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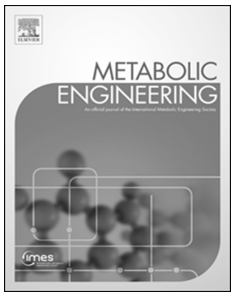

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Corrigendum to "Engineering *Pseudomonas putida* KT2440 for efficient ethylene glycol utilization"

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The sequence of the promoter used to drive expression of *glcDEF* operon in the engineered strain MFL185 in the original article was incorrect. As described here, we performed additional experiments that indicate expression of this operon was increased in MFL185, as intended. Ultimately, this error is immaterial with respect to the findings and conclusions reported in the original article.

The intended sequence of the plasmid used to integrate the the *tac* promoter upstream of the *glcDEF* operon was provided in the supplementary material in the original manuscript and included a 107 bp region 5' of the start codon for *glcD* (Fig. 1a). The *lac* operator, which serves to repress expression in *Escherichia coli* during plasmid construction, was accidentally omitted. Likely as a result of expression levels that were toxic in *E. coli*, a mutation deleting 49 bp and the intended promoter (Fig. 1b) occurred and was overlooked from earlier sequencing results. As a result, the actual sequence of MFL185 is missing portions of the *soxR* terminator and the *tac* promoter. Despite this inadvertent omission, another potential promoter fortuitously introduced by this deletion was identified by an online bacterial promoter predictor program (BPROM – www.softberry.com) is underlined in Fig. 1b. In the original article, MFL185 exhibited greater ethylene glycol utilization relative to MFL168, which is identical to MFL185 except that does not have the additional promoter driving expression of *glcDEF*, which we attributed to improved metabolic flux of ethylene glycol intermediates caused by overexpression of the *glcDEF* operon. Upon discovery that the incorrect sequence was integrated 5' of these genes, however, we sought to determine whether the *glcDEF* operon is more highly expressed in MFL185 than MFL168 and whether expression of that operon driven by the *tac* promoter as originally intended could results in better growth on ethylene glycol. Thus, we constructed a new strain (MFL213) which included the correct *tac* promoter and is shown in Fig. 1c. We compared growth of three strains: MFL168 (*P. putida* KT2440 *fpvA*::P_{tac}:*gcl*), MFL185 (*P. putida* KT2440 *fpvA*::P_{tac}:*gcl* $P_{tac(49\ bp\ deletion)}$:*glcD)* and MFL213 *P. putida* KT2440 *fpvA*::P_{tac}:*gcl* P_{tac}:*glcD)* in ethylene glycol, both in a Bioscreen C microplate reader used in the original study and in shake flasks. Additionally, we performed quantitative real-time PCR to analyze expression of *glcD* in these strains as well as in wild-type *P. putida* KT2440 strain.

a) CGCAAAAAACCGCACCCAGGTGCGGTTTTTTGAATTCGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTCAGACTCAATAATAATAATAAGGAGG
TATCGAATG
b) CGCAAAAAACCGCACCCAGGTGCGGTT-
----------AGACTCAATAATAATAATAAGGAGGTATCGAATG

c) CGCAAAAAACCGCACCCAGGTGCGGTTTTTTGAATTCGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCA
CACCGGAGGGAGTTTTGCGATG

Figure 1. (a) 107 bp region 5' of *glcD* of pLJ030 used to integrate the promoter upstream of *glcD*. **(b)** Engineered plasmid sequence of pLJ030 used for construction of strain MFL185 and MFL186 which contained a 49 bp deletion, and a potential, inadvertently introduced promoter. **(c)** 120 bp sequence of region 5' of *glcD* which contains the correct tac promoter. The soxR terminator is double underlined. The -35 and -10 regions of the promoters are underlined. The Shine Delgarno sequence is underscored by a broken line. The lac operator sequence (*trp* repressor site) is underscored by a wavy line.

Evaluation of growth on ethylene glycol: Assays comparing growth of MFL168, MFL185, and MFL213 on ethylene glycol in 1X M9 medium in a plate reader and shake flasks were performed as described in the original article. Similar results were observed in the microplate reader (Fig. 2) and shake flask (Fig. 3) experiments; both MFL185 and MFL213 exhibited similarly improved growth an ethylene glycol relative to MFL168.

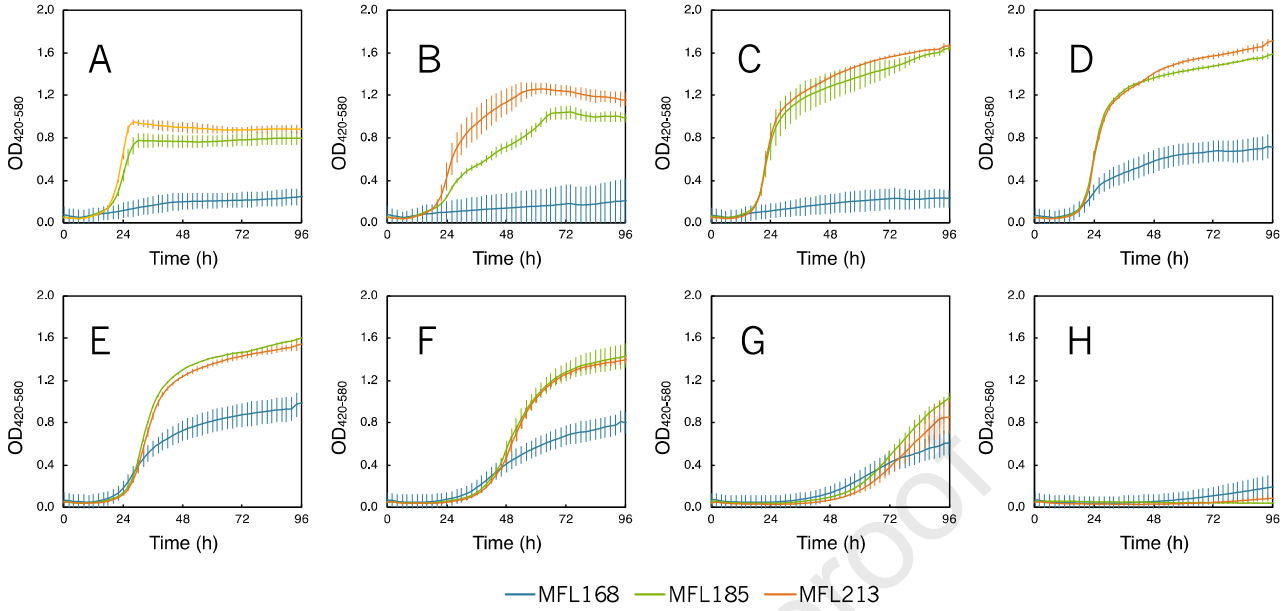

Figure 2. Growth comparison of MFL168 (blue), MFL185 (green) and MFL213 (orange) in a microplate reader on various concentrations of ethylene glycol (A) 0.05 M (B) 0.1 M (C) 0.25 M (D) 0.5 M (E) 1.0 M (F) 1.5 M (G) 1.8 M and (H) 2.0 M

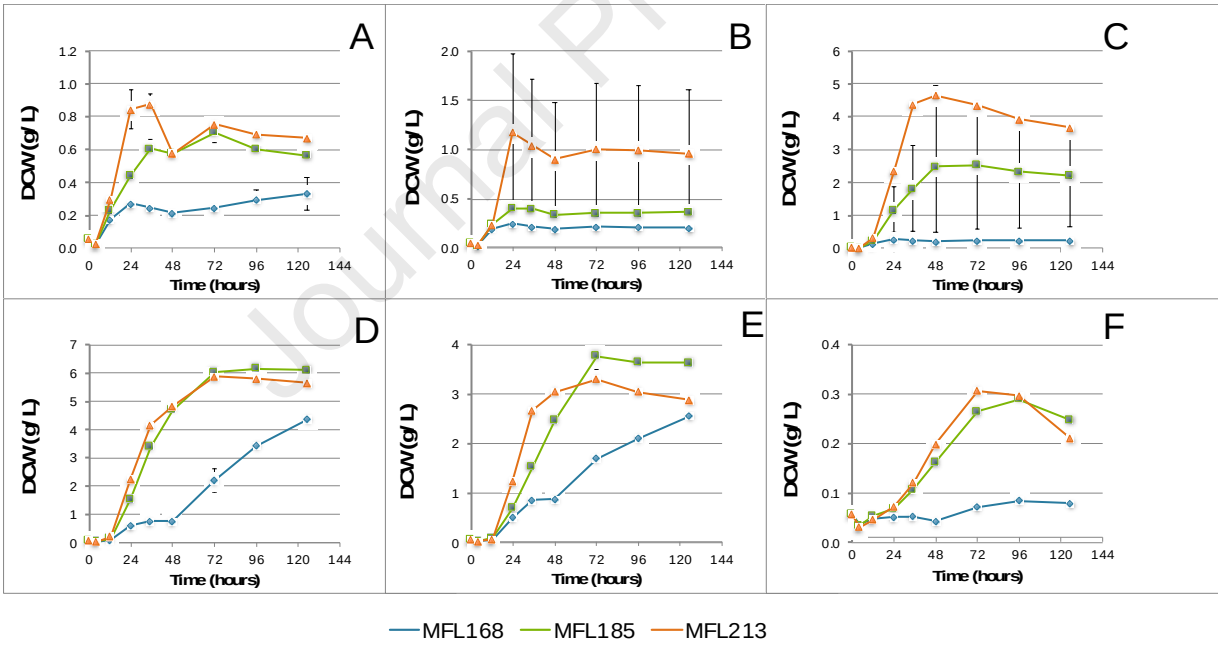

Figure 3. Growth comparison using dry cell weight (DCW) of MFL168 (blue), MFL185 (green), and MFL213 (orange) in shake flasks on various concentrations of ethylene glycol (A) 0.1 M (B) 0.25 M (C) 0.5 M (D) 1.0 M (E) 1.5 M and (F) 2.0 M.

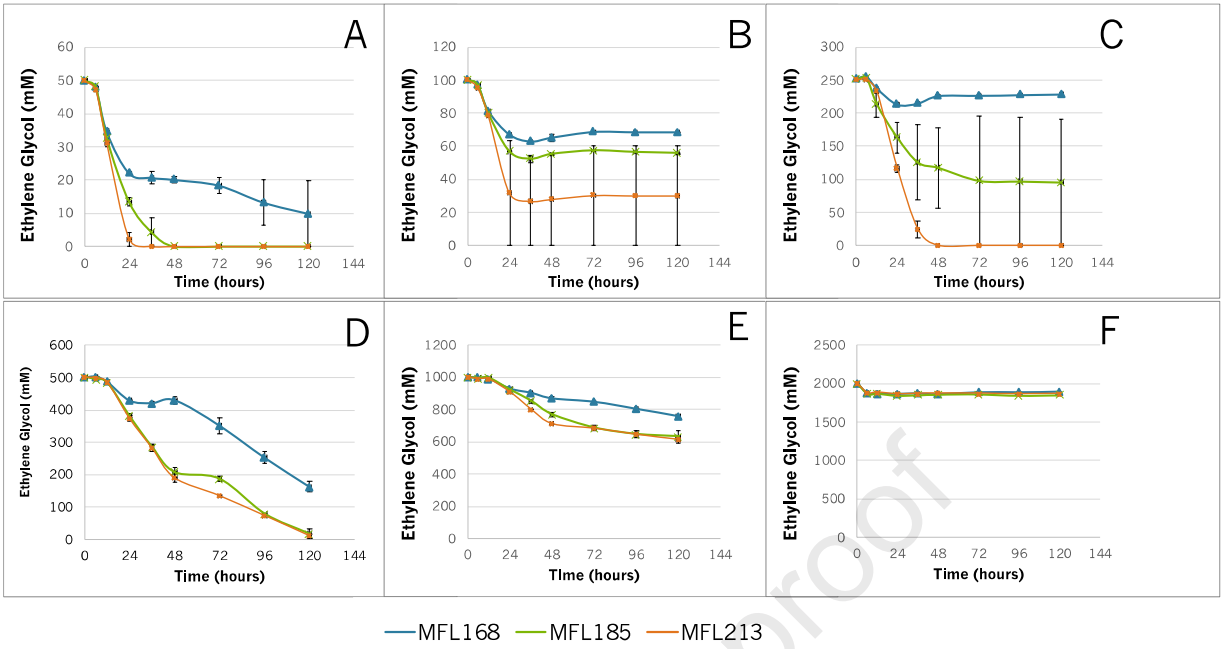

Figure 4. Ethylene glycol consumption of MFL168 (blue), MFL185 (green), and MFL213 (orange) in shake flasks on various concentrations of ethylene glycol (A) 0.1 M (B) 0.25 M (C) 0.5 M (D) 1.0 M (E) 1.5 M, and (F) 2.0 M.

Quantitative real-time PCR: To evaluate expression of the *glcDEF* operon in MFL185 compared to the base strain (MFL168), we conducted quantitative real-time PCR experiments to measure transcript levels of *glcD*. *P. putida* cultures for RNA extraction were prepared and RNA extracted as described in the original article, except that seed cultures were grown in LB, prior to being washed, and resuspended in triplicate shake flasks containing M9 minimal medium with 20 mM glucose and harvested when OD_{600} reached ~2-3. RNA extraction, cDNA synthesis, primer and probe design, and quantitative PCR, and analysis were performed as described in the original article, with the housekeeping gene *rpoD* serving to normalize expression levels.

Transcript levels for *glcD* in MFL168 were similar to the wild-type parent strain, as anticipated. Relative to the wild-type strain and parent strain, transcript levels for *glcD* were ~20X higher in MFL185 and ~ 370X in MFL213, the strain with the corrected *tac* promoter. Together, these results demonstrate that the altered sequence 5' to *glcDEF* in MFL185 did increase its transcript levels and confer improved growth on ethylene glycol, even though the intended sequence was not present. The conclusions stated in our original article are consistent with the results described in this corrigendum.

Table 1. Primers used for gene expression of *glcD* and the housekeeping gene *rpoD*

Primer ID	Primer Sequence	Gene Target
oLP395	TCTGGAGCTGTGTGTGAAAG	MF_GlcD FWD
oLP396	AGGGTAATTTCGTCGCTGTT	MF_GlcD REV
oLP397	/56-FAM/ACATCTGGT/ZEN/TGATCTTCTCGCGCC/3IABkFQ/	MF_GlcD PRB
oLP398	AACCTGCGTCTGGTGATTT	MF_rpoD FWD
oLP399	ACTTGTCCACCGCTTTCAT	MF_rpoD REV
oLP400	/56-FAM/ATCGCCAAG/ZEN/AAGTACACCAACCGT/3IABkFQ/	MF_rpoD PRB

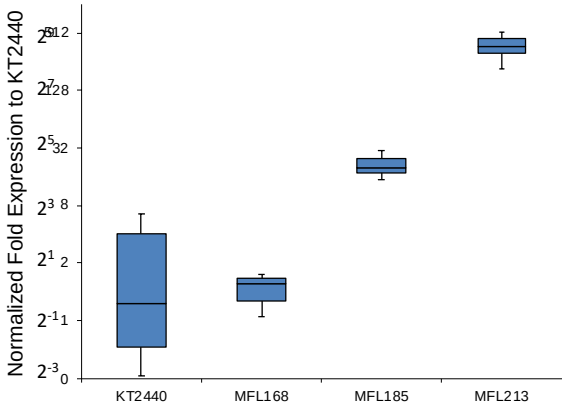

Figure 5. Box plot figure representing fold change in gene expression of *glcD* compared to the control *P. putida* KT2440. Expression levels of MFL168, MFL185, and MFL213 were normalized to the housekeeping gene *rpo*D and then normalized to expression levels present in the wild-type strain *P. putida* KT2440.

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