# SUPPLEMENTAL INFORMATION

## Genomic and physiological characterization of the chromate-reducing, aquifer-derived firmicute *Pelosinus* sp. strain HCF1

Harry R. Beller*, Ruyang Han, Ulas Karaoz, Hsiao Chien Lim, Eoin L. Brodie

Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

***Corresponding author:**

Harry R. Beller
Lawrence Berkeley National Laboratory
1 Cyclotron Rd., MS 70A-3317
Berkeley, CA 94720

E-mail HRBeller@lbl.gov
Phone (510) 486-7321
Fax (510) 486-5686

TABLE S1. PCR primers for qPCR analysis of the *rpoB*, *narG*, *nrfH1*, and *nrfH2* genes in strain HCF1.

| Primer names | Primer sequences | Target sequences | Purpose |
|---|---|---|---|
| rpoB_116F | TAAAGGAAGGGCTGCAAGAA | Hcf1DRAFT_04198 rpoB | PCR[a] |
| rpoB_1841R | TTTGCATCATCGTTTTCCAA | Hcf1DRAFT_04198 rpoB | PCR |
| rpoB_1179F | ATTGCTGCAAAACCAATTCC | Hcf1DRAFT_04198 rpoB | qPCR |
| rpoB_1313R | TTGATAGCAGCCACAACAGG | Hcf1DRAFT_04198 rpoB | qPCR |
| narG_294F | GGGAGCTAGCTTCTCCTGGT | Hcf1DRAFT_02301 narG | PCR |
| narG_1834R | CATACTGCCACTGGTTGGTG | Hcf1DRAFT_02301 narG | PCR |
| narG_551F | CTTATGGACCTGACCGCATT | Hcf1DRAFT_02301 narG | qPCR |
| narG_687R | GGGCAAATCTGCATACCAGT | Hcf1DRAFT_02301 narG | qPCR |
| nrfH1_10F | GGGAAATTCCTGGAGCGTAG | Hcf1DRAFT_00451 nrfH1 | PCR |
| nrfH1_471R | TTCAACGTTAACCCCTCCTG | Hcf1DRAFT_00451 nrfH1 | PCR |
| nrfH1_89F | GCGCAGGGTTTGTATATTCC | Hcf1DRAFT_00451 nrfH1 | qPCR |
| nrfH1_218 | TCATGAGGAACATGGCAGTC | Hcf1DRAFT_00451 nrfH1 | qPCR |
| nrfH2_13F | CAGTTTTTAAACCGCAATGCT | Hcf1DRAFT_02324 nrfH2 | PCR |
| nrfH2_463R | TTATTCCCCCTTTGCTCTTG | Hcf1DRAFT_02324 nrfH2 | PCR |
| nrfH2_115F | CCAGGGTTTTGTGGAAGTTG | Hcf1DRAFT_02324 nrfH2 | qPCR |
| nrfH2_226 | CAAAATTCTGCTGTGGCAAA | Hcf1DRAFT_02324 nrfH2 | qPCR |

[a] PCR used to generate templates for calibration standards.

TABLE S2. PCR primers for qPCR analysis of hydrogenase genes in strain HCF1.

| Primer names | Primer sequences | Target sequences | Purpose[a] |
|---|---|---|---|
| hyd1_232F | GCTCAGCAGGCTTTAAATGG | Hcf1DRAFT_00661 | PCR[a] |
| hyd1_1368R | ACCAGGTCCTCCAACACATC | Hcf1DRAFT_00661 | PCR |
| hyd1_485F | TCACACGTCCCTGTGAAAGA | Hcf1DRAFT_00661 | qPCR |
| hyd1_612R | TGCACCAAAAGGACATGCTA | Hcf1DRAFT_00661 | qPCR |
| hyd2_352F | GGCATAAAACAGGTGCGATT | Hcf1DRAFT_01773 | PCR |
| hyd2_1564R | TATAAAGCCCTGCGCCTCTA | Hcf1DRAFT_01773 | PCR |
| hyd2_631F | ACAGCGAAACTCTGGAAGGA | Hcf1DRAFT_01773 | qPCR |
| hyd2_745R | CATTTTGCCCCTCTGAAAAA | Hcf1DRAFT_01773 | qPCR |
| hyd3_69F | CTCGGAAGTCTGCCCTGTAG | Hcf1DRAFT_02066 | PCR |
| hyd3_1272R | CTTTCGATAGCGCTGCTCTT | Hcf1DRAFT_02066 | PCR |
| hyd3_824F | CGGCGATTGATTTTAATGCT | Hcf1DRAFT_02066 | qPCR |
| hyd3_935R | ATCGCTGCTTCCATAACACC | Hcf1DRAFT_02066 | qPCR |
| hyd4_139F | CGCCAAGTAGCAAACCAAAT | Hcf1DRAFT_02349 | PCR |
| hyd4_1165R | TACAAGCCATGCCTTCAATG | Hcf1DRAFT_02349 | PCR |
| hyd4_794F | AACCAGATGCCAAAGTGGTC | Hcf1DRAFT_02349 | qPCR |
| hyd4_902R | AGCTCCTGAAAGGTCAGCAC | Hcf1DRAFT_02349 | qPCR |
| hyd5_24F | TCAACTGTGCATGTCAGCAA | Hcf1DRAFT_02598 | PCR |
| hyd5_1095R | TAATTCCTGCTTTGCCTGCT | Hcf1DRAFT_02598 | PCR |
| hyd5_720F | CCCTGGCAGTACAGGATGTT | Hcf1DRAFT_02598 | qPCR |
| hyd5_863R | AAATGAGGACTGGCACAACC | Hcf1DRAFT_02598 | qPCR |
| hyd6_45F | TGTAAGTCGGCGAAACTTCTT | Hcf1DRAFT_03510 | PCR |
| hyd6_1046R | TGAATCGTAGAAAGTGCACCA | Hcf1DRAFT_03510 | PCR |
| hyd6_442F | GCTAAAGGAGCTGCTGCAAT | Hcf1DRAFT_03510 | qPCR |
| hyd6_584R | GGGCATCCCGGTACTTTAAT | Hcf1DRAFT_03510 | qPCR |

[a] PCR used to generate templates for calibration standards.

TABLE S3. Relative transcription of six hydrogenase genes in strain HCF1.

| Electron donor[a] | Relative expression (transcript copy number normalized to that of *rpoB*) | | | | | |
|---|---|---|---|---|---|---|
| | **hyd1[b]** | **hyd2[b]** | **hyd3[b]** | **hyd4[b]** | **hyd5[b]** | **hyd6[b]** |
| Lactate | 0.25±0.036[c] | 0.19±0.037 | 0.21±0.061 | 0.27±0.055 | 0.08±0.018 | 1.50±0.182 |
| Fructose | 0.19±0.037 | 0.12±0.114 | 0.21±0.098 | 0.18±0.238 | 0.04±0.024 | 1.54±0.461 |
| $H_2$ | 0.31±0.123 | 0.23±0.157 | 0.41±0.252 | 0.29±0.225 | 0.12±0.074 | 1.90±0.203 |

a The sole electron donors and headspace compositions were: (a) lactate (80% $N_2$/10% $CO_2$), (b) fructose (80% $N_2$/10% $CO_2$), and (c) 90% $H_2$/10% $CO_2$.

b hyd1 = Hcf1DRAFT_00661 [FeFe]; hyd2 = Hcf1DRAFT_01773 [FeFe]; hyd3 = Hcf1DRAFT_02066 [FeFe]; hyd4 = Hcf1DRAFT_02349 [FeFe]; hyd5 = Hcf1DRAFT_02598 [NiFe]; hyd6 = Hcf1DRAFT_03510 [NiFe]

c Mean ± standard deviation

TABLE S4. Relative transcription of three nitrate or nitrite reductase genes in strain HCF1[a]

| **Conditions**[b] | **Relative expression (transcript copy number normalized to that of *rpoB*)** | | |
|---|---|---|---|
| | *narG*[c] | *nrfH1*[d] | *nrfH2*[e] |
| No nitrate, 36 hr | 0.018 ± 0.002[f] | 0.053 ± 0.013 | 0.308 ± 0.067 |
| No nitrate, 72 hr | 0.018 ± 0.002 | 0.087 ± 0.028 | 0.097 ± 0.018 |
| Nitrate, 36 hr | 0.308 ± 0.039 | 0.062 ± 0.012 | 0.794 ± 0.235 |
| Nitrate, 72 hr | 0.472 ± 0.148 | 0.156 ± 0.072 | 0.807 ± 0.245 |

a This table includes the experimental error for the data presented in Figure 3B.

b See text and Figure 3.

c *narG* = Hcf1DRAFT_02301

d *nrfH* - copy 1 = Hcf1DRAFT_00451

e *nrfH* - copy 2 = Hcf1DRAFT_02324

f Mean ± standard deviation

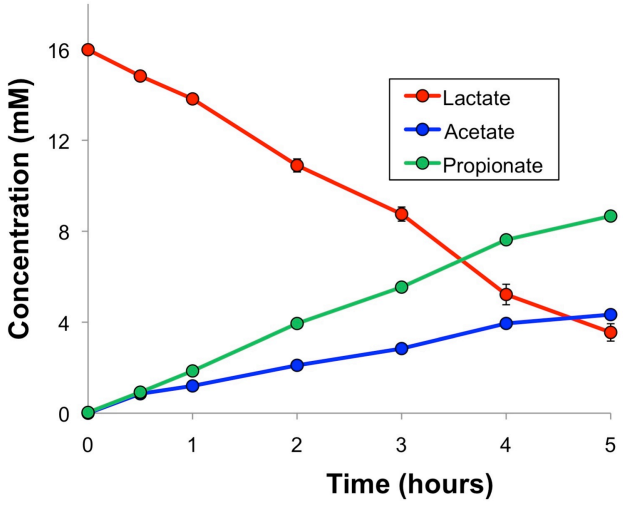


**FIGURE S1**. Fermentation of lactate to acetate and propionate in anaerobic cell suspensions of strain HCF1. Cell densities were approximately 1.3 x $10^9$ cells/mL. Data points represent averages of duplicates and error bars represent 1 standard deviation.

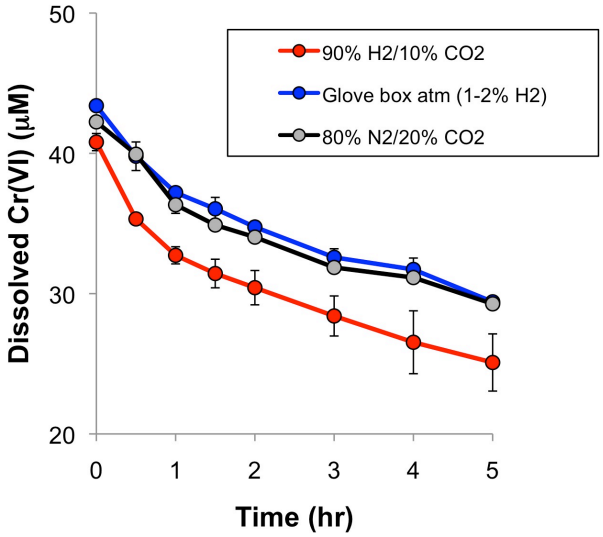


**FIGURE S2**. Results of cell suspension assays to determine the ability of strain HCF1 to use $H_2$ as the sole electron donor for Cr(VI) reduction. Three hydrogen concentrations were used: (1) no $H_2$ (80% $N_2$/20% $CO_2$), (2) the glove box atmosphere (1-2% $H_2$), and (3) 90% $H_2$/10% $CO_2$. Cell densities were approximately 5.6 x $10^8$ cells/mL. Data points represent averages of duplicates and error bars represent 1 standard deviation.

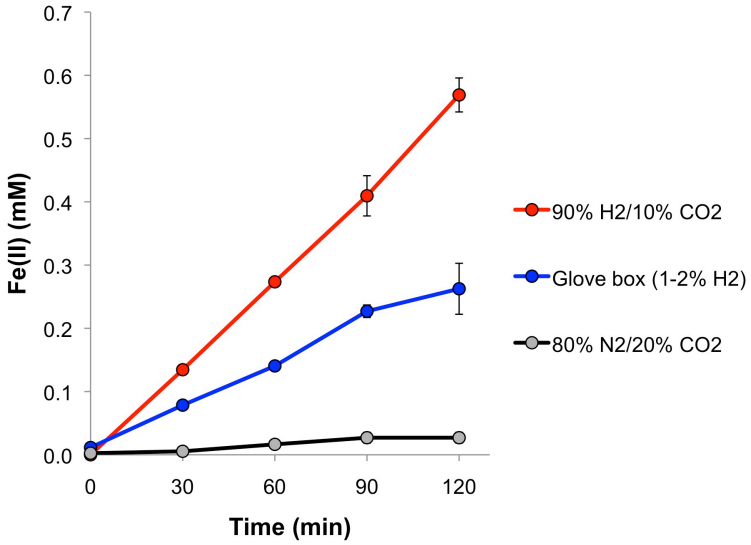


**FIGURE S3**. Results of cell suspension assays to determine the ability of strain HCF1 to use $H_2$ as the sole electron donor for Fe(III)-NTA reduction. Hydrogen concentrations used were the same as those described in Supplementary Figure 2. Cell densities were approximately 4.6 x $10^8$ cells/mL. Data points represent averages of duplicates and error bars represent 1 standard deviation.

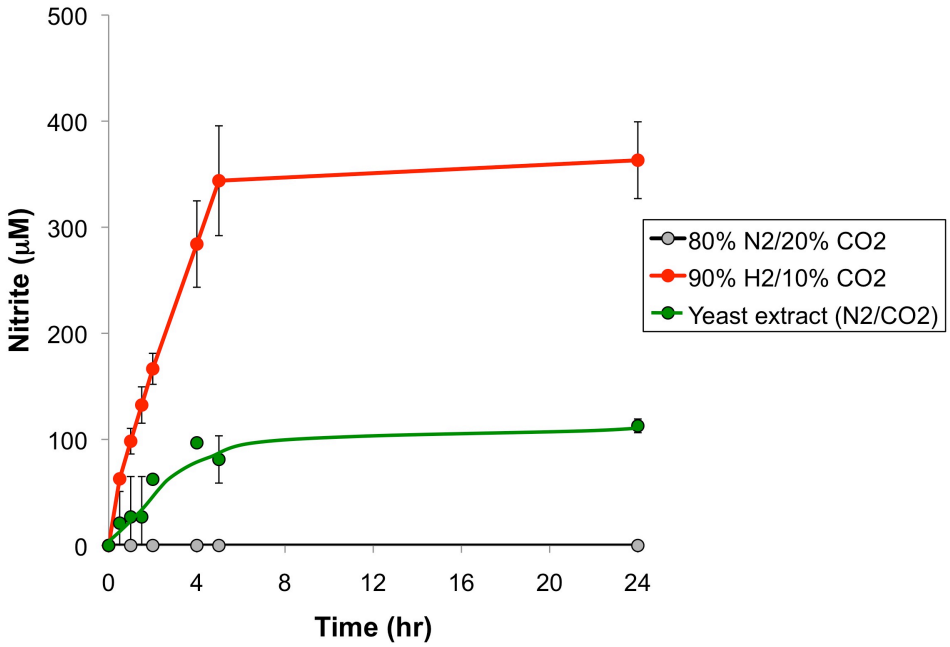


**FIGURE S4**. Results of cell suspension assays to determine the ability of strain HCF1 to use $H_2$ or yeast extract as the electron donor for nitrate reduction. Two hydrogen concentrations were used: (1) no $H_2$ (80% $N_2$/20% $CO_2$) and (2) 90% $H_2$/10% $CO_2$. When present, yeast extract was at the same concentration as in the growth medium used for the experiment represented in Figure 3 (0.5 g/L). Data points represent averages of duplicates and error bars represent 1 standard deviation. It is clear from the data that yeast extract could only account for a small portion of the nitrite accumulated in the experiment represented in Figure 3A.

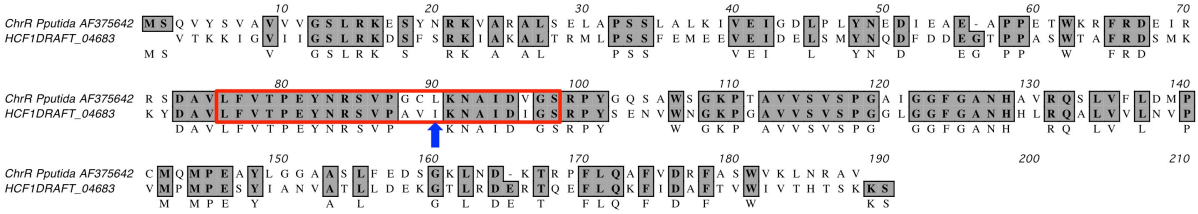


**FIGURE S5**. Alignment of the predicted amino acid sequence of the putative flavoprotein Hcf1DRAFT_04683 and the sequence of the chromate reductase ChrR from *P. putida* (GenBank AF375642). Identical residues are shaded in gray. The red box highlights a motif characteristic of the NADH_dh2 family of putative flavin-binding quinone reductases and the blue arrow indicates an L90I deviation in the Hcf1DRAFT_0468 sequence (see text).