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 pMALc2 and pET23d vectors for heterologous expression in Escherichia coli BL21. Maltose binding proteintagged 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.