

CORRECTION

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Correction: Adipic acid production from lignin

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Correction for 'Adipic acid production from lignin' by Derek R. Vardon et al., *Energy Environ. Sci.*, 2015,
8, 617–628, <https://doi.org/10.1039/C4EE03230F>.

We have discovered that pMFL22, the plasmid that was used to replace *catRBC* with the *tac* promoter to cause accumulation of muconic acid and drive constitutive expression of the catechol dioxygenase gene *catA* in *Pseudomonas putida* KT2440, contains a mutation. Consequently, this mutation is also present in the muconate production strain MFL30 (*P. putida* KT2440 $\Delta catRBC::Ptac:catA$) and strains derived from it. The mutation occurs just upstream of *catA*, likely in or near its ribosome binding site (Fig. 1).

To determine if this mutation might affect expression and, thus, activity of CatA in our muconate production strains, we repaired this mutation in the muconate production strain CJ200 (*P. putida* KT2440 $\Delta catRBC::Ptac:catA$ $\Delta pcaHG::Ptac:aroY:ecdB:asbF$), a strain derived from MFL30 that was further engineered to produce muconate from sugars as well as aromatic molecules by the introduction of genes encoding a protocatechuate decarboxylase, *aroY*, an accessory protein required to produce a prenylated FMN cofactor required by AroY, *ecdB*, and a 3-dehydroshikimate dehydratase, *asbF*.¹ The performance of CJ200 was compared to the repaired strain, GB221, by growing the strains in M9 minimal media supplemented with 10 mM glucose and 30 mM benzoate at 30 °C, shaking at 225 rpm, and feeding an additional 10 mM glucose after 12 h (Fig. 2).

tac promoter A→C *catA*
TTGACAATTATCATCGGCTCGTATAATGTGTGAATTGTGAGCGGATAACAATTTACACACAATACGAGGTCAGCACGATGACCGTG...

Fig. 1 The sequence of *tac* promoter, the 5' end of the *catA* gene, and the intervening sequence showing the mutation in plasmid pMFL22 and, consequently, MFL30 and other muconate production strains derived from it.

Surprisingly, repairing the mutation back to the wild-type sequence in GB221 resulted in the accumulation of catechol that was not observed in CJ200. Since the strains are otherwise identical, this suggests that the mutation likely results in increased expression and, subsequently, activity of CatA that enables catechol to be metabolized more quickly. Going forward, we intend to incorporate this mutation in subsequent *P. putida* KT2440 strains engineered to produce muconate and would recommend others interested in similar strain engineering to do the same by introducing the mutation into primer LP32 (5'-AATTTACACAATACGAGGTCAGCACGATG-3') when constructing the $\Delta catRBC::Ptac:catA$ plasmid as described in our original manuscript. We apologize to readers for not being aware of this mutation sooner, and we thank Dr Gayle Bentley for making strain GB221 and conducting these experiments.

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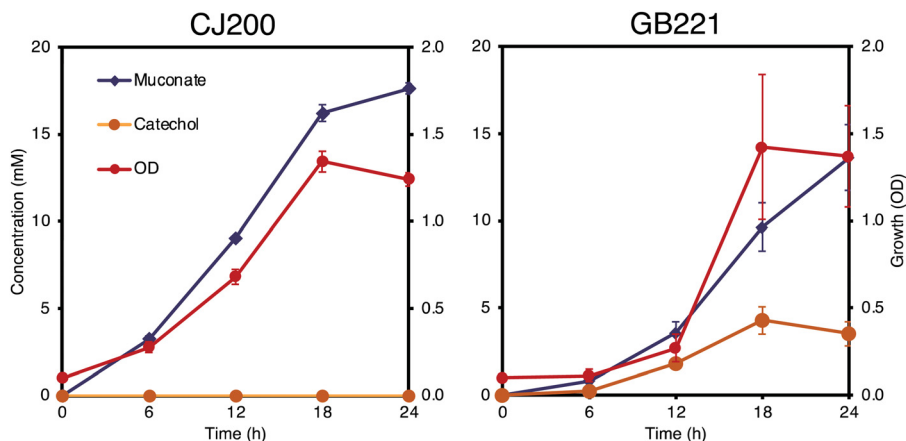


Fig. 2 Production of muconate from benzoate and glucose by CJ200 and the equivalent strain with the mutation repaired, GB221. Strains were cultivated in M9 minimal media supplemented with 10 mM glucose and 30 mM benzoate at 30 °C, shaking at 225 rpm, and fed an additional 10 mM glucose after 12 h. The cultures were sampled periodically to evaluate growth (OD₆₀₀) and muconate and catechol accumulation by HPLC according to methods in the original publication.

The Royal Society of Chemistry apologises for these errors and any consequent inconvenience to authors and readers.

References

- 1 C. W. Johnson, D. Salvachúa, P. Khanna, H. Smith, D. J. Peterson and G. T. Beckham, Enhancing muconic acid production from glucose and lignin-derived aromatic compounds via increased protocatechuate decarboxylase activity, *Metab. Eng. Commun.*, 2016, **3**, 111–119.

