

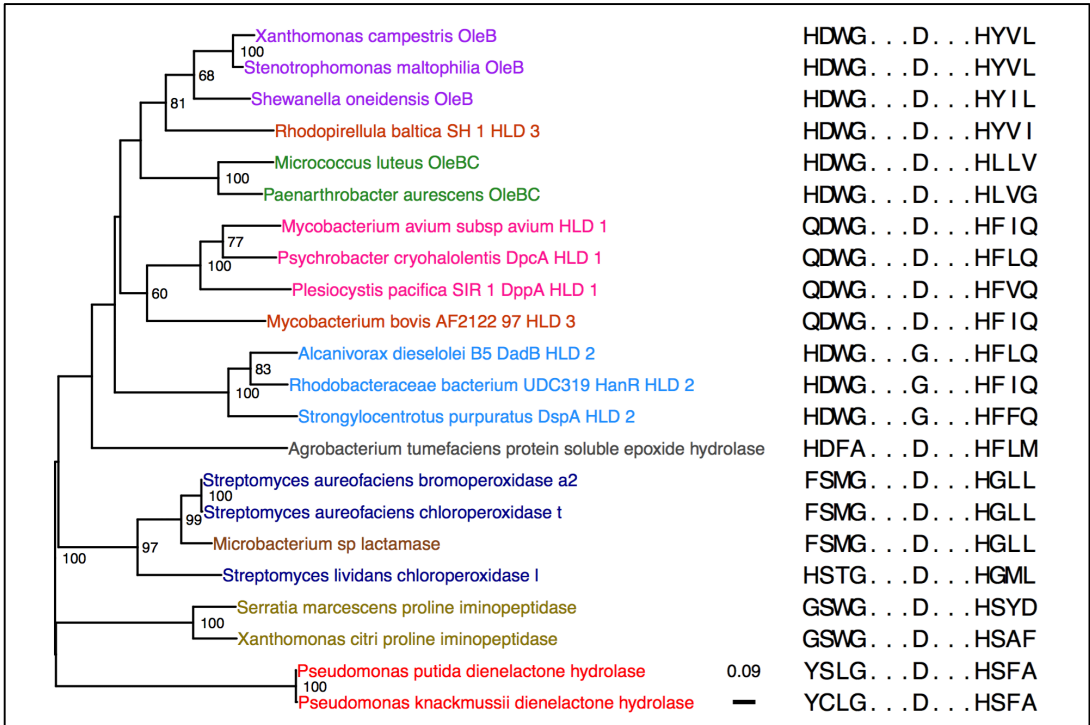
# OleB from bacterial hydrocarbon biosynthesis is a $\beta$ -lactone decarboxylase sharing key features with haloalkane dehalogenases

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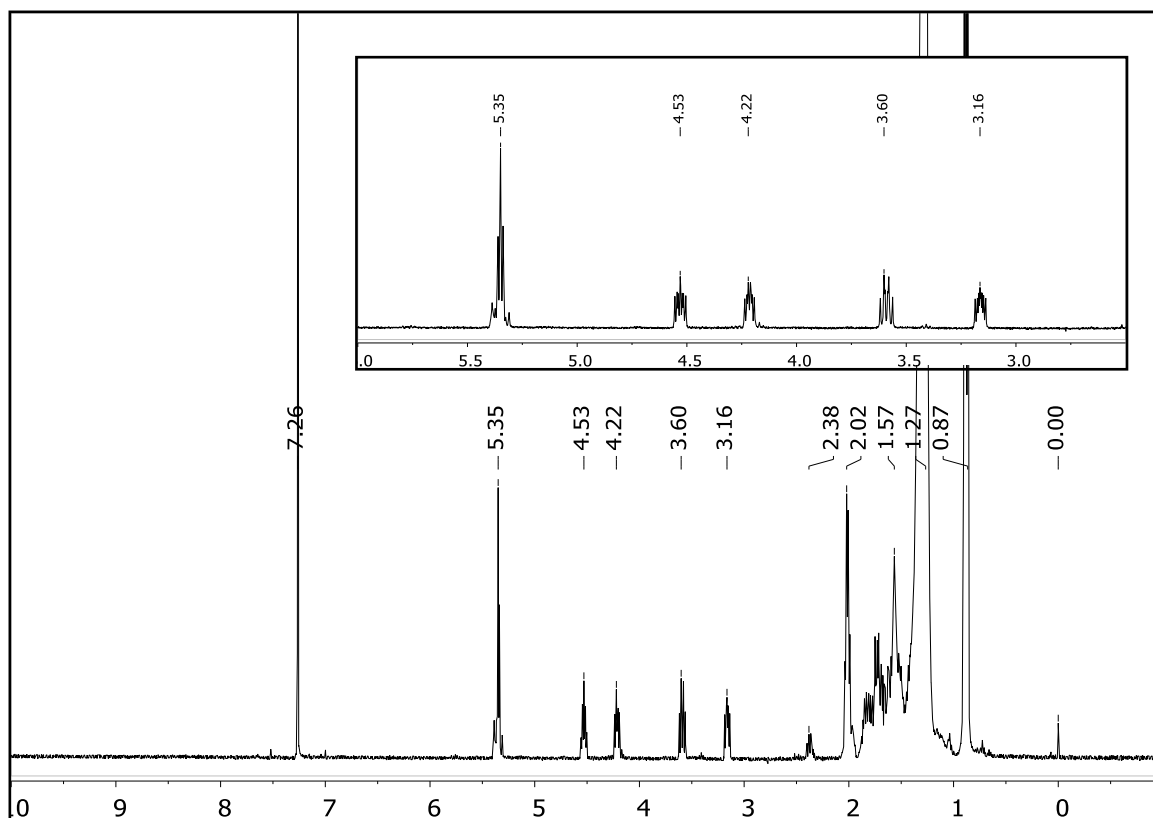
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Running Title:  $\beta$ -lactone decarboxylase

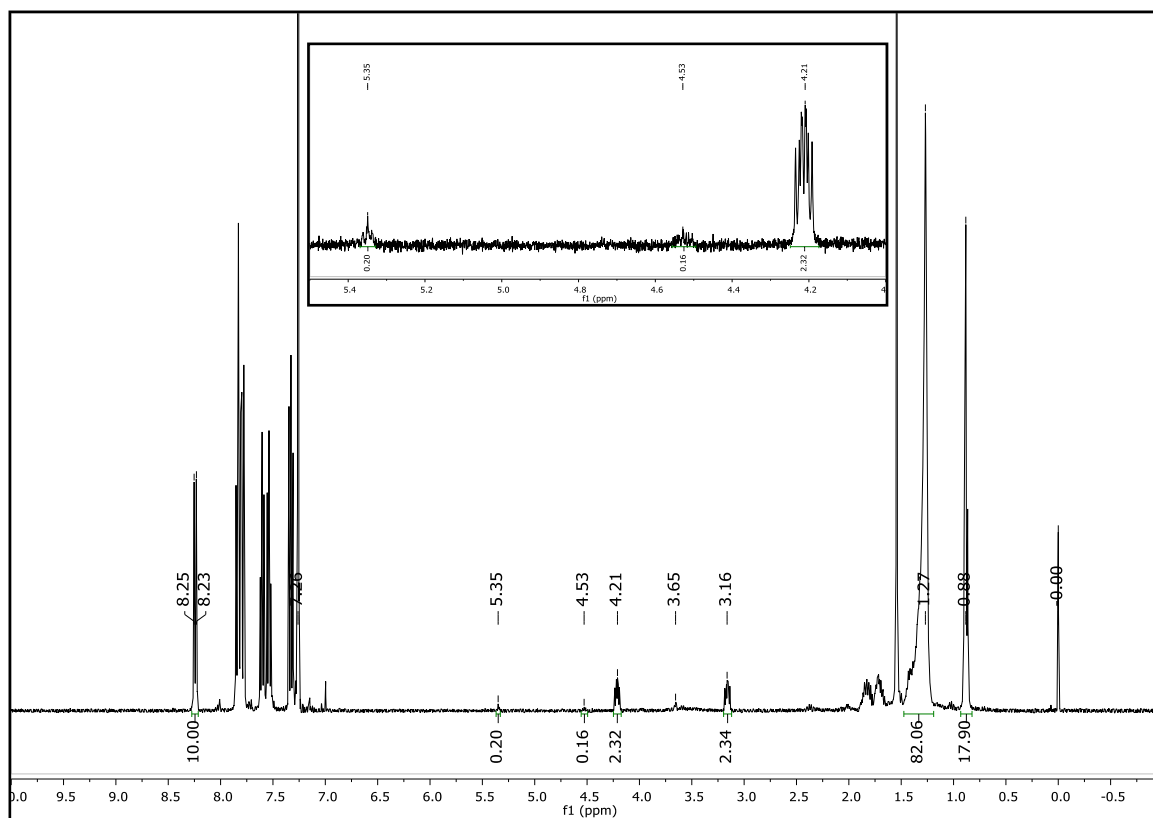
## Supplemental Figures



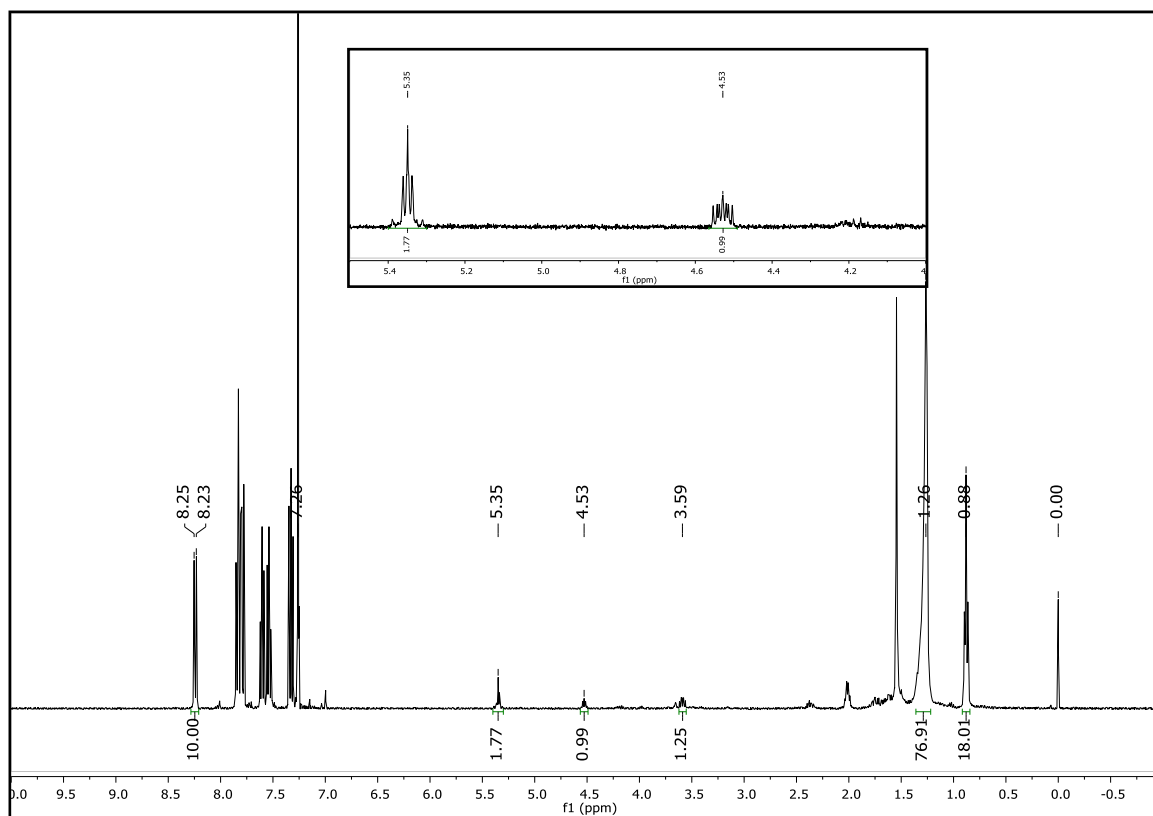
**Figure S1.** Alignment of OleB and OleBC fusion proteins with bacterial  $\alpha/\beta$ -hydrolase enzymes.



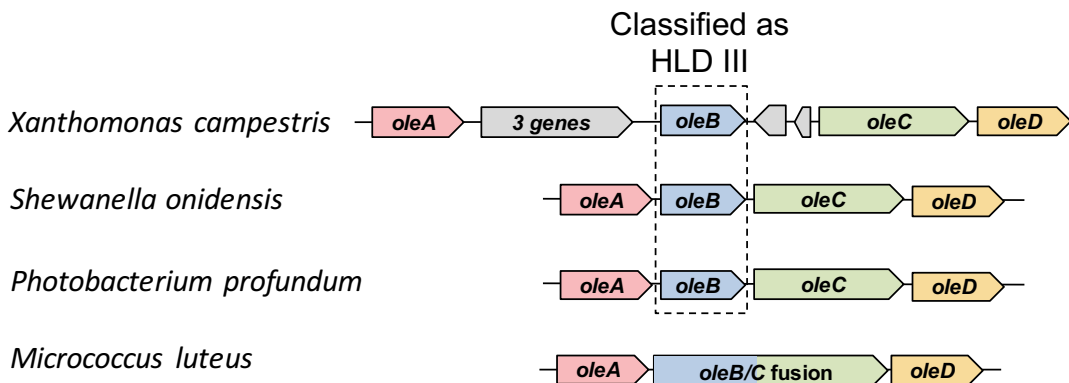
**Figure S2** Mix of the synthetic standards of *cis*- and *trans*-3-octyl-4-nonyl-2-oxetanone (*cis*- and *trans*- $\beta$ -lactone) with *cis*-9-nonadecene (olefin). The single protons from the 4 and 3 positions of the *trans*-lactone ring have resonances at 4.21 ppm and 3.16 ppm respectively. The single protons from the 4 and 3 positions of the *cis*-lactone ring have resonances at 4.53 ppm and 3.60 ppm respectively. The two vinyl protons of the *cis*-olefin appear at 5.35 ppm.



**Figure S3** <sup>1</sup>H-NMR of *X. campestris* OleB reacted with >90% pure *trans*-β-lactone. The protons from the 4 and 3 positions of the *trans*-lactone ring have resonances at 4.21 ppm and 3.16 ppm respectively. Small resonances for *cis*-β-lactone contaminant can be seen at 4.53 ppm and 3.65 ppm. Resonances from 1-bromonaphthalene at 8.25 ppm and 8.23 ppm were used as integration references.

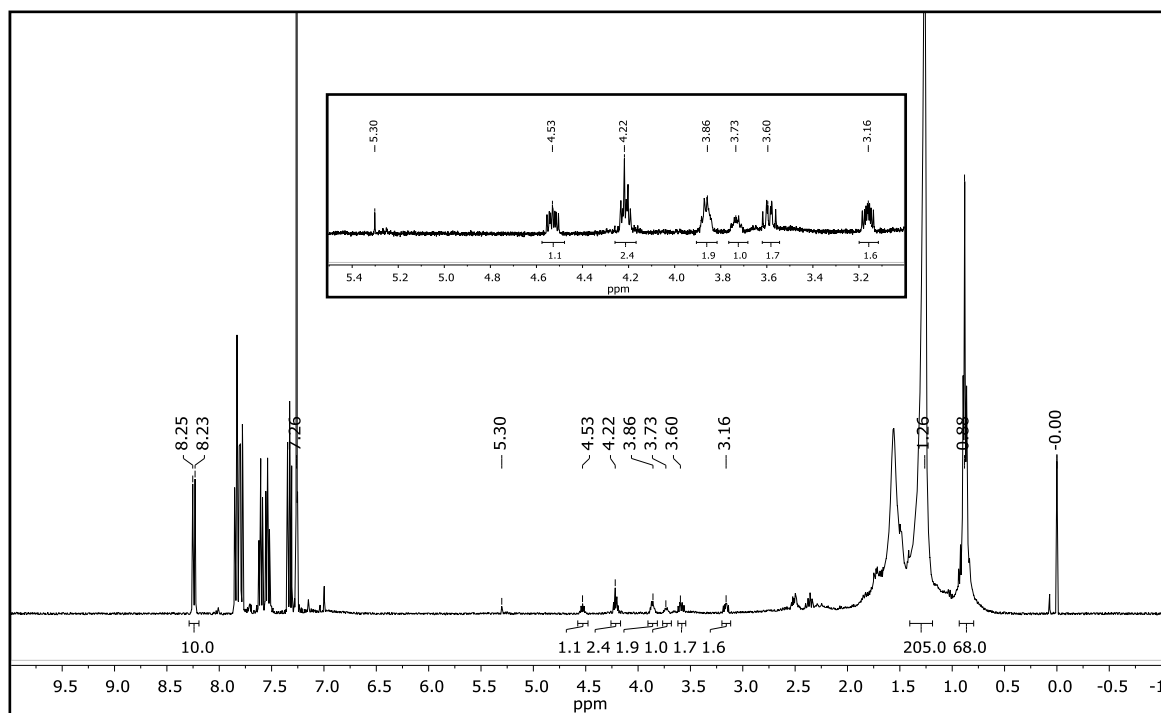


**Figure S4**  $^1\text{H}$ -NMR of *X. campestris* OleB reacted with >90% pure *cis*- $\beta$ -lactone. The protons from the 4 and 3 positions of the *cis*-lactone ring have resonances at 4.53 ppm and 3.59 ppm respectively and the two vinyl protons of the *cis*-olefin appear at 5.35 ppm. Resonances from 1-bromonaphthalene at 8.25 ppm and 8.23 ppm were used as integration references.

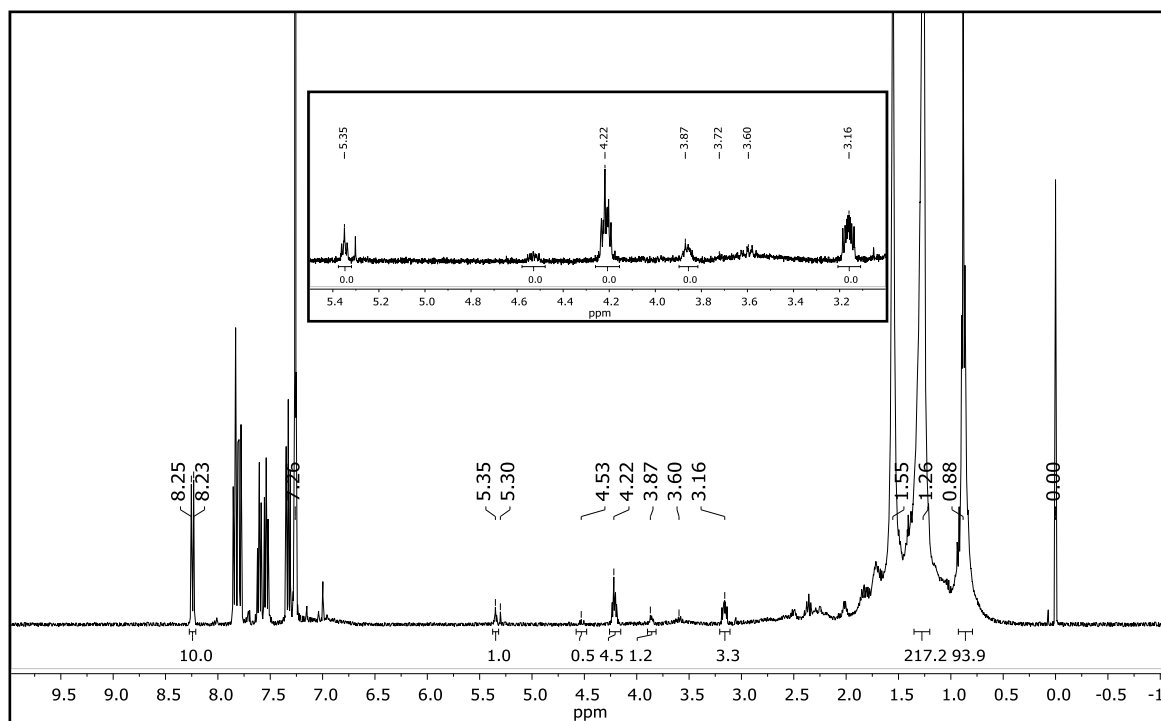


**Figure S5.** *OleB* proteins are encoded in *oleABCD* gene clusters; however, many were annotated haloalkane dehalogenase (HLD) subfamily III in work by Chovancova et al.<sup>1</sup> The *OleB* domain of *OleBC* fusion proteins like *Micrococcus luteus* was not included in Chovancova's alignments, but clusters within the HLD III subgroup.

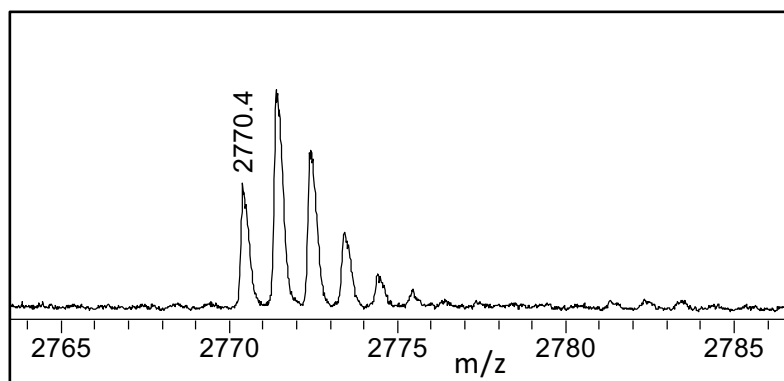
1) Chovancova E, Kosinski J, Bujnicki JM, Damborsky J. Phylogenetic analysis of haloalkane dehalogenases. *Proteins Struct Funct Genet.* 2007;67(2):305-316. doi:10.1002/prot.21313.



**Figure S6**  $^1\text{H}$ -NMR of *M. luteus* OleBC<sub>D163A</sub> fusion (inactive OleB domain) reacted with a racemic diastereomeric mixture of  $\beta$ -hydroxy acids (3-hydroxy-2-octyldodecanoic acid). The protons from the 4 positions of the *cis*- and *trans*- $\beta$ -lactone ring have resonances at 4.53 ppm and 4.21 ppm respectively and no olefin is observed. Resonances for the *syn*- and *anti*- $\beta$ -hydroxy acid starting material are 3.86 ppm and 3.73 ppm respectively. Resonances from 1-bromonaphthalene at 8.25 ppm and 8.23 ppm were used as integration references.



**Figure S7**  $^1\text{H}$ -NMR of *M. luteus* OleBC fusion reacted with a racemic diastereomeric mixture of  $\beta$ -hydroxy acids (3-hydroxy-2-octyldodecanoic acid). The protons from the 4 positions of the *cis*- and *trans*- $\beta$ -lactone ring have resonances at 4.53 ppm and 4.21 ppm respectively and the two vinyl protons of the *cis*-olefin appear at 5.35 ppm. Resonances for the *syn*- and *anti*- $\beta$ -hydroxy acid starting material are 3.87 ppm and 3.72 ppm respectively. Resonances from 1-bromonaphthalene at 8.25 ppm and 8.23 ppm were used as integration references.



**Figure S8.** Peptide fragment of *M. luteus* OleBC containing Asp<sub>163</sub>. The OleBC fusion was incubated with *cis*- $\beta$ -lactone in unlabeled H<sub>2</sub><sup>16</sup>O water, trypsin digested, and run on MALDI-TOF to serve as a control for no <sup>18</sup>O incorporation.