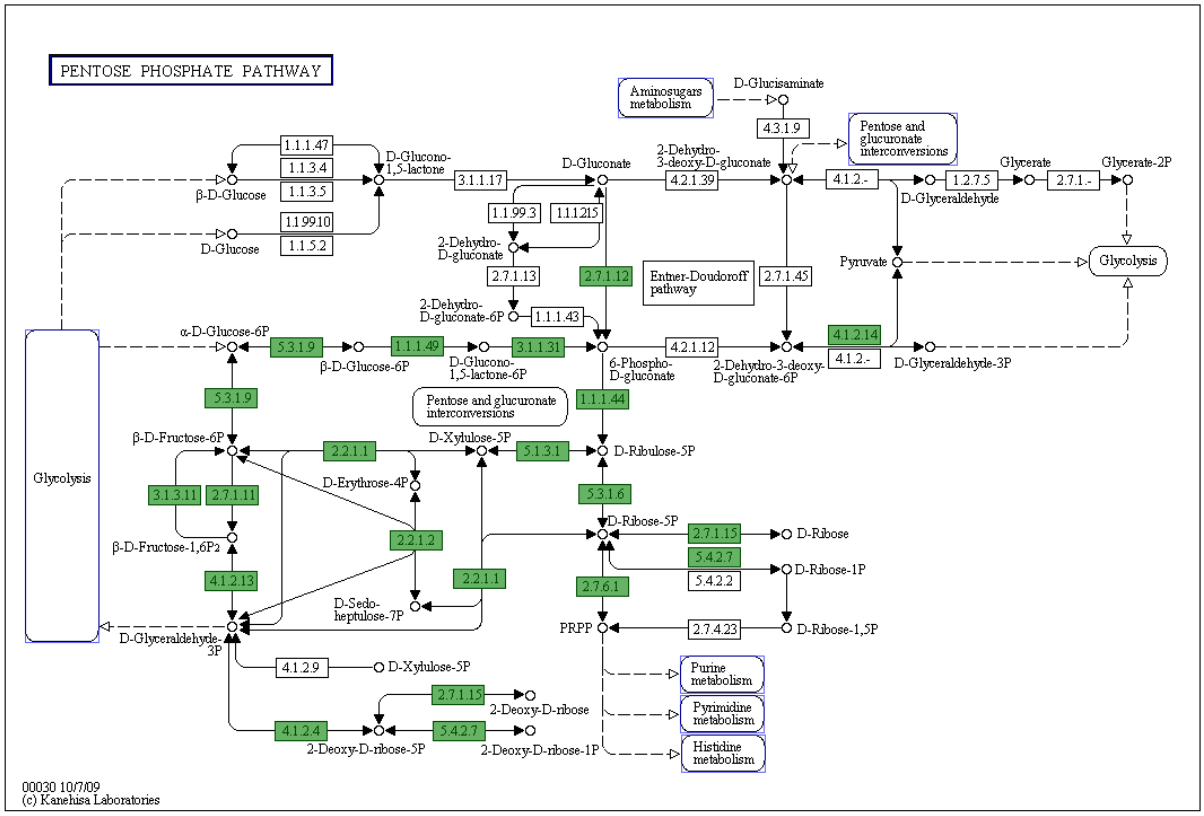

Figure S3. KEGG map of the pentose-phosphate pathway in *Brochothrix*.

Enzymes that are present in *Brochothrix* species are shown in green boxes.

Enzymes that are absent are represented in uncoloured boxes.

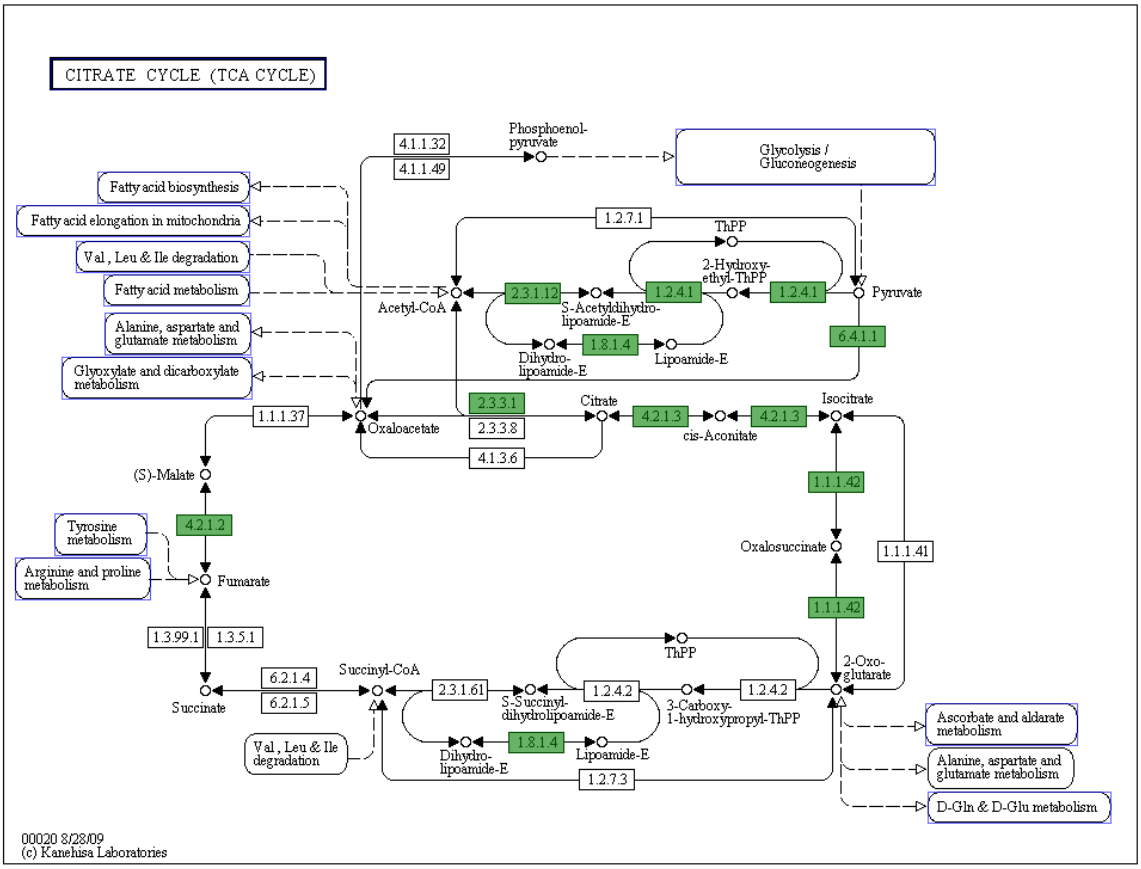

Figure S4. KEGG map of the citrate cycle shows key enzymes are missing in *Brochothrix*. Enzymes that are present in *Brochothrix* are shown in green boxes and enzymes that are absent are represented in uncoloured boxes. Although not shown here, a gene encoding an adihydrolipoamide S-succinyltransferase enzyme (EC 2.3.1.61) was also identified in the genome of *B. campestris* DSM 4712.

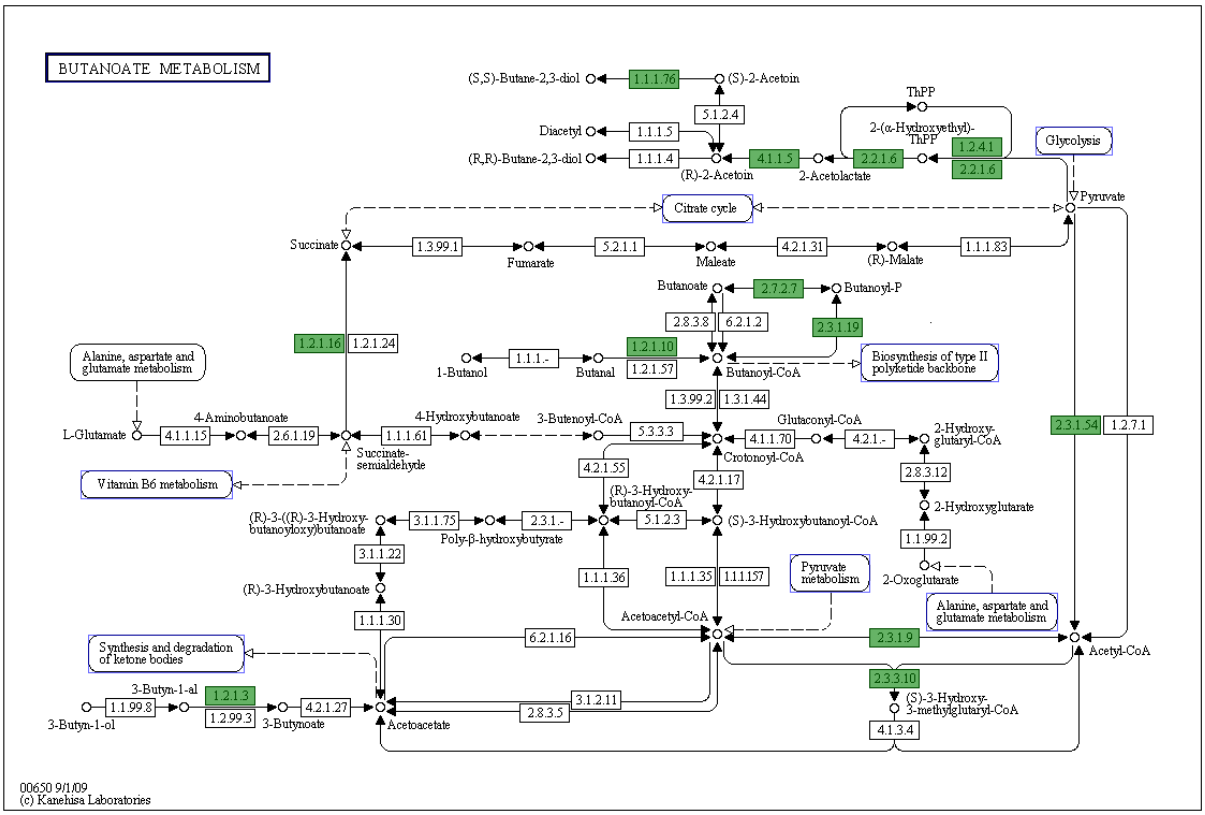

Figure S5. KEGG map of butanoate metabolism indicates that enzymes are present in *Brochothrix* for the conversion of pyruvate to acetoin and 2,3-butanediol. Enzymes that are present in *Brochothrix* species are shown in green boxes, while enzymes that are absent are represented in uncoloured boxes.

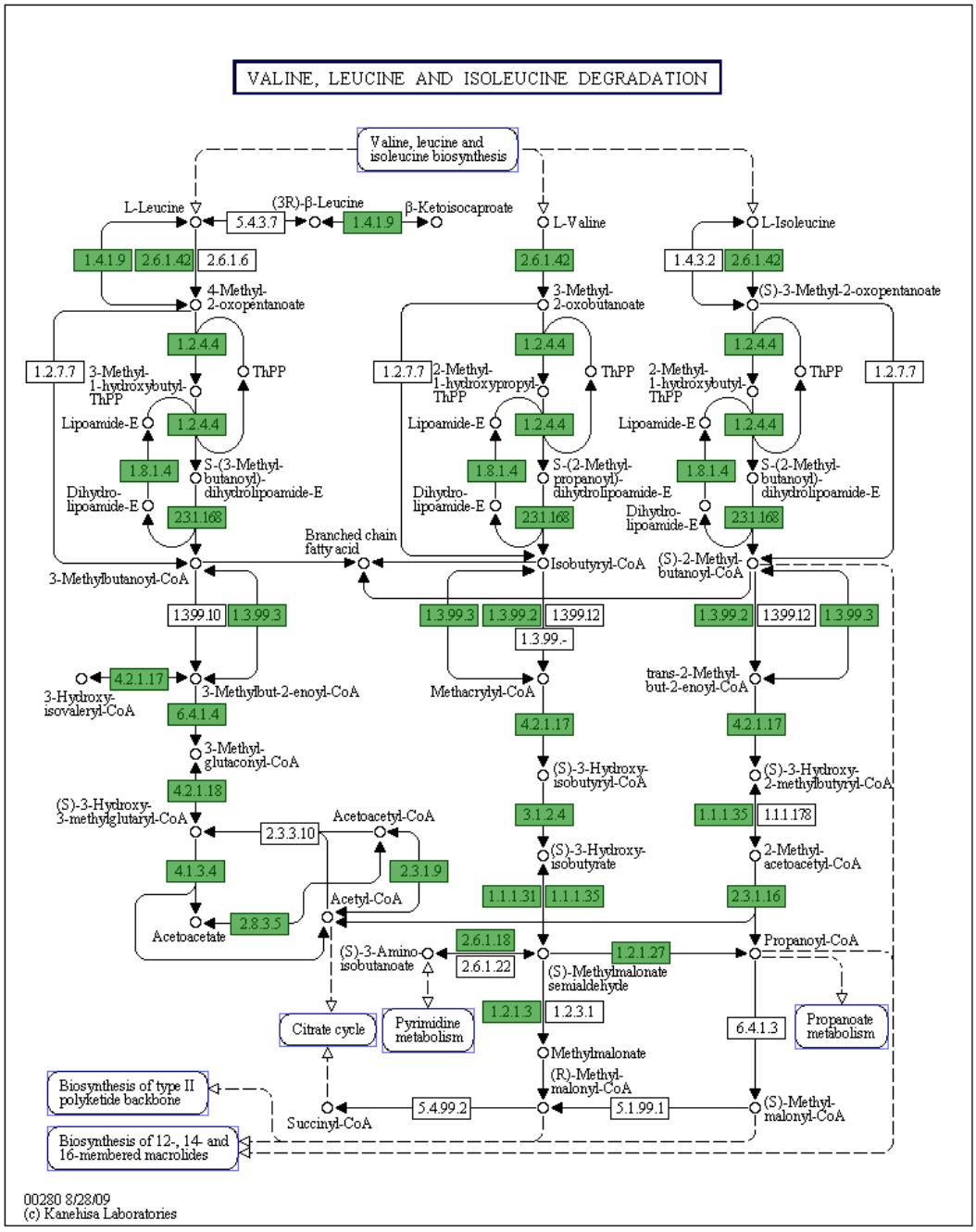

Figure S6. KEGG map of valine, leucine and isoleucine degradation pathways indicates all enzymes are present for the conversion of these amino acids to the acyl-CoA derivatives isobutyryl-CoA, isovaleryl-CoA and 2-methylbutananoyl-CoA, and propanoyl-CoA. Enzymes present in the *B. thermosphacta* strains are marked in green boxes and enzymes that are absent are in uncoloured boxes. While all enzymes required for the conversion of valine, leucine and isoleucine to their branched chain acyl-CoA derivatives are present in *B.*

campestris DSM 4712, many of the enzymes downstream of these are missing including enzymes required for propanoyl-CoA production.