Supplement to:

# Expression and Activity of the BioH Esterase of Biotin Synthesis is Independent of Genome Context

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**Table S1. Bacterial strains and plasmids**

| Strains | Relevant Genotype or Description | Ref. or Derivation |
|---|---|---|
| *E. coli* | | |
| MG1655 | *E. coli* K-12 wild type | Lab stock |
| BL21(DE3) | *E. coli* B *ompT hsdSB gal dcm* (DE3) | Lab stock |
| S17.1 | $F^-$ *thi pro hsd*R [RP4-2 Tc::Mu Km:: Tn] | Lab stock |
| STL14 | BL21 (DE3)/pSTL6 | [1] |
| STL111 | MG1655 Δ*bioD::kan*$^r$ | Lab stock |
| XC.037 | BL21 (DE3)/pXC.039 | This study |
| XC.047 | STL111/pKD46 | This study |
| XC.052 | MG1655 Δ*bioD bioH::His*$_6$-FRT | This study |
| *P. aeruginosa* | | |
| PAO1 | | Lab stock |
| XC.059 | PAO1 *bioD::Gm* | This study |
| XC.109 | PAO1 *bioD::Gm bioH::His*$_6$*-Tet* | This study |
| **Plasmids** | | |
| pET28b | T7 promoter expression vector, KanR | Novagen |
| pKD3 | Template plasmid for FRT-flanked cat cassette | [2] |
| pKD46 | Encodes recombineering phage lambda Red genes | [2] |
| pCP20 | Encodes the yeast Flp recombinase gene | [3] |
| pSTL6 | pET28b encoding C-terminal His-tagged *E. coli* BioH | [1] |
| pSTL42 | pET28b encoding *E. coli* BioH plus $His_6$-tagged *E. coli* BioC | Lab stock |
| pXC.039 | pET28b encoding C-terminal His-tagged *P. aeruginosa* BioH | This study |
| pXC.042 | pET28b encoding C-terminal His-tagged *P. aeruginosa* BioC | This study |

| | | |
|---|---|---|
| pXC.040 | A 1,000 bp PCR fragment containing the *P. aeruginosa* *bioD*::Gm$^r$ cassette in pK19mobsacB, Gm$^r$, Kan$^r$ | This study |

**Table S2. Oligonucleotides (5'-3')**

| | | |
|---|---|---|
| BioH-F | BioH Forward | ATACTACCATGGATGCGTGACCACTTGATCCTGTTGCCGGGCT |
| BioH-R | BioH Reverse | ACTACTCGAGGGCATCGTCACCCTCGCGAAGGAATCTCA |
| BioC-F | BioC Forward | ATACTACCATGGATGCCTGACGATTCCTCG |
| BioC-R | BioC Reverse | ACTACTCGAGGGAATCCTTGCGCAGCAC |
| P1 | P1 priming site for *E. coli* genomic | CGAGTTTTGTCACCTGCTGGTGGCGTTGAAGCAGAGGGTGCATCACCATCACCA |
| | bioH-$His_6$ | TCACTAATGTGTAGGCTGGAGCTGCTTC |
| P2 | P2 priming site for *E. coli* genomic | GCGAGTCAGTAAAGTTCTGTCTCGCCATTTCAAAAGCCACCATATGAATATCCT |
| | bioH-$His_6$ | CCTTA |
| oXC48 | UpbioD Forward (EcoRI) | ACATGAATTCCACAACCTCAACCCCGGGC |
| oXC49 | DownbioD Reverse (HindIII) | ATACAAGCTTCGCGTTGAGGCCACTGACGAAA |
| oXC50 | DownbioD Forward (BamHI) | TTCACCCTGGTGGATCCGGAGGGCGC |
| oXC51 | UpbioD Reverse (BamHI) | GCGCCCTCCGGATCCACCAGGGTGAA |
| oXC53 | *E. coli bioH* Reverse | GCGAGTCAGTAAAGTTCTGTCTCGCCATTTC |
| oXC54 | *E. coli bioH* Forward | AACTCAGTGATGATTTTCAGCGTAC |

| | | |
|---|---|---|
| oXC92 | PAO1 *bioC* Forward (q-RT) | TTCGTCCACGTCAATCGCT |
| oXC93 | PAO1 bioC Reverse (q-RT) | GCCTTGAGTTCGTGGGTCA |
| oXC116 | *E. coli* 16S Forward (q-RT) | TACCGCATAACGTCGCAAGA |
| oXC117 | *E. coli* 16S Reverse (q-RT) | TTCCAGTGTGGCTGGTCATC |
| oXC118 | *E. coli bioH* Forward (q-RT) | ATTGCGTTAACCCATCCCGA |
| oXC119 | *E. coli bioH* Reverse (q-RT) | GTTTGTAACGCCAGGAACCG |
| oXC124 | *E. coli bioC* Forward (q-RT) | CGACGTTCGATCTTGCATGG |
| oXC125 | *E. coli bioC* Reverse (q-RT) | GATAATGCACGCCGTTCAGC |
| oXC126 | PAO1 bioH::$His_6$-Tet Forward | ATATAGAATTCAACTCGACGATAACCTGCCGCGCGATA |
| oXC131 | PAO1 bioH::$His_6$-Tet Reverse | ATGCAGGAGCCGGTCCTCGT |
| oXC184 | PAO1 *bioH* Forward (q-RT) | TGCGTGACCACTTGATCCTG |
| oXC185 | PAO1 *bioH* Reverse (q-RT) | CGGCAGGTTATCGTCGAGTT |
| oXC188 | PAO1 *16S* Forward (q-RT) | CAAGGCGACGATCCGTAACT |
| oXC189 | PAO1 16S Reverse (q-RT) | ATCAGGCTTTCGCCCATTGT |
| oXC201 | pET28b Kan Forward (q-RT) | CGGTTTGGTTGATGCGAGTG |
| oXC202 | pET28b Kan Reverse (q-RT) | GTGACGACTGAATCCGGTGA |

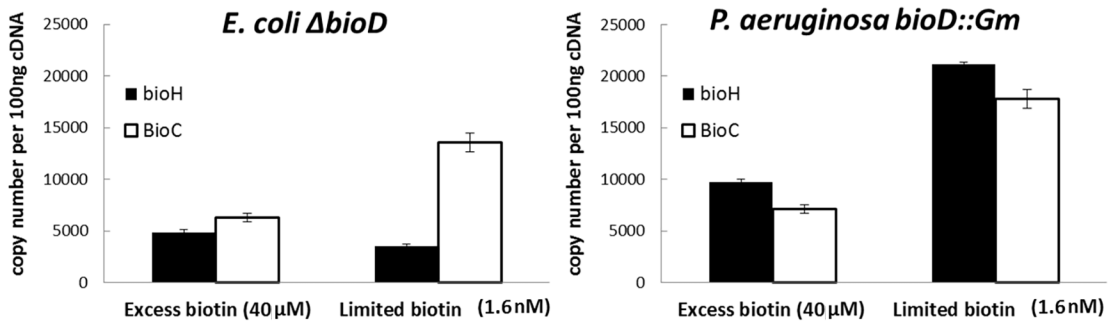


**Figure S1.** Transcriptional profiles of *bioH* and *bioC* in an *E. coli* Δ*bioD* (strain STL111) and in *P. aeruginosa bioD::Gm* (strain XC.059). Cultures were grown to mid-log phase in defined medium supplemented with excess biotin (40 μM) or limited biotin (1.6 nM), respectively. Absolute transcript values for each gene were obtained individually. Error bars indicate standard deviation from three independent experiments. The amplification efficiency for each pair of primers was evaluated (Materials and Methods). A linear equation of "Ct-value vs gene concentration" for *bioC* and *bioH* was generated with the corresponding primer pairs.

Supplemental References

1 Lin, S., Hanson, R. E. & Cronan, J. E. Biotin synthesis begins by hijacking the fatty acid synthetic pathway. *Nat Chem Biol* **6**, 682-688,

2 Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A* **97**, 6640-6645.

3 Cherepanov, P. P. & Wackernagel, W. Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* **158**, 9-14 (1995).