SUPPLEMENTAL MATERIAL Mao and Grogan, 2017

This document:

Associated data files:

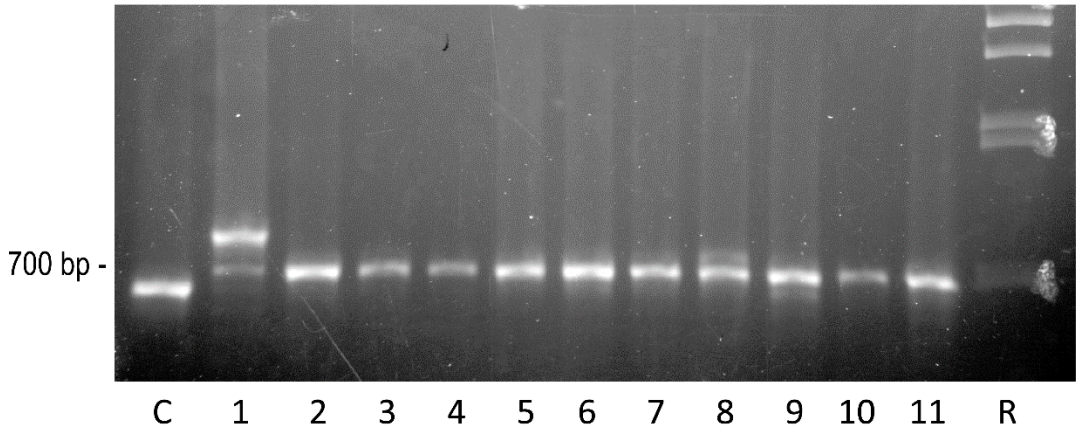

FIGURE S1. Phenotypic revertants of strain GB8-9

Gel shows PCR products that span the tandemly repeated SR unit of strain DG185 (lane C, 5x allele), clones of GB8-9 selected as phenotypic revertants (lanes 1-11), and reference markers (lane R). All phenotypic revertants show the contracted (6x) allele of this TR (co-migrating with the 700 bp marker); culture 1 also retained cells with the original 7x allele of GB8-9 (see Fig. 2).

Table S1. Nutritional supplementation of strain GB8-9[a]

Supplement[b]	Relative growth response
C+D	+
D+P	+
D+V	+
I+V	+
AE+K+S	+
R+H+M+R	0
D+I+F+Y	0
C+L+P+V	0
A+R+D+C	0
E+H+I+L	0
K+M+F+P	+
S+T+Y+V	++
acid-hydrolyzed casein	+++
DIFY+CLPV	+
EHIL+KMFP	++

[a]Maximal growth of strain GB8-9 in defined medium had been observed previously to be supported by a pool of amino acids (Bell GD. 2001. "Genetic Diversity of Natural *Sulfolobus* Populations and Mutator Mutants of *Sulfolobus acidocaldarius*". *Thesis, M S*). We conducted additional growth tests with pure amino acids (see Materials and Methods), and found that the growth increase tended to increase with the complexity of the mixture added, and none of the defined mixtures tested was as effective as acid-hydrolyzed casein. Although the metabolic basis of this dependence remains to be defined, it is consistent with the criterion used initially to isolate strain GB8-9, namely, dependence on supplementation of defined medium with 0.05% yeast extract (see Results).

[b]Amino acids (listed by single-letter abbreviations) were added to chemically defined liquid medium containing only ammonium sulfate as N source (see Materials and Methods).

[c]Growth was measured turbidometrically, relative to an unsupplemented control. Other binary combinations of pure amino acids yielded no detectable growth stimulation, and certain amino acids seemed to counteract the growth-stimulating effects of others (not listed). Symbols: 0, no net increase in cell density; +, increase of approximately 5 $x10^7$ cells/mL

Table S2. Spontaneous *pyrE* mutations of wild-type and GB8 strains[a]

	Number		Percent of total	
Type of Event	WT	GB8	WT	GB8
+A	6	6	6.1	8.3
-A	53	27	53.5	37.5
A -> G	0	1	--	1.4
A -> T	2	2	2.0	2.8
+C	0	2	--	2.8
-C	1	0	1.0	--
C -> G	1	0	1.0	--
C -> T	2	0	2.0	--
+G	5	11	5.1	15.3
-G	8	2	8.1	2.8
G -> A	6	5	6.1	6.9
+T	4	3	4.0	4.2
-T	0	2	--	2.8
T -> A	1	4	1.0	5.6
T -> C	1	0	1.0	--
deletion	1	1	1.0	1.4
triplet expansion	3	0	3.0	--
TD	5	5	5.1	6.9
transposition	0	1	--	1.4

[a]Seventy-two independent spontaneous FOA-resistant mutants were selected, and the *pyrE* mutation in each was identified by sequencing. The resulting spectrum of mutations resembled that of wildtype *S. acidocaldarius* in most respects; in particular G:C to A:T transitions were not elevated relative to wild-type.

Table S3. Sequence differences not analyzed as polymorphisms[a]

Position	Base	Observed[b]	Predicted impact of correction
201457		A added	converts 152aa ORF to 145aa ORF
224370	T	C	
359301		T removed	extends 149aa ORF to 219aa
396514	A	G	
419146		C added	negligible effect on ORFs
542545		A removed	converts 70aa ORF to 108aa ORF
627121	G	T	
627222	A	T	
627265	A	T	removes TAA stop codon
628472		AAA > AAAA	removes TAG codon, fuses flanking ORFs
673663		A removed	removes 192aa ORF
700719	G	A	
863049		T removed	
863062		T removed	
863069		T removed	converts 49aa ORF to 48aa ORF
963993		T added	removes 98aa ORF
1095706	T	C	
1133248		T added	removes 53aa ORF
1225698		T removed	converts 95aa ORF to 185aa ORF

1225967		T added	
1269571	A	G	
1270078	C	T	
1271203	C	T	
1278928	A	G	
1351450	C	T	
1755721	T	C	
1791106		G removed	converts 34aa ORF to 50aa ORF
2002815		C removed	shortens Saci_2168

[a] Differences observed between the genome sequence published by Chen et al. () and deposited as CP000077, and independent analysis of DSM 639 or ATCC 33909 (see Materials and Methods)

[b] Refers to the sequence observed in ATCC33909 and all other *S. acidocaldarius* strains examined