Biochemical characterization of two nitrilotiracetate monooxygenases from *Gordonia* NB4-1Y thought to mediate the transformation of 6:2 fluorotelomer sulfonate to 6:2 fluorotelomer aldehyde

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The way we understand bacterial transformation of sulfonated hydrocarbons traditionally focuses on three groups of enzymes: dibenzothiophene monooxygenases (DszA/B), taurine dioxygenases (TauD) and alkanesulfonate monooxygenases (SsuD). Bacteria may use these enzymes to break carbon-sulfur bonds, releasing sulfite and an aldehyde. In recent years, fluorinated surfactants, such as 6:2 fluorotelomer sulfonamide alkyl betaine (6:2 FTAB) in aqueous film-forming foams (AFFF) for firefighting, have emerged as contaminants of concern due to their resistant chemical properties. A 6:2 FTSA breakdown product, 6:2 fluorotelomer sulfonate (6:2 FTSA), is a partially fluorinated version of octane sulfonate often observed in the environment following AFFF deployment. The bacterium *Gordonia* NB4-1Y was found to be able to partially degrade 6:2 FTSA to short chain aldehydes and carboxylic acids, presumably via the production of the yet to be detected 6:2 fluorotelomer aldehyde (6:2 FTUA). Proteomic analysis of NB4-1Y revealed that two flavin-dependent nitrilotriacetate monooxygenases (NtaA) were produced under sulfur-limiting conditions with 6:2 FTSA as the sole added source of sulfur. In order to know if NB4-1Y is able to use the carbon-nitrogen bond cleavage activity of NtaAs to remove sulfur from 6:2 FTSA, two NB4-1Y NtaAs (ISGA 1218 and 1222) were cloned into pMAL-c2 and pET23d vectors for heterologous expression in *Escherichia coli* BL21. Maltose binding protein-tagged ISGA 1218 and 1222 have been expressed and purified as shown by proteins of 96 and 91 kDa on SDS-PAGE gels. Expression of His-tagged ISGA 1218 and 1222 is currently underway. Purified SsuD from *E. coli* BL21 and NB4-1Y are being used to develop kinetic assays against octanesulfonate as a positive control. Simultaneously, 6:2 FTSA transformation by NB4-1Y NtaAs is being tested in the presence of *E. coli* flavin reductase and anoxic photoreduction of flavin. The generation of ISGA 1218 and 1222 protein crystals will shed light on how NtaAs may accommodate 6:2 FTSA into active sites.