Covalent Incorporation of Fluorine into Cellular Lipids in *Pseudomonas* sp. Strain 273

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Background/Objectives. Synthetic organofluorine chemicals, especially per- and polyfluoroalkyl substances (PFAS), have essential roles in private household, industrial, military, and fire-fighting applications, and have emerged as widespread, persistent environmental pollutants. Specialized microbes possess enzyme systems that can break C-F bonds; however, the fate of fluorinated alkanes and PFAS is uncertain. We recently demonstrated that *Pseudomonas* sp. strain 273 can grow with α and α,ω-fluorinated C_7-C_{10} alkanes with 90 to 95% of fluorine in fluoroalkanes released as inorganic fluoride; however, the fluorine mass balance could not be closed. In search for the missing fluorine, this work applied metabolomic approaches to identify fluorinated metabolites in *Pseudomonas* sp. strain 273 cells grown with fluoroalkanes.

Approach/Activities. The soil isolate *Pseudomonas* sp. strain 273 was grown in completely synthetic, defined basal salts medium with different non-halogenated and terminally fluorinated alkanes provided as a sole source of carbon and energy. To assess breakage of C-F bonds, fluoride concentrations in cultures were monitored over time with a Dionex ion chromatography system equipped with an IonPac AS18 hydroxide-selective anion-exchange column and a conductivity detector. The consumption of the growth substrate (i.e., alkanes) was measured by gas chromatography in sacrificial cultures extracted with hexane. To shed light on the (fluoro)metabolites in strain 273, ultra-performance liquid chromatography (UPLC) – tandem mass spectrometry (MS/MS) and gas chromatography (GC) – mass spectrometry (MS) was applied to strain 273 cells grown under different conditions. Growth of strain 273 was followed by 16S rRNA gene-targeted qPCR.

Results/Lessons Learned. *Pseudomonas* sp. strain 273 grows with decane, 1-fluorodecane (FD) and 1,10-difluorodecane (DFD) as the sole carbon and energy sources under oxic conditions. During growth with FD and DFD, both compounds were completely consumed, and 92.2% ± 2.2% and 94.5% ± 2.8% of fluorine, respectively, was accounted for as inorganic fluoride. Fatty acid analysis in strain 273 cells grown with fluoroalkanes identified fluorinated catabolites such as shorter-chain fatty acids. Intriguingly, fluorinated C_{16} and C_{18} longer-chain fatty acids were also detected. Untargeted lipidomic analysis revealed that the majority of phospholipids in strain 273 cells grown with DFD carried a fluorine substitution. Detailed MS/MS investigations conclusively demonstrated that fluorine was covalently incorporated into the fatty acid tail of different phospholipid species. This work demonstrates that covalent incorporation of fluorine into the bacterial membrane is a novel, heretofore unknown sink for organofluorine with consequences for our understanding of the fate and transport of organofluorine chemicals, including PFAS, in the environment.