Identification of Biomarker Genes to Predict Biodegradation of 1,4-Dioxane

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SUPPORTING INFORMATION

The genome of *Pseudonocardia dioxanivorans* CB1190 was used to identify biomarkers targeting genes encoding monooxygenase enzymes. *In silico* analysis of the CB1190 genome revealed 2,267 monooxygenase-associated genes (Figure S1). A group of bacterial multicomponent monooxygenases consisting of the dioxane/tetrahydrofuran monooxygenase (DXMO), Phenol-2 monoxygenase (PHE), propane monooxygenase (PrMO) were identified within this set and evaluated to serve as candidate biomarkers for biodegradation of 1,4-dioxane. In addition to these gene targets, the aldehyde dehydrogenase (ALDH) associated with the dioxane/tetrahydrofuran degrading gene cluster (1) and an alcohol dehydrogenase (ADH) associated with alkane oxidation pathways (2) were also selected as a potential biomarkers because these targets may play important roles in metabolic or cometabolic dioxane biodegradation.

Primer Design:

Primers were designed using Primer3 Plus (3). Specificity of each target was examined using blastp and blastn against sequences deposited in NCBI Genbank protein and nucleotide databases, respectively. Phylogenetic trees were constructed to determine the specificity of primer sets to both gene function and to known dioxane-degrading bacteria. Once targeted regions were

screened for specificity, primer amplicons were evaluated for secondary structure using mfold (4). Primers passing all criteria were used to examine gene abundance and expression in pure cultures of CB1190 and activated sludge samples.

Growth on an Alternative Monooxygenase-Inducing Substrate

To determine the influence of toluene on the expression of our biomarker candidate genes, CB1190 was grown in 50 mL AMS medium supplemented with 100 mg/L toluene in triplicate sterile 250 mL boston bottles equipped with Mininert valves® at 30°C with 150 rpm agitation. 0.3 mL of sample were collected over time and toluene was quantified using a gas chromatograph equipped with a flame ionization detector (GC-FID). 100 µL of headspace was collected from the bottles and injected into a Hewlett-Packard 6890 GC-FID (Hewlett-Packard, Atlanta, GA) with a Restek® Stabilwax-DB capillary column (30 m x 0.53 mm ID x 1 µm; Restek, Bellefonte, PA). The injector and detector were maintained at 220°C and 250°C, respectively. The oven was programmed to ramp from 100°C to 150°C at 25°C/min and held for 1.5 min at 150°C. The retention time of toluene was 2.5min. Samples were collected during growth on toluene and RNA was isolated and qRT-PCR was performed as described in the Materials and Methods.

REFERENCES

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4. **Zuker M.** 2003. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic acids research **31:**3406-3415.

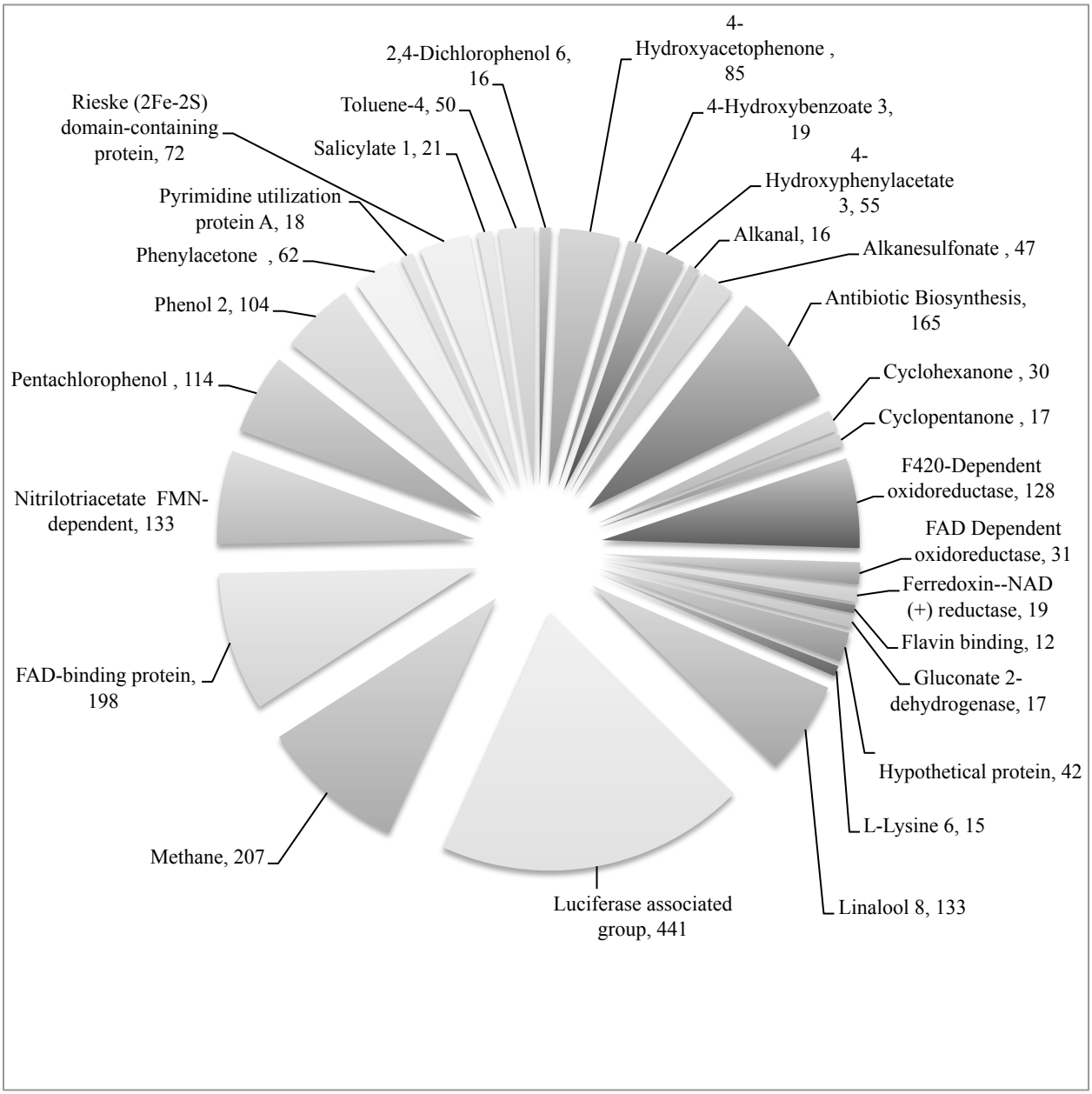

Figure S1. Monooxygenase associated functional groups identified within the genome of *Pseudonocardia dioxanivorans* CB1190.

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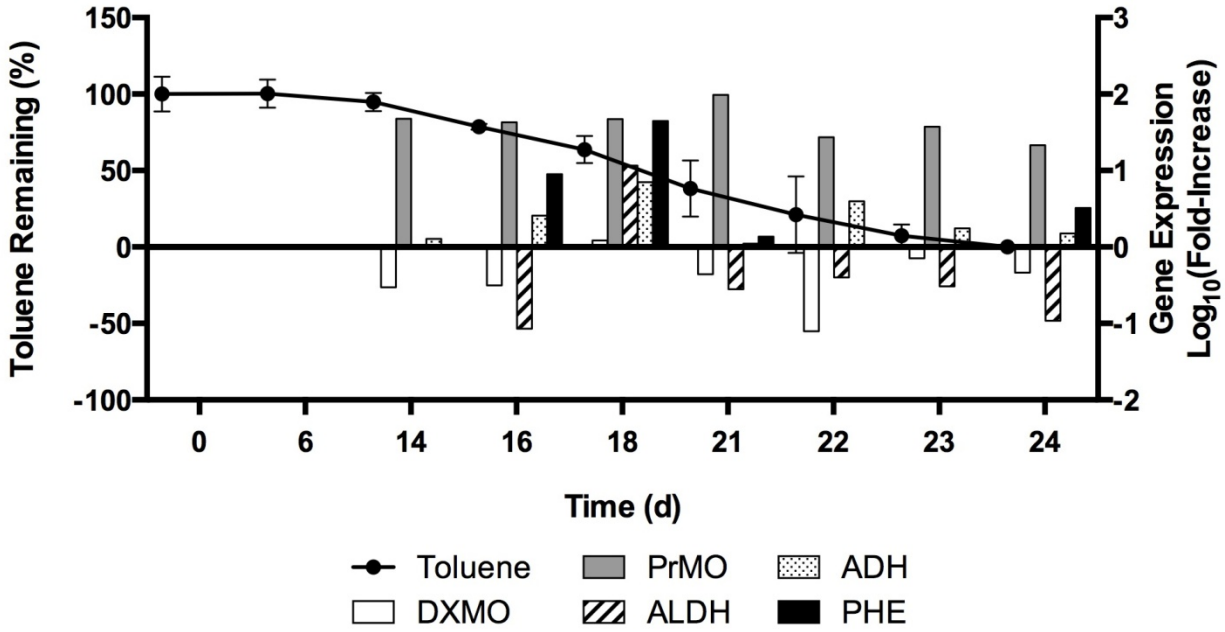

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63 **Figure S2.** Time-dependent gene expression profile for candidate biomarkers in pure cultures of
64 *Pseudonocardia dioxanivorans* CB1190 grown on toluene as the sole carbon and energy source.
65 DXMO was the only target without significant expression during growth on toluene.