Report for grant SG183: The first fluorination enzyme from the sea

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Background

The introduction of fluorine into organic molecules can substantially modulate their physicochemical properties, such as lipophilicity and basicity, as well as slow down their metabolic degradation. This effect has been exploited by the pharmaceutical industry because fluorinated compounds frequently show enhanced binding affinity to target proteins. Well over 20% of current drugs in clinical trials contain fluorine, thus demonstrating the importance of this modification.[1]

Despite the major commercial success of selectively fluorinated compounds, fluorinated natural products from natural sources are extremely rare.[2] Dr Hai Deng is part of the team that discovered the first fluorination enzyme from the soil bacterium *Streptomyces cattleya*. This enzyme has been fully characterised, crystallised and is reasonably well understood at the mechanistic level. [3]

There are clear prospects of transferring the fluorinase gene and related gene clusters into other organisms. Success would enable a fermentation technology approach to access novel organofluorine products rather than using challenging chemical methods. Additionally biotechnology has the potential to offer structurally complex and high value products such as fluorinated antibiotics and anticancer agents. [4]

However, the biological scope is limited to this single pathway in *S. cattleya* so far. Therefore, the discovery of new fluorometabolites and fluorometablism would be the key for the success of using synthetic biology for the fermentation technology.[4]

Marine-derived Streptomyces xinghaiensis

Nature has hardly evolved a biochemistry of fluorine.[1] One of the main reasons is that the extremely low concentration of fluoride occurs in water. Many fluoride minerals are hardly dissolved in water. Fluoride concentration in marine environment is, however, 6-100 times higher than that in the fresh water, suggesting that marine organisms may evolve to harness the higher bioavailability of fluoride and develop novel biochemistry of fluorine than the terrestrial counterparts.

Streptomyces xinghaiensis NRRLB24674 is a novel marine-derived bacterium, first isolated in 2009 from a marine sediment sample around Xinghai Bay, Dalian, China.[5] Due to the unique phenotype and genotype, it was subjected to the genome sequencing in 2011.[6] Its draft genome contains approximately 7.6 Mbp with a GC content of 72.5%. The sequence was annotated in RAST server. A recent study indicated that this strain has capacity to produce a novel alkaloid compound xinghaiamine A.[7]

Data collected under grant

Genome mining analysis allowed identification of a putative fluorinase gene in the contig with the NCBI access no. (AFRP01002228.1) and the encoded protein sequence shared high sequence identity (84%) with known fluorinases, including a 21 amino acids loop, a unique signature of the fluorination enzymes identified so far, suggesting that S. xinghaiensis may be the first fluorometabolite producer from the marine origin. To investigate further, S. xinghaiensis was grown in shake flask culture supplemented with fluoride (2 mM) in fresh water. It did not behave like other Streptomyces and failed to produce healthy cell mass. No organofluorine signal was observed in ^{19}F NMR in these samples. However when the medium was supplemented with artificial sea salt (30 g L^{-1}) a healthy growth was established suggesting a sea salt dependency for this marine bacterium. The

supernatant of a 10-day culture was analysed by 19F{1H}-NMR. The organism produced fluoroacetate. Gene inactivation experiments indicated that the identified gene cluster indeed direct the biosynthesis of fluoroacetate. The results were published in the international journal: *Organic and Biomolecular Chemistry*, 2014, 12, 4828-4831.
Later, Dr Deng collaborated with Dr Long Ma in Tianjin University of Science and Technology, China and characterized the identified fluorination enzyme from S. xinghainesis. The results were published in the international journal, RSC Advances, 2016, 6, 27047-27051.

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