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Degradation and defluorination of 6:2 fluorotelomer sulfonamidoalkyl betaine and 6:2 fluorotelomer sulfonate by *Gordonia* sp. strain NB4-1Y under sulfur-limiting conditions

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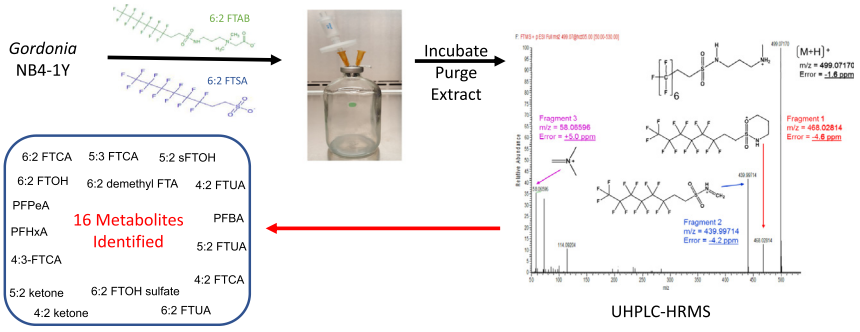
HIGHLIGHTS

- Biotransformation of 6:2 FTAB & 6:2 FTSA was studied under sulfur-limiting conditions.
- *Gordonia* sp. NB4-1Y rapidly metabolized 6:2 FTAB (70.4%) & 6:2 FTSA (99.9%).
- 16 metabolites were identified using high resolution Orbitrap mass spectrometry.
- Two distinct degradation pathways were utilized by *Gordonia* sp. NB4-1Y.
- Major breakdown products included 6:2 FTCA, 6:2 FTUA, and 5:2 fluorotelomer ketone.

GRAPHICAL ABSTRACT



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ABSTRACT

6:2 fluorotelomer sulfonamidoalkyl betaine (6:2 FTAB) is a major component of aqueous film-forming foams (AFFFs) used for firefighting and is frequently detected, along with one of its suspected transformation products, 6:2 fluorotelomer sulfonate (6:2 FTSA), in terrestrial and aquatic ecosystems impacted by AFFF usage. Biochemical processes underlying bacterial biodegradation of these compounds remain poorly understood due to a lack of pure culture studies. Here, we characterized the water-soluble and volatile breakdown products of 6:2 FTSA and 6:2 FTAB produced using *Gordonia* sp. strain NB4-1Y cultures over seven days under sulfur-limited conditions. After 168 h, 99.9% of 60 μM 6:2 FTSA was degraded into ten major breakdown products, with a mol% recovery of 88.2, while 70.4% of 60 μM 6:2 FTAB was degraded into ten major breakdown products, with a mol% recovery of 84.7. NB4-1Y uses two pathways for 6:2 FTSA metabolism, with 55 mol% of breakdown products assigned to a major pathway and <1.0 mol% assigned to a minor pathway. This work indicates that rapid transformation of 6:2 FTSA and 6:2 FTAB can be achieved under controlled conditions and improves the bacterial metabolism of these compounds.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a group of aliphatic fluorinated chemicals in which all (per-) or nearly all (poly-)

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carbon atoms are fluorinated, exclusive of the terminal functional group (Buck et al., 2011). Incorporating fluorine into organic compounds can increase chemical and thermal stability, and alter chemical properties; consequently, PFASs usually exhibit unique characteristics that impart oil and water repellency, and reductions in surface tension (Murphy, 2010; Smart, 1994). These properties make them useful in a wide range of industrial and commercial products (Wang et al., 2014a, 2014b). However, the enduring properties of PFASs also make them persistent organic pollutants that accumulate in wildlife (Sedlak et al., 2017) and humans (Olsen et al., 2007), and there is growing concern for the impact these compounds may pose to human health (Grandjean and Clapp, 2015).

In recent years, aqueous film-forming foam (AFFF) products have been identified as major sources of PFAS compounds in the environment (Baduel et al., 2015; Houtz et al., 2016; Milley et al., 2018). AFFFs are used to extinguish Class B hydrocarbon-fuel fires, and see frequent use at military bases, aviation facilities, and in the petroleum industry (Moody and Field, 2000). Though AFFF formulations are proprietary, recent studies using advanced mass spectrometry techniques have revealed the identity of dozens of new classes of fluorosurfactant compounds (Baduel et al., 2017; Barzen-Hanson et al., 2017; D'Agostino and Mabury, 2014; Moe et al., 2012; Place and Field, 2012). 6:2 fluorotelomer sulfonamidoalkyl betaine (6:2 FTAB) is a component of several AFFF formulations, while 6:2 FTSA is a possible degradation intermediate of 6:2 FTAB and other precursors present in AFFFs (D'Agostino and Mabury, 2014; Moe et al., 2012). Both compounds are among the most frequently detected PFASs at AFFF-impacted sites (Baduel et al., 2017; Boiteux et al., 2016; Mejia-Avendaño et al., 2017), contaminating nearby terrestrial and aquatic ecosystems (Boiteux et al., 2017; D'Agostino and Mabury, 2017a; Dauchy et al., 2017; Munoz et al., 2017). These compounds and their metabolites can persist in soils (Liu and Liu, 2016) and sediments (Ahrens et al., 2015), and be mobilized through surface and subsurface hydrologic processes (Weber et al., 2017). Human or ecological risks associated with 6:2 FTSA and 6:2 FTAB have been rarely examined. The only study that assesses the health risks associated with 6:2 FTSA found that 6:2 FTSA exhibits moderate hepatotoxicity relative to that reported for legacy perfluorooctane sulfonate (PFOS) and carboxylate (PFOA) (Sheng et al., 2017).

To date, understanding of the microbial metabolism of these compounds has relied predominantly on analyses of their transformation by largely uncharacterized mixed microbial communities. Recently, microbial degradation of 6:2 FTAB by wastewater treatment plant sludge inocula was monitored under aerobic conditions (D'Agostino and Mabury, 2017b). The results indicated an incomplete degradation of 6:2 FTAB after 109 days of incubation, resulting in the generation of 6:2 fluorotelomer alcohol (6:2 FTOH), 5:3 fluorotelomer acid (5:3 FTCA), perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA) and perfluorohexanoic acid (PFHxA) as the major biodegradation products, along with the potential abiotic generation of 6:2 fluorotelomer sulfonamidoalkyl amine (6:2 FTSAm) in both microbially-active experiments and sterilized controls. Similar studies of the microbial degradation of 6:2 FTSA by wastewater treatment plant sludge inocula also showed an incomplete biodegradation, with 63.7% of the 6:2 FTSA being detected in cultures after 90 days, and 7% detected as transformation products, which included 5:3 FTCA, PFBA, PFPeA, PFHxA, 5:2 fluorotelomer ketone (5:2 ketone), and 5:2 fluorotelomer secondary alcohol (5:2 sFTOH), at the conclusion of the experiment (Wang et al., 2011). Conversely, the near complete transformation of 6:2 FTSA in aerobic river sediments to stable transformation products (5:3 Acid, PFPeA, PFHxA) has been demonstrated after 90-days with a half-life <5 days (Zhang et al., 2016).

While an understanding of the transformation of these compounds by mixed microbial communities provides clues to their environmental fate, analyses of the biodegradation of these compounds by pure microbial cultures are essential to understand the fundamental biochemistry underlying PFAS metabolism. There is a need for improved understanding of the microbial metabolism of these compounds, and the search for unknown metabolites will be driven by improved mass-balance analyses that account for experimental artifacts such as sorption to glassware, septa and biological materials. Elucidating metabolic pathways in pure cultures will facilitate the identification of genes and proteins required for such processes, and can form the basis for biotechnological applications aimed at biocatalysis and bioremediation (Eibes et al., 2015; Sharma et al., 2018; Sheldon and Woodley, 2018). Pure cultures additionally provide opportunities for improved control and manipulation of conditions in order to understand the metabolic regulation mechanisms involved and to determine optimal transformation conditions. A few pure culture analyses of PFAS metabolism have been described, largely focusing on the degradation of fluorotelomer alcohols (FTOHs) by bacteria (*Pseudomonas* spp. and *Mycobacterium vaccae*) (Kim et al., 2014, 2012) and fungi (*Fusarium* sp., *Penicillium* sp., and *Aspergillus* sp.) (Merino et al., 2018), as well as the derivatives of FTOHs such as 6:2 polyfluoroalkyl phosphates (Lewis et al., 2016). The labile hydroxyl group on FTOHs allows their fast transformation in various aerobic microbial systems with or without carbon co-substrates. In contrast, sulfur-limiting condition seems to be necessary for the breakdown of 6:2 FTSA, as demonstrated with a *Pseudomonas* sp. (Key et al., 1997), as well as with the vermicompost bacterial isolate *Gordonia* sp. strain NB4-1Y (Van Hamme et al., 2013).

No pure culture has been demonstrated for its abilities to break down 6:2 FTAB, although the slow biotransformation of the PFAS previously observed in activated sludge suggests that desulfonation might also be the rate-limiting step. Therefore, the purpose of this study was to improve our understanding of the broad organosulfur compound catabolic abilities of *Gordonia* strain NB4-1Y in metabolizing 6:2 FTAB, in comparison to 6:2 FTSA, when the compounds were provided as sole-added sulfur sources. By identifying and quantifying the volatile and water-soluble metabolites using high-resolution mass spectrometry, we are able to describe a major and a minor degradation pathway used by NB4-1Y. As the most comprehensive pure culture analysis of the degradation of 6:2 FTSA, and the first such description of the degradation of 6:2 FTAB completed to date, this work provides fundamental insight into the bacterial metabolism of these highly fluorinated compounds.

2. Materials and methods

2.1. Chemicals and materials

The ammonium salt of 6:2 FTSA was obtained from Synquest Laboratories (certified 98.0% pure by titration with NaOH). A technical grade solution of 6:2 FTAB (0.27 g mL^{-1}) was obtained from Shanghai Kingpont Industrial Co. Ltd. (Shanghai, China). Calibration standards for PFBA, PFPeA, PFHxA, PFHpA, 3:3 fluorotelomer acid (3:3 FTCA), 4:3 fluorotelomer acid (4:3 FTCA), 5:3 FTCA, 6:2 fluorotelomer acid (6:2 FTCA), 6:2 FTOH, 5:2 ketone, 5:2 sFTOH, 6:2 FTSA, and 6:2 FTAB were obtained from Wellington Laboratories (Guelph, ON, Canada), DuPont USA (Wilmington, DE, U.S.A.), or Synquest Laboratories (Alachua, FL, U.S.A.). Isotope-labelled internal standards (IS) obtained from Wellington Laboratories (Guelph, ON, Canada) included $^{13}C_4$-PFBA, $^{13}C_3$-PFPeA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_2$-6:2 FTCA, $^{13}C_2$-6:2 FTSA, and $^{13}C_2$-6:2FTUA (chemical purities > 98% and isotopic purities > 99%). Maxi-Clean 600 mg SPE C18 cartridges were purchased from GRACE (Deerfield IL, USA). The chemical names, formula and corresponding acronyms are listed in the Supplementary material (SM), along with the details of other reagents.

2.2. Gordonia strain NB4-1Y growth conditions

Isolation and characterization of *Gordonia* strain NB4-1Y have been described previously (Van Hamme et al., 2013). For this study, pure culture