Short-Chain Alkane Monooxygenase (SCAM) and Cometabolic 1,4-Dioxane Biodegradation by Gaseous Alkane-Oxidizing Bacteria

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Background/Objectives.
Soluble diiron monooxygenases (SDIMOs) are a broad group of structurally-related enzymes that are responsible for initiating the bacterial metabolism of diverse substrates, including most gaseous hydrocarbons (methane, propane, *n*-butane, ethene, propylene, isobutylene, and isoprene). Our recent research has focused on characterizing the cometabolic degradation of 1,4-dioxane (14D) by gaseous alkane-oxidizing bacteria. We have demonstrated that isobutane (2-methylpropane) is consistent in its ability to support the growth of bacteria that can degrade low concentrations of 14D (≤1 μg L^{-1}). Here we report the results of proteomic analyses used to identify the various monooxygenases that are expressed by four of these bacterial strains when grown on isobutane and other alkane substrates that support their 14D-degrading activity.

Approach/Activities.
Cells of several gaseous alkane-oxidizing bacteria were grown on ranges of alkanes (C_2-C_{14}) or non-inducing substrates such as dextrose. Their 14D-degrading activities were subsequently determined under standardized reactions conditions. The relative abundance of all monooxygenases produced by these different organisms was then determined by shotgun proteomic analyses of whole cell extracts.

Results/Lessons Learned.
Shotgun proteomic analyses of four strains (*Mycobacterium vaccae* JOB5, *Mycobacterium* sp. 1A, *Rhodococcus aetherivorans* BCP1 and *R. rhodochrous* ATCC 21198) revealed that these 14D-degrading strains all express an unusual and highly conserved SDIMO when grown on gaseous alkanes. The alpha hydroxylase component of this enzyme, short chain alkane monooxygenase (SCAM), was detected at high levels (≤3.5% of all detected proteins) in alkane-grown cells. Only low levels of this component (≤0.1% of all detected proteins) was observed in dextrose-grown cells that do not degrade 14D. In addition to SCAM, all four strains also expressed lower levels of one of two other gaseous alkane-oxidizing monooxygenases. Both *Mycobacterium* strains expressed a particulate methane monooxygenase (pMMO)-like enzyme after growth on all on the alkanes tested. Unlike *M. vaccae* JOB5, the genome of *Mycobacterium* 1A also encodes a propane monooxygenase (PrMO) but components of this enzyme were not detected in cells of strain 1A grown on any of the tested substrates (dextrose, propane, or isobutane). In contrast, both *Rhodococcus* strains expressed a PrMO although components of this enzyme were only detected at substantial levels (≤1% of all detected proteins) in cells grown on propane. While our physiological and molecular studies indicate SCAM is responsible for oxidizing 14D, our current research aims to determine what roles the secondary monooxygenases (pMMO and PrMO) expressed by gaseous alkane-oxidizing bacteria have in the broader contaminant-oxidizing activities exhibited by these four bacterial strains. The significance of these findings to our understanding of 14D biodegradation and methods to stimulate and detect 14D-degrading bacteria in the field will be discussed.