Supplemental Information

Microbial survival in an extreme Martian analog ecosystem: Poás volcano, Costa Rica

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Table S1. Summary of Laguna Caliente environmental data from our sampling campaigns. All concentrations are in parts per million.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Temp.** **(°C)** | **pH** | **[Na+]** | **[K+]** | **[Ca2+]** | **[Mg2+]** | **[Fe]T** | **[A13+]** | **[Br-]** | **[F-]** | **[Cl-]** | **[SO42-]** |
| 2013 Sampling | 45 | 0.29 | 256 | 181 | 227 | 222 | 631 | 1220 | 7 | 570 | 14381 | 57230\* |
| 2017 Sampling | 35 | 1.45 | 101 | 59 | 1103 | 113 | 276 | 507 | 7 | 270 | 4815 | 17672\* |

 \*likely includes sulfite and thiosulfate

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **[Ag]** | **[As]** | **[Cd]** | **[Co]** | **[Cr]** | **[Cu]** | **[Ni]** | **[Zn]** |
| 2013 Sampling | 0.7 | 164 | 2.7 | 26 | 22 | 122 | 12 | 126 |
| 2017 Sampling | DL | 51 | 6.5 | 89 | 72 | 17 | 215 | 490 |

Table S2. Concentrations of select metals in Laguna Caliente from 2013 and 2017. All concentrations are in parts per billion. (DL = machine detection limit)

Table S3. Genes identified in the metagenomes that may be utilized for survival and environmental adaptation.

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene, Operon, or Protein Name** | **Function** | **Environmental Adaptation** | **Note for Transporter Genes** |
| *Nuo*  | NADH Dehydrogenase | Acid Resistance  |  |
| *Cyo*  | Cytochrome bo | Acid Resistance |  |
| F1Fe-ATPase | ATPase/synthase | Acid Resistance | Primary Active Transport |
| AR2 (*GadA, GadB, GadC*) and AR3 (*AdiA, AdiC*) | Amino acid decarboxylases and coupled antiporter systems | Acid Resistance | *GadC*: Glu/GABA Antiporter (in AR2, Tertiary Active Transport)*AdiC*: Arg/Agm Antiporter (in AR3) |
| *hrcA-grpE-dnaJ* | ATP-dependent molecular chaperone protein  | Heat Resistance |  |
| *groES-groEL* | ATP-dependent molecular chaperone protein | Heat Resistance |  |
| *dnaK-sHSP* | ATP-dependent molecular chaperone protein | Heat Resistance |  |
| *fbpABC* | ABC Transporter | Iron Uptake | Primary Active Transport |
| *Cyc2* | Outer membrane-bound cytochrome c. Responsible for iron oxidation activity in *Acidithiobacillus ferrooxidans* (Castelle *et al.,* 2008). | Iron Oxidation |  |
| *Ton* system (*TonB-ExbB-ExbD*) | ABC Transporter | Iron Transport | Primary Active Transport |
| NAD(P)H-dependent chromate reductase | Reductase | Chromate Resistance |  |
| *Ars* Operon | Detoxification of arsenic (arsenate, & arsenite) by reduction (*ArsC*) and expulsion (*ArsAB*) | Arsenic Resistance | arsAB - Primary Active Transport |
| *arsM* | Arsenite methyltransferase  | Arsenic Resistance |  |
| *Aox* | Arsenite Oxidase (energy yield) | Arsenite Oxidation |  |
| *Cnr/Czc/Cus System*  | Cation-proton antiporter | Cu(I), Ag(I), Co(II), Ni(II), Co(II), Zn(II), and Cd(II) Export | Secondary Active Transport (H+) |
| *Cue* System | *CopA*: P-type ATPase*CueO*: Periplasmic multicopper oxidase | Cu(I) Detoxification | CopA - Primary Active Transport |
| Multidrug efflux pumps | ABC Transporter | Resistance for Multiple Substrates | Primary Active Transport |

Table S4. KEGG IDs identified through metagenomic sequencing of the Laguna Caliente samples that are associated with genes that have been identified as being part of the Entner-Doudoroff Pathway, Methylglyoxal Pathway, and Serine-Glyoxylate Pathway. Abundance for each gene has been normalized based on the presence of single copy genes (see methods for more details).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Pathway** | **KEGG ID** | **Gene** | **2013 Lake Fluid** | **2017 Lake Bottom Sediment** | **2017 Sulfur Clump** |
| Entner-Doudoroff  | K00844 | HK; hexokinase [EC:2.7.1.1] | 0.018651832 | 0.039331783 | 0.026228143 |
| Entner-Doudoroff | K00012 | UGDH, ugd; UDPglucose 6-dehydrogenase [EC:1.1.1.22] | 1.685209424 | 2.010150137 | 2.024979184 |
| Entner-Doudoroff | K00033 | PGD, gnd, gntZ; 6-phosphogluconate dehydrogenase [EC:1.1.1.44 1.1.1.343] | 1.336387435 | 1.497568196 | 1.43796836 |
| Entner-Doudoroff | K17463 | dgaF; 2-dehydro-3-deoxy-phosphogluconate aldolase [EC:4.1.2.14] | 0 | 0.000845845 | 0 |
| Entner-Doudoroff | K01625 | eda; 2-dehydro-3-deoxyphosphogluconate aldolase / (4S)-4-hydroxy-2-oxoglutarate aldolase [EC:4.1.2.14 4.1.3.42] | 0.866164921 | 0.787481497 | 0.758534555 |
| Methylglyoxal  | K01734 | mgsA; methylglyoxal synthase [EC:4.2.3.3] | 0 | 0 | 0.001248959 |
| Methylglyoxal  | K08234 | yaeR; glyoxylase I family protein | 0.001963351 | 0.561218016 | 0.495004163 |
| Methylglyoxal  | K18471 | ydjG; methylglyoxal reductase [EC:1.1.1.-] | 0.003599476 | 0.000845845 | 0.002914238 |
| Methylglyoxal  | K00128 | ALDH; aldehyde dehydrogenase (NAD+) [EC:1.2.1.3] | 1.861910995 | 8.999365616 | 9.229392173 |
| Methylglyoxal  | K00149 | ALDH9A1; aldehyde dehydrogenase family 9 member A1 [EC:1.2.1.47 1.2.1.3] | 0 | 0.000422922 | 0 |
| Methylglyoxal  | K14085 | ALDH7A1; aldehyde dehydrogenase family 7 member A1 [EC:1.2.1.31 1.2.1.8 1.2.1.3] | 0.005562827 | 0.008458448 | 0.006244796 |
| Methylglyoxal  | K01803 | TPI, tpiA; triosephosphate isomerase (TIM) [EC:5.3.1.1] | 0.912958115 | 1.021780503 | 1.009575354 |
| Serine-Glyoxylate | K14272 | GGAT; glutamate--glyoxylate aminotransferase [EC:2.6.1.4 2.6.1.2 2.6.1.44] | 0.001636126 | 0.000422922 | 0 |
| Serine-Glyoxylate | K00814 | GPT, ALT; alanine transaminase [EC:2.6.1.2] | 0.039267016 | 0.001268767 | 0.002081599 |
| Serine-Glyoxylate | K14260 | alaA; alanine-synthesizing transaminase [EC:2.6.1.66 2.6.1.2] | 0.005562827 | 0.037217171 | 0.006244796 |
| Serine-Glyoxylate | K00827 | AGXT2; alanine-glyoxylate transaminase / (R)-3-amino-2-methylpropionate-pyruvate transaminase [EC:2.6.1.44 2.6.1.40] | 0.007198953 | 0.000422922 | 0.001248959 |
| Serine-Glyoxylate | K00830 | AGXT; alanine-glyoxylate transaminase / serine-glyoxylate transaminase / serine-pyruvate transaminase [EC:2.6.1.44 2.6.1.45 2.6.1.51] | 2.913939791 | 2.324804398 | 2.340133222 |
| Serine-Glyoxylate | K15893 | HPR1; glycerate dehydrogenase [EC:1.1.1.29] | 0.002617801 | 0 | 0 |
| Serine-Glyoxylate | K00018 | hprA; glycerate dehydrogenase [EC:1.1.1.29] | 0.022905759 | 0.057940368 | 0.06286428 |
| Serine-Glyoxylate | K13870 | SLC7A12\_13, AGT1; solute carrier family 7 (L-type amino acid transporter), member 12/13 | 0 | 0 | 0 |