

HIGHLIGHT

Toward a molecular understanding of fluoride stress in a model *Pseudomonas* strain

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Chemical and biological understanding of fluorine in its many forms has lagged behind that of the other halogens, but studies on fluorine have recently come to the forefront. Thousands of commercial fluorine-containing materials are now found throughout the fabric of society, prompting new concerns regarding their health and environmental impacts. The utility and fate of many fluorine-containing products are inextricably linked to microbial responses to the element in its various forms. In this issue, the paper by Calero et al. (2022) provides new insights into the response of the model soil bacterium *Pseudomonas putida* KT2440 to fluoride anion. The study takes a broad approach to paint a picture of how fluoride impacts the bacterium and the microorganism's physiological reaction. Methods employed include fluoride toxicity assays, *Tn*-Seq, genetic knockouts, fluorescent sensing of fluoride intracellularly and metabolomics. Clearly, microbes like *P. putida* KT2440 have evolved multiple mechanisms to protect themselves from toxic fluoride anion.

Fluorine is the 13th most abundant element in the Earth's crust but the unavailability of mineral forms and its cellular toxicity has limited the element in bacteria. A review article compiling information on the 33 most abundant elements in prokaryotes did not detect fluorine, showing it to be less prevalent than cadmium, tin and barium in the organisms examined (Novoselov et al., 2013). However, a select few bacteria and plants have learned to sequester fluoride for the purpose of biosynthesizing monofluorinated anti-metabolites as toxins that ward off predators (Chan & O'Hagan, 2012; Walker & Chang, 2014). There is currently interest in producing new fluorinated compounds using the enzyme fluorinase (Calero et al., 2020; O'Hagan &

Deng, 2015; Pardo et al., 2022) and this is one of the potential outgrowths of the present work by Calero et al.

The scarcity of naturally biosynthesized fluorinated compounds by microbes and plants likely emanates at least partly from cellular toxicity of the mineral acid hydrogen fluoride, HF, and its dissociated anion, fluoride. Humans tragically experienced the toxicity of fluorine gas (F₂) and HF in 19th-century laboratories (Weeks, 1932). Henri Moissan was honoured with the Nobel Prize in Chemistry in 1906 for his innovations in safer handling of fluorine but unfortunately died several months after receipt of the award. Subsequent to Moissan, there was a great expansion in industrial uses of fluorine, leading to Freon refrigerants, Teflon-type polymers, specialized surfactants, and most recently, agricultural and pharmaceuticals (Dolbier Jr, 2005; Lombardo, 1981). Unlike F₂ and HF, many commercial organofluorine compounds are largely unreactive with microbial enzymes, leading to an undesirable environmental persistence (Wackett, 2021).

Most industrial fluorine today derives from the mineral fluorite (CaF₂). CaF₂ is converted to HF and salts of the conjugate base, fluoride anion. Fluoride is used in the types of organofluorine synthesis previously mentioned, as well as aluminium extraction, steel hardening and water fluoridation (Pelham, 1985). Related to the latter, fluoride is added to toothpastes to harden teeth and inhibit caries-causing oral bacteria such as *Streptococcus mutans* (Marquis, 1995). The application of inhibiting microbes points to the major differences in microbiological response to fluoride compared to chloride anion. Chloride anion is abundant in many bacterial cells at concentrations greater than 50 mM and some halophiles prefer molar levels of the anion

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(Chen, 2005). By contrast, fluoride anion shows toxicity at intracellular levels of 0.1 mM (Ji et al., 2014; McIlwain et al., 2021). At that level and above, fluoride coordinates to metal centres in key enzymes, such as ATPases, pyrophosphatase and enolase. Fluoride stress is increased by low pH as HF is the species that enters cells, which then dissociates to fluoride anion at the near-neutral pH values of the cytoplasm in most bacteria (Ji et al., 2014).

To respond, cells must initially sense potentially toxic levels of fluoride intracellularly and that can be mediated, in some cases, by a fluoride-responsive riboswitch which controls gene expression (Baker et al., 2012; Breaker, 2012). Among the most important genes are those encoding membrane proteins that transport fluoride out of the cytoplasm (Ji et al., 2014; Last et al., 2018; Stockbridge et al., 2012). One such protein is a fluoride-proton antiporter and another is a fluoride exporter in which fluoride expulsion is driven by an electrochemical gradient. Indeed, a major gene upregulated in *Pseudomonas putida* KT2440 during fluoride exposure, as shown in the paper by Calero et al. (2022), is *crcB* that encodes the gradient-driven fluoride exporter. The X-ray structure of the CrcB protein from *Bordetella pertussis* has been solved to a maximum resolution of 2.1 Å (Stockbridge et al., 2015) and CrcB transporters have been shown to export fluoride anion at an astounding rate of 10^5 per second (McIlwain et al., 2021).

Basic and directed fluoride response work has previously been conducted with *E. coli* and *Streptococcus* spp., respectively, the latter due to the cavity-prevention angle (Ji et al., 2014; Liao et al., 2017). The current study by Calero et al. (2022) is important in the context of it focusing on a model soil bacterium, *P. putida* KT2440 (Belda et al., 2016), and using multiple molecular genetic approaches to obtain new insights into responses to fluoride. The article describes the use of a Tn-seq library and making scar-less deletion mutants of fluoride-responsive genes thus identified. The authors constructed an intracellular fluoride biosensor to determine internal fluoride levels under different environmental conditions with multiple mutants. The study also explores pH and metabolic perturbations occurring with fluoride insult. Previous work had shown fluoride sensitivity increases with lower pH (Ji et al., 2014) suggesting that metabolic rerouting, as described in Calero et al. (2022), might raise pH in the cell's local environment and thus mitigate against HF import.

Pseudomonas putida is naturally quite resistant to fluoride and a deeper understanding of its metabolism and genetics is emerging. In this context, *P. putida* is a good platform for producing novel fluorinated compounds via the fluorinase pathway, which will require high levels of fluoride in the medium, which might be damaging to host organisms lacking stout defence mechanisms (Calero et al., 2020). Moreover,

Pseudomonas spp. are recognized as important for biodegradation and may help remediate polyfluorinated pollutants, which will release fluoride from C-F bond cleavage reactions (Wackett, 2022). There are some indicators from environmental studies that *Pseudomonas* spp. may be prevalent community members in fluoride-laden waters (Zhang et al., 2019).

As all good studies do, the paper by Calero et al. (2022) opens as many new questions as it answers. The identification of genes that respond to fluoride begs new questions regarding their physiological function. The response and importance of the CrcB protein were underscored in the present study. But some upregulated genes are denoted as hypotheticals and even for 'known' proteins, the annotated function appears disconnected from fluoride resistance. Indeed, the CrcB protein, which clearly functions as a fluoride exporter, was once designated as a 'camphor resistance protein' in *E. coli* (Hu et al., 1996). This illustrates a fundamental issue in genome annotation, many gene function designations are misleading, some are verifiably wrong (Schnoes et al., 2009). But the paper by Calero et al. (2022) provides the fodder for new discovery, and more precision in annotation related to bacterial response to fluorine, exactly the process by which the science will advance.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest related to this work.

DATA AVAILABILITY STATEMENT

This is a commissioned Highlight article, to comment on other people's data.

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