

Supplementary Material

Table S1. Occurrence of TK2268 homologs in Thermococcales species

Thermococcales	Homologs of TK2268
<i>Thermococcus kodakarensis</i>	TK2268
<i>Thermococcus onnurineus</i>	Ton_1259
<i>Thermococcus gammatolerans</i>	TGAM_1838
<i>Thermococcus sibiricus</i>	TSIB_1328
<i>Thermococcus barophilus</i>	TERMP_01282
<i>Thermococcus</i> sp. 4557	GQS_09360
<i>Thermococcus</i> sp. AM4	TAM4_36
<i>Thermococcus cleftensis</i>	CL1_1464
<i>Thermococcus litoralis</i>	OCC_08839
<i>Thermococcus paralvinellae</i>	TES1_1311
<i>Thermococcus nautili</i>	BD01_1614
<i>Thermococcus eurythermalis</i>	TEU_00135
<i>Thermococcus guaymasensis</i>	X802_01935
<i>Thermococcus</i> sp. 2319x1	ADU37_CDS13830
<i>Thermococcus chitonophagus</i>	CHITON_1226
<i>Thermococcus peptonophilus</i>	A0127_04025
<i>Thermococcus piezophilus</i>	A7C91_01730
<i>Thermococcus gorgonarius</i>	A3K92_02075
<i>Thermococcus celer</i>	A3L02_05740
<i>Thermococcus barossii</i>	A3L01_06925
<i>Thermococcus</i> sp. 5-4	CDI07_06895
<i>Thermococcus siculi</i>	A3L11_01055
<i>Thermococcus thioeducens</i>	A3L14_09295
<i>Thermococcus profundus</i>	A3L09_03190
<i>Thermococcus radiotolerans</i>	A3L10_06370
<i>Thermococcus pacificus</i>	A3L08_00190
<i>Thermococcus</i> sp. P6	A3L12_02125
<i>Thermococcus indicus</i>	tFH039_10410
<i>Thermococcus camini</i>	TIRI35C_1690
<i>Thermococcus aciditolerans</i>	FPV09_11205
<i>Thermococcus argininiproducens</i>	K1720_09755
<i>Pyrococcus furiosus</i> DSM 3638	PF0522
<i>Pyrococcus furiosus</i> COM1	PFC_01730
<i>Pyrococcus horikoshii</i>	PH0771
<i>Pyrococcus abyssi</i>	PAB1523
<i>Pyrococcus</i> sp. NA2	PNA2_1412
<i>Pyrococcus yayanosii</i>	PYCH_02880
<i>Pyrococcus</i> sp. ST04	Py04_0704
<i>Pyrococcus kukulkanii</i>	TQ32_03330
<i>Palaeococcus pacificus</i>	PAP_05920

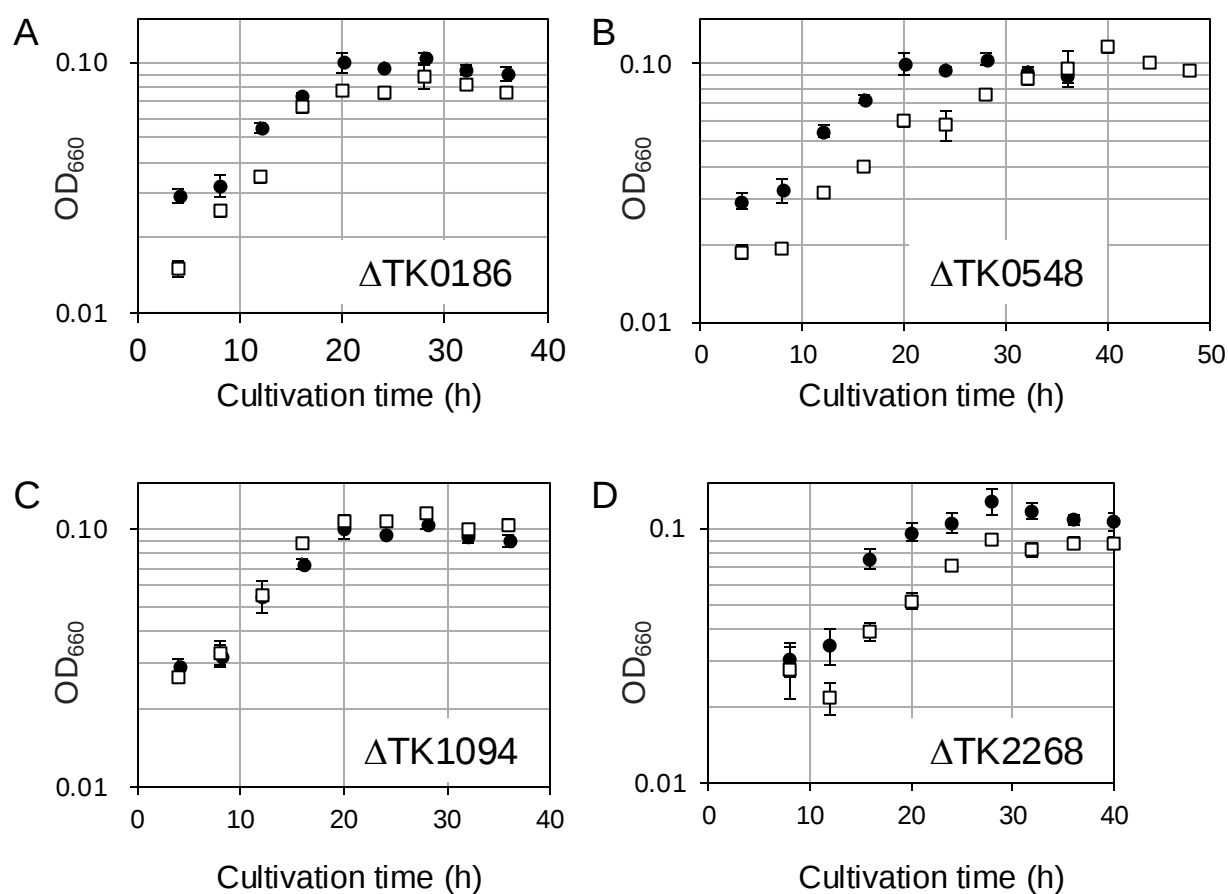


FIG S1. Growth properties of *T. kodakarensis* KU216 and different aminotransferase gene disruption strains in synthetic medium ASW-AA-m1-S⁰(Ura⁺). Growth of KU216 is indicated with closed circles and those of the disruption strains are indicated with open squares in panels A, Δ TK0186; B, Δ TK0548; C, Δ TK1094; D, Δ TK2268. Error bars indicate the standard deviation values of three biologically independent culture experiments.

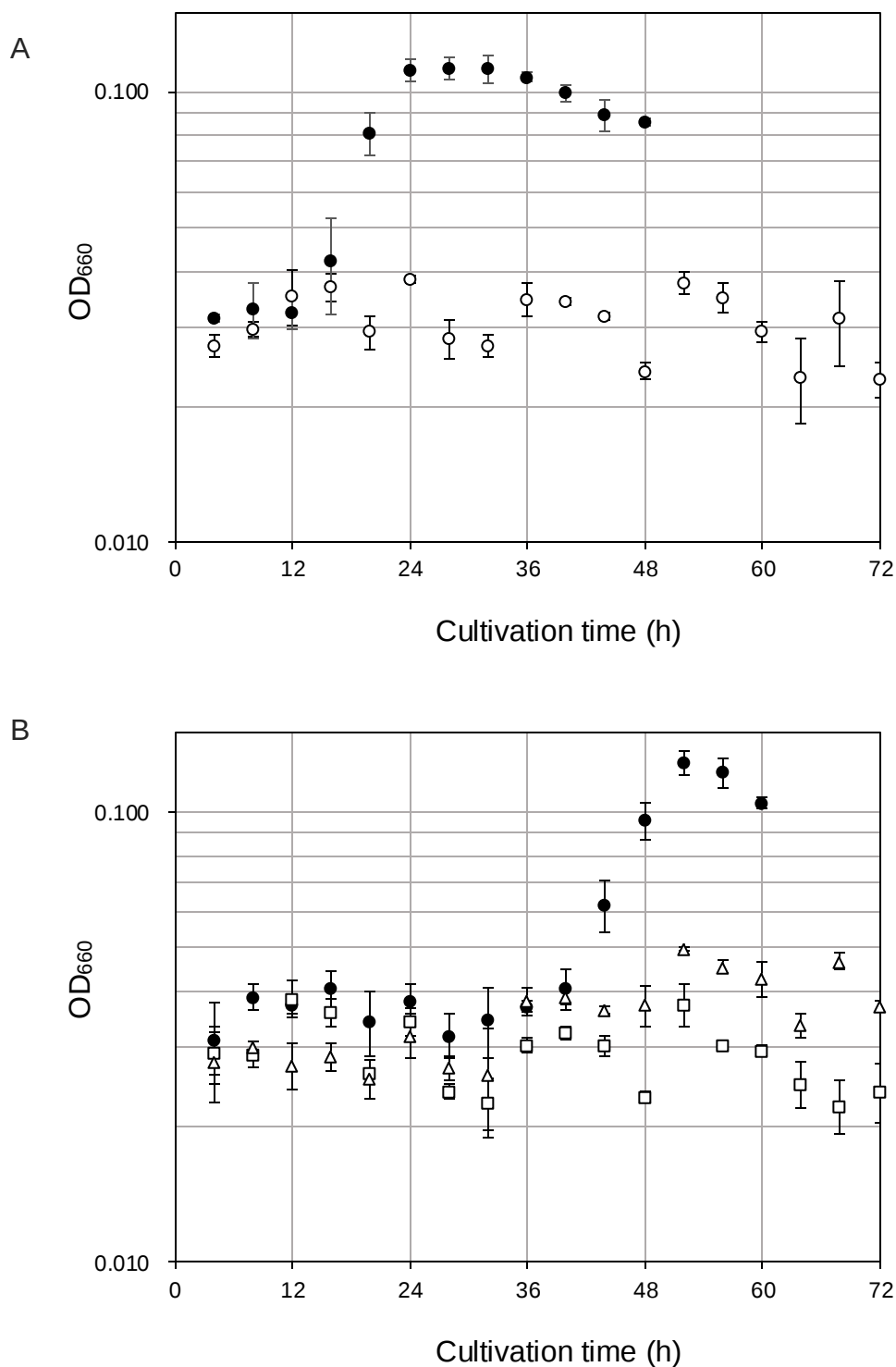
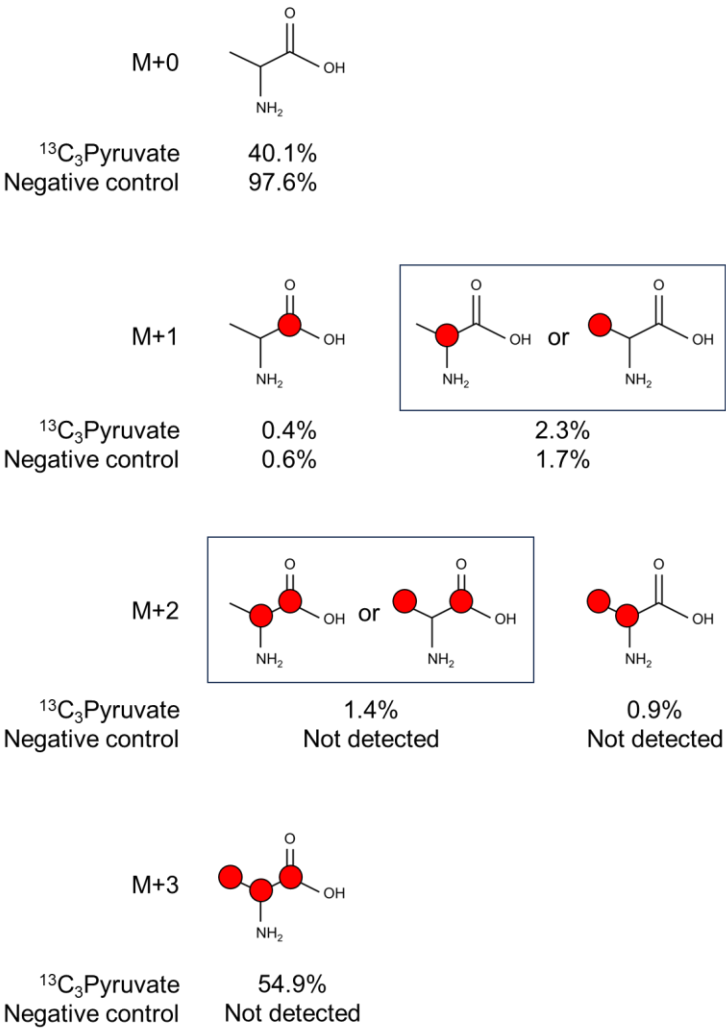


FIG S2. Growth properties of *T. kodakarensis* KU216 in various synthetic media. (A) *T. kodakarensis* KU216 was cultivated in synthetic medium ASW-AA-m1-S⁰(Ura⁺) (closed circles) and in ASW-AA(D-N)-m1-S⁰(Ura⁺) (open circles). (B) KU216 was cultivated in synthetic medium ASW-AA(D-N)-m1-S⁰(Ura⁺) supplemented with Glu alone (open squares), OAA alone (open triangles), or with both Glu and OAA (closed circles). Error bars indicate the standard deviation values of three biologically independent culture experiments.

alanine

 ¹³C



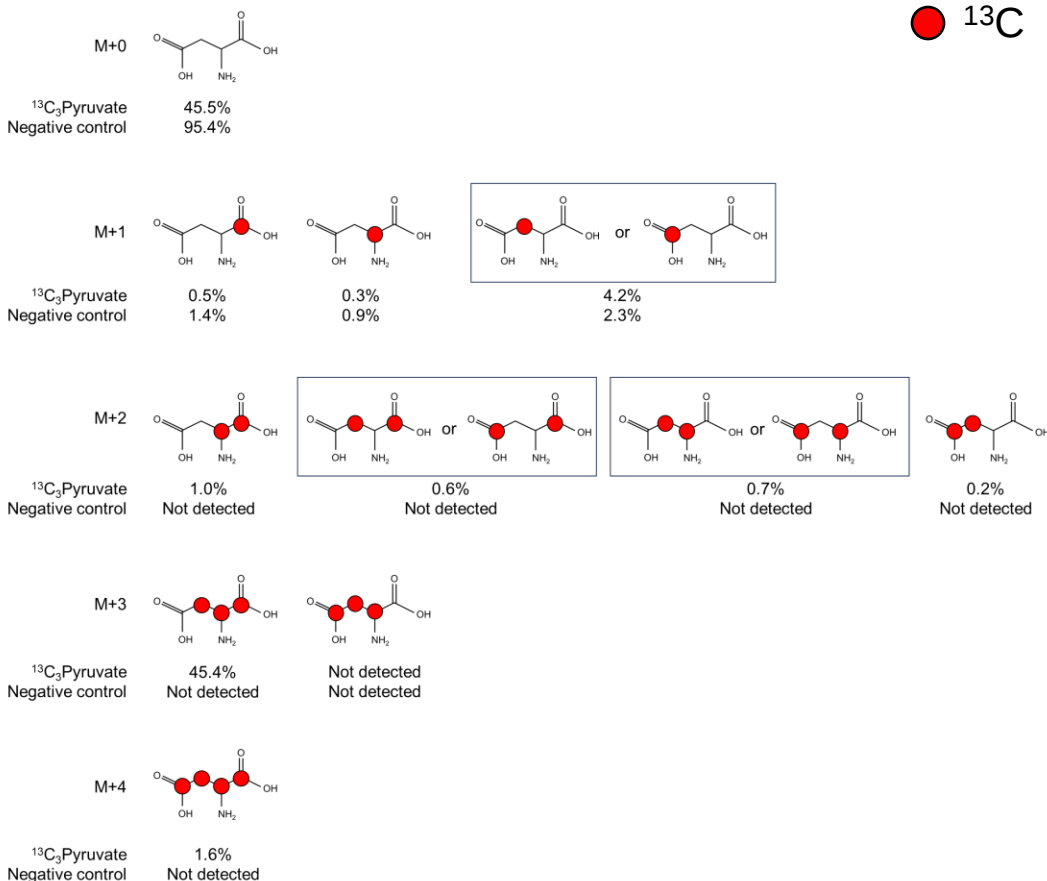
	Isotopomer pattern		Natural abundances of isotopes	<i>T. kodakaraensis</i> KU216	
	No. of ¹³ C			¹³ C ₃ pyruvate Na	Negative control
Amino acid	C-1	Others	Abundance (%)	Abundance (%)	Abundance (%)
¹³ C ₀ Ala	0	0	96.7	40.1	97.6
¹³ C ₁ Ala	1	0	1.1	0.4	0.6
	0	1	2.2	2.3	1.7
¹³ C ₂ Ala	1	1	0.0	1.4	N.D.
	0	2	0.0	0.9	N.D.
¹³ C ₃ Ala	1	2	0.0	54.9	N.D.

N.D., not detected

FIG S3. The relative abundances of each isotopomer pattern from Ala. The mass fractions for M+0, M+1, M+2, and M+3 represent amino acids containing 0-3 ¹³C-labeled carbons, respectively. Red circles show ¹³C-labeled carbon atoms in the carbon skeleton.

aspartate

 ¹³C



	Isotopomer pattern		Natural abundances of isotopes	<i>T. kodakaraensis</i> KU216	
	No. of ¹³ C			¹³ C ₃ pyruvate Na	Negative control
Amino acid	C-1	Others	abundance (%)	Abundance (%)	Abundance (%)
¹³ C ₀ Asp	0	0	95.7	45.5	95.4
¹³ C ₁ Asp	1	0	1.1	0.5	1.4
	0	1	3.2	4.5	3.2
¹³ C ₂ Asp	1	1	0.0	1.5	N.D.
	0	2	0.0	1.0	N.D.
¹³ C ₃ Asp	1	2	0.0	45.4	N.D.
	0	3	0.0	N.D.	N.D.
¹³ C ₄ Asp	1	3	0.0	1.6	N.D.
Amino acid	C-1, 2	C-3, 4	Abundance (%)	Abundance (%)	Abundance (%)
¹³ C ₀ Asp	0	0	95.7	45.5	95.4
¹³ C ₁ Asp	1	0	2.1	0.8	2.2
	0	1	2.1	4.2	2.3
	2	0	0.0	1.0	N.D.
¹³ C ₂ Asp	1	1	0.0	1.3	N.D.
	0	2	0.0	0.2	N.D.
¹³ C ₃ Asp	2	1	0.0	45.4	N.D.
	1	2	0.0	N.D.	N.D.
¹³ C ₄ Asp	2	2	0.0	1.6	N.D.

N.D., not detected

FIG S4. The relative abundances of each isotopomer pattern from Asp. The mass fractions for M+0, M+1, M+2, M+3, and M+4 represent amino acids containing 0-4 ¹³C-labeled carbons, respectively. Red circles show ¹³C-labeled carbon atoms in the carbon skeleton.