Expanding the Limits of Thermoacidophily in the Archaeon *Sulfolobus solfataricus* by Adaptive Evolution

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Running title: Extremophile Trait Evolution

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Figure S1. Increasing thermoacidophily by adaptive passage in SARC strain SULI. Bars indicate individual passaged cultures of *S. solfataricus*. Clonal populations adapted for specific pH values are circled (SULG, green; SULH, yellow; SULI, red; and SULJ, purple). Only SULG, the wild type parental strain, was pH downshifted without adaptation (green). Bar height is proportional to generation time and decreases with increasing passage.
Figure S2. Increasing thermoacidophily by adaptive passage in SARC strain SULO. Bars indicate individual passaged cultures of *S. solfataricus*. Clonal populations adapted for specific pH values are circled (SULM, green; SULN, yellow; SULO, red; and SULP, purple). Only SULM, the wild type parental strain, was pH downshifted without adaptation (green). Bar height is proportional to generation time and decreases with increasing passage.
Figure S3. Growth of *S. solfataricus* strains SULA and SULC in 1mM sodium sulfite. Closed symbols (SULA), open symbols (SULC). Squares (no sodium sulfite), circles (1mM sodium sulfite).
Figure S4. Comparison of SULA and the $merI$ mutant physiology at low pH. The growth rates of SULA (closed circles) and the $merI$ mutant (open circles) across a range of acidities from 1 mM (pH 3.0) to 158 mM (pH 0.8) are shown in doublings per hour.
Figure S5: TLC analysis of SARC and parental lipids. Total lipid TLC (chloroform/methanol/water [65:25:4]) for parental *S. solfataricus* grown in pH 3 media, SARC grown in pH 3 media, and SARC grown in pH 1 media. Total lipids of the parental strain were extracted and exposed to pH 3 or pH 1 media to account for abiotic contributions to different lipid compositions. The plates were stained with either 8-anilino-1-naphthalene sulfonic acid to visualize all lipids (panel A), or with alpha-napthol to visualize sugar containing lipids (panel B) as indicated.
### Table S1. 2-fold or greater RNAseq changes in SARC strain SULC

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**Transporters**

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**Oxidoreductases**

<p>| SULA_0140   | -- | +3  | Alcohol dehydrogenase |
| SULA_0298   | -- | +8  | Alcohol dehydrogenase |
| SULA_0434   | -- | +2.6 | Pyridine nucleotide-disulfide oxidoreductase |
| SULA_0443   | -4.1 | +3.8 | Aldehyde oxidase |
| SULA_0493   | -- | +17 | Mercuric reductase |
| SULA_0559   | -- | +2.5 | Pyruvate ferredoxin oxidoreductase |
| SULA_0568   | -- | -5  | 4Fe-4S ferredoxin |
| SULA_0583   | -- | +4  | Aldo/keto reductase |
| SULA_0602   | -2  | -2  | Oxidoreductase |
| SULA_0623   | -- | +3.4 | 2-oxoacid:ferredoxin oxidoreductase subunit alpha |
| SULA_0624   | -- | +2.4 | 2-oxoacid:ferredoxin oxidoreductase subunit beta |
| SULA_0631   | +3  | +6.7 | Oxidoreductase |
| SULA_0671   | -- | +8  | Alcohol dehydrogenase |
| SULA_0698   | -5  | -10 | Nitrite reductase |
| SULA_0863   | -- | -4.7 | Peroxiredoxin |
| SULA_0867   | -- | -4  | Sulfur reduction protein DsrE |
| SULA_0783   | +3  | +6  | Alcohol dehydrogenase |
| SULA_0974   | -- | +2.8 | Alcohol dehydrogenase |
| SULA_1003   | -- | +2.3 | Alcohol dehydrogenase |
| SULA_1020   | -- | -2.4 | FAD-dependent oxidoreductase |
| SULA_1347   | -- | -5.3 | NADH ubiquinone oxidoreductase subunit 4L |
| SULA_1348   | -- | -3.7 | Oxidoreductase |
| SULA_2025   | +10.5 | +6.7 | NADH ubiquinone oxidoreductase |
| SULA_2128   | -2  | +7.5 | Peroxiredoxin |
| SULA_2130   | -2  | +8.8 | Heterodisulfide reductase subunit C |
| SULA_2134   | -2  | +3  | Heterodisulfide reductase subunit C |
| SULA_2135   | -- | +3.4 | Disulfide reductase |
| SULA_2209   | +2.5 | +18 | Aldehyde oxidase |
| SULA_2213   | -- | +5  | FAD-linked oxidase |
| SULA_2287   | -- | +5  | Alcohol dehydrogenase |
| SULA_2454   | -- | +7  | Alcohol dehydrogenase |
| SULA_2719   | +4  | +30 | NADH oxidase |
| SULA_2825   | +6.5 | +8.8 | Aldehyde oxidase |
| SULA_2889   | -- | +2  | Aldehyde oxidase |</p>
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