SUPPLEMENTAL MATERIALS

A genetic examination of initial amino acid oxidation and glutamate catabolism in the hyperthermophilic archaeon *Thermococcus kodakarensis*

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Table S1. Primers used in this study.

Primer names	Primer sequences
D*gdh*-F1	5’- AAA**GGATCC**TTGACCCAGCGCTCGATACCCTTGCT -3’
D*gdh*-R1	5’- AAAAAA**GAATTC**ATCAACTCCAAGTCTCTTCCGCT -3’
D*gdh*-F2	5’- TTTCTTCTCCCTTTTCTCTTGTCCT -3’
D*gdh*-R2	5’- GTTTTGAACAAAAAGTTAAATAGTGAAA -3’
D*gdh*-F3	5’- GGAAAGAGAGTGAGCTGAGAGATG -3’
D*gdh*-R3	5’- GATGATAAGGGCGATGGTGGAAG -3’
D*gdh*-F4	5’- CGAGGGACAGCGTGAAGAATATCTG -3’
D*gdh*-R4	5’- AATTCTGGAGGGGTGTGCTGAAATGA -3’
D*gdh*-F5	5’- ATGGTCGAGATTGACCCGTTTGAGAT -3’
D*gdh*-R5	5’- TTCTTCTCCTTGTGGGTGTTGAAG -3’
D*ogor*-F1	5’- AAA**GGATCC**GGCAAAAACGGAGGGAATCGCAGT -3’
D*ogor*-R1	5’- AAA**GGATCC**CGAGCTTAACTTTTCCGTGCCATGCT -3’
D*ogor*-F2	5’- GTGAGCAGGGAAGCGATAGAAAAGGC -3’
D*ogor*-R2	5’- TATCGGGTAGCCAGCGTAGAAGCGGCA -3’
D*ogor*-F3	5’- GCGGAGGAAACGACGGGACTTG -3’
D*ogor*-R3	5’- CCCTGAAATCCGAGAGGAGAAT -3’
D*ogor*-F4	5’- CGAGATAGGAAAGCTGAGTGCGGAG -3’
D*ogor*-R4	5’- GCTGGGAGGACGAAAACCAGTGC -3’
D*ogor*-F5	5’-ACAGATGGAGGACGAGATAGC -3’
D*ogor*-R5	5’- CGCGTAGTCGGGGCTGAGGGTCTTG -3’
D*scs*-F1	5’- GAC**GAATTC**GAACTCCTCGATTGTTCTGACG -3’
D*scs*-R1	5’- GGG**GAATTC**CATCGCCGTCTTCGCCCACATA -3’
D*scs*-F2	5’- GCTTTTAGCCCAAGCGCTCTAAAATCT -3’
D*scs*-R2	5’- TGAAATCACCTCCGAAGCTTTT -3’
D*scs*-F3	5’- AGATTGTCTGAGGCGTCTTTCCGAG -3’
D*scs*-R3	5’- GGATATGTTGAAATAGACAAAACCG -3’
D*scs*-F4	5’- TCATTGGCCTCTCTCTGAGCAGTT -3’
D*scs*-R4	5’- CCAAGCACCTATCGGCCATCTTCCT -3’
D*scs*-F5	5’- CGTCGCAGTCATAGGTGCCTCAAACGTGC -3’
D*scs*-R5	5’- GTTCCTTGCCATCTTGTGCGGGTAGTCTC -3’
E*gdh*-F1	5’- TTTGTCACTTTTTCAAATCTATCTTG -3’
E*gdh*-R1	5’- CTTCTGTTTCGTTTTTATTTAGTTT -3’
E*gdh*-F2	5’- GAGACCATAATGAGGAGAAAGGGC -3’
E*gdh*-R2	5’- CAGCTTTTTGAGAGGCATATGCATCACC -3’

Bold sequences represent restriction sites..

Table S2. Amino acid dehydrogenase activity in *T. kodakarensis*.

Strain	KUW1				KGDH1		KGDH1C	
Medium	ASW-YT-S^0		ASW-YT-Pyr		ASW-YT-Pyr		ASW-YT-Pyr	
Cofactor	NAD^+	$NADP^+$	NAD^+	$NADP^+$	NAD^+	$NADP^+$	NAD^+	$NADP^+$
Glu	39.5 ± 4.8 [*1]	24700 ± 300	26.9 ± 1.2	21900 ± 300	< 2	< 2	39.6 ± 4.6	27800 ± 100
Gln	< 2	4.7 ± 0.3	< 2	12.3 ± 0.6	< 2	< 2	N.D. [*2]	26.0 ± 1.4
Asp	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Asn	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Ala	< 2	< 2	< 2	6.7 ± 0.5	< 2	< 2	N.D.	11.0 ± 0.6
Val	< 2	< 2	< 2	4.5 ± 2.4	< 2	< 2	N.D.	7.9 ± 0.8
Leu	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Ile	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Gly	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Ser	< 2	< 2	< 2	< 2	12.3 ± 1.0	< 2	< 2	N.D.
Thr	51.6 ± 2.0	< 2	98.3 ± 5.8	< 2	163 ± 14	< 2	55.4 ± 2.2	N.D.
Pro	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Arg	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Lys	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
His	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Phe	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Tyr	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Trp	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Met	< 2	< 2	< 2	< 2	< 2	< 2	N.D.	N.D.
Cys	< 2	8.1 ± 3.2	< 2	10.8 ± 3.9	< 2	< 2	N.D.	23.8 ± 1.0

[*1] The unit of all activity values is nmol min^{-1} mg $protein^{-1}$.

[*2] N.D., not determined.

FIGURE S1A

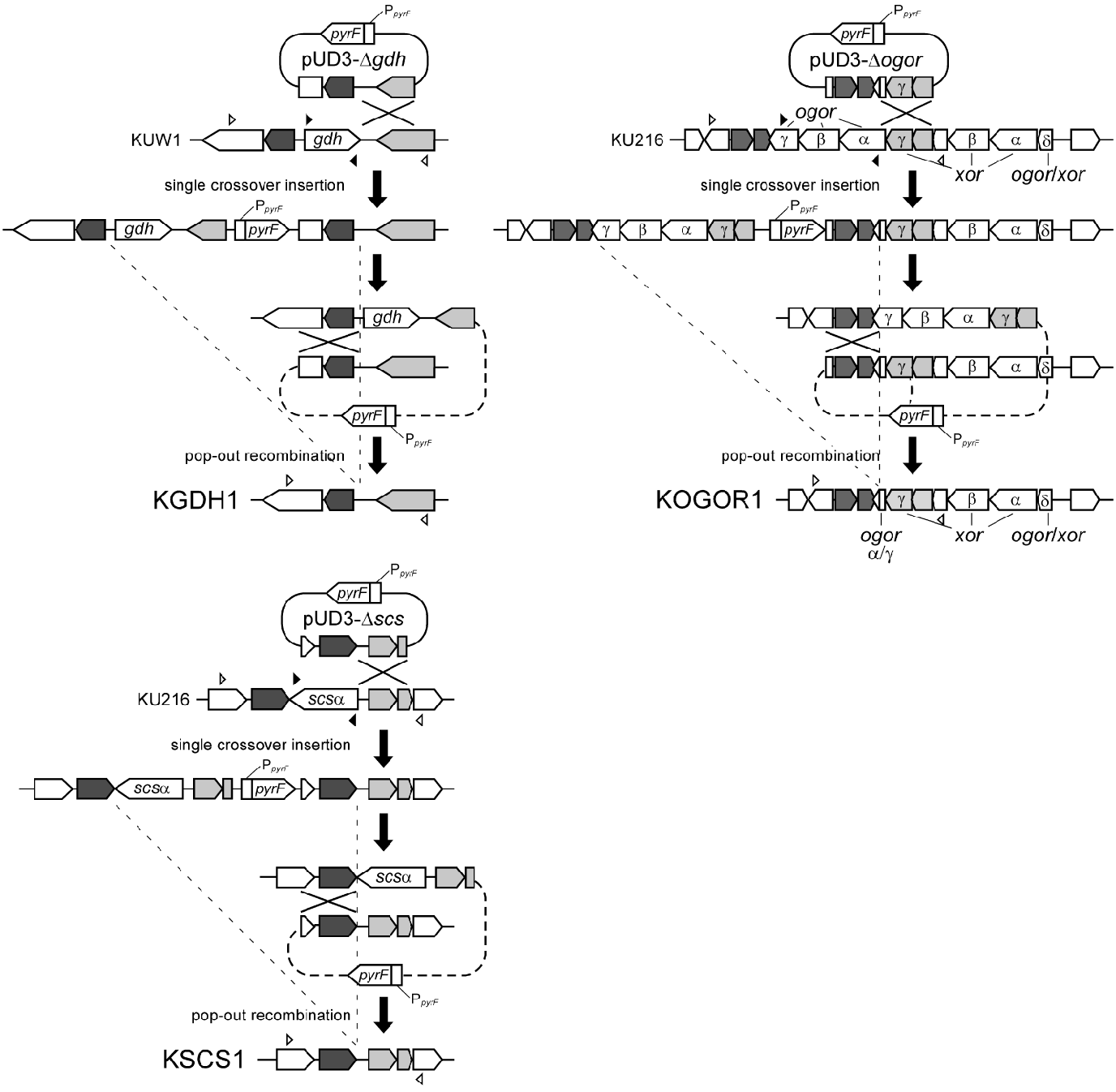
pUD3-Δgdh
pyrF
KUW1
gdh
single crossover insertion
pop-out recombination
KGDH1
pUD3-Δogor
ogor
xor
ogorlxor
KOGOR1
pUD3-Δscs
KU216
scsα
KSCS1

FIGURE S1B

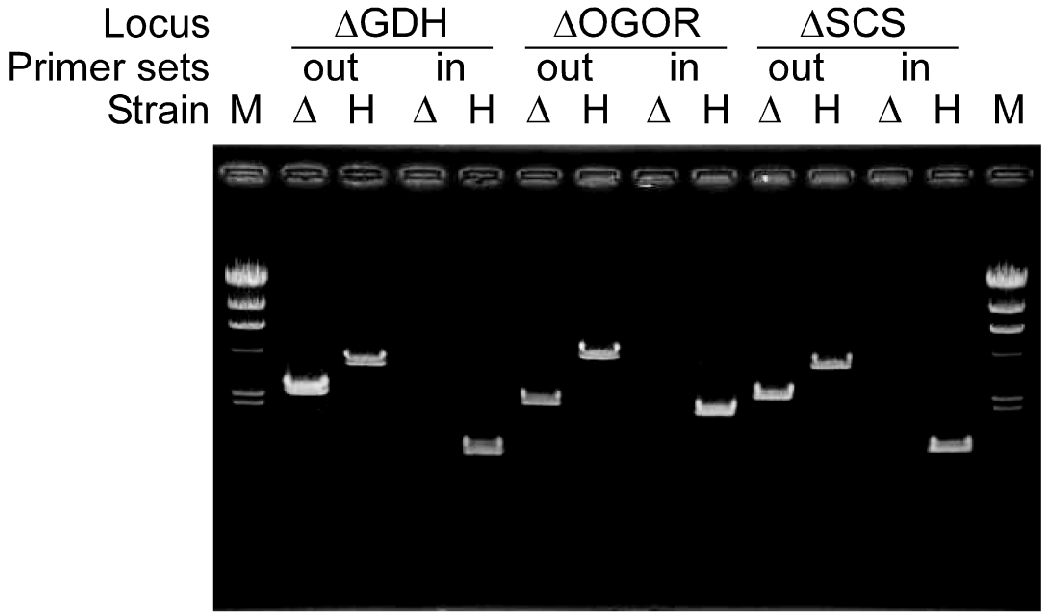

Fig. S1. Construction of gene disruption strains of GDH, OGOR and SCS in *T. kodakarensis*. (A) Design of the plasmids and recombination strategies for the disruption of the GDH, OGOR and SCS. In the case of GDH, the TK1431 gene was entirely deleted. In the case of OGOR and SCS, for details see Materials and Methods. Arrowheads indicate the position of the primers used for PCR analyses in (B). Open arrowheads represent primer sets that anneal outside of the homologous regions and closed arrowheads represent primer sets that anneal within the coding region of the gene disrupted. (B) PCR analyses of the three loci confirming gene disruption. The specific loci, primer sets and strains for template DNA are indicated above the gel. Out/in represent primer sets indicated by open/closed arrowheads in (A); Δ, disruption strain; H, host strain; M, marker.