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Exploration of urease-mediated biomineralization for defluoridation by *Proteus columbae* MLN9 with an emphasis on its genomic characterization

Moitri Let [a], Krishnendu Majhi [a], Urmi Halder [a], Ayan De [b], Dipnarayan Saha [c], Annapurna Chitikineni [d e], Tarit Roychowdhury [b], Rajeev K. Varshney [d e], Rajib Bandopadhyay [a]

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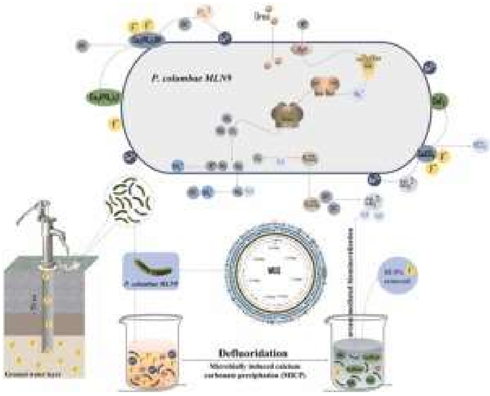
Abstract

Fluoride (F^-) contamination of groundwater is a silent killer of human health that has now become a global problem. In the present study, we sought to isolate fluoride-resistant, urease producing bacteria from F^- contaminated groundwater to evaluate their potential F^- biomineralization. Strain MLN9, isolated from the fluoride-contaminated village of Madhabpur in Birbhum, could resist up to 5600 mg L^{-1} F^- concentration. Taxonomic classification of strain MLN9 based on whole genome sequence identified it as *Proteus columbae* MLN9. Strain MLN9 showed 88.9% of maximum F- removal efficiency at pH 8.0, 1.0 g L^{-1} $CaCl_2$, and 10.0 g L^{-1} urea concentration. Scanning electron micrographs showed dense and porous biological crystal precipitates surrounding the surface of the bacterial cells, and the adherence of F^- to the cell surface was confirmed by the energy dispersion spectrum. Moreover, the X-ray diffraction pattern showed that the F^- precipitates on the cell surface were biological crystals of $Ca_5(PO_4)_3F$ and CaF_2. Genomic analysis of strain MLN9 revealed the presence of *crcB* gene homologs possibly encoding F^- transporters and urease regulatory proteins such as UreA, UreB, UreC, UreD, and UreG suggesting a role in defluoridation. Our findings provide new insights into the urease based MICP technology using a noble bacterium *Proteus columbae* MLN9 for fluoride removal.

Graphical Abstract



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Introduction

Groundwater is often referred to as the "hidden sea," which is the most important water resource for sustaining life. However, due to the presence of several toxic contaminants in groundwater, including fluoride (F^-), access to clean and safe drinking water is becoming a prime concern. The major factors for the increase of F^- concentration in groundwater are weathering and dissolution of F^- containing rocks and minerals, discharge of F^- bearing industrial wastes, and overexploitation of fluoride-rich phosphate fertilizers and pesticides [1]. The threshold limit for F^- in drinking water is $1.5\,mg\,L^{-1}$ [2], which is a double-edged sword as both inadequate (> $0.5\,mg\,L^{-1}$) and excessive (> $1.5\,mg\,L^{-1}$) intake of F^- can lead to serious health problems [3], [4]. Ingestion of various doses of excessive F^- causes chronic dental fluorosis (> $1.5\,mg\,L^{-1}$), skeletal fluorosis (> $0.4\,mg\,L^{-1}$), paralysis (> $10\,mg\,L^{-1}$), impaired intelligence quotient (IQ), and a decrease in growth hormone production in school-aged children [5], [6]. Consequently, more than 200 million people in 25 countries around the world are struggling with F^- related problems [7]. Southeast Asian countries such as China, Bangladesh, and India are heavily polluted with fluoride. In India, Birbhum district in West Bengal is considered a fluoride endemic area where the population consumes F^- containing groundwater daily and faces serious health problems [8].

Various conventional technologies are used to remove F^-, such as adsorption, precipitation, ion exchange, reverse osmosis, nanofiltration, and electrodialysis [9], [10]. These technologies not only require a large amount of energy, expensive instrumentation, and water desalination but also generate secondary waste [11], [12]. Recently, researchers have focused their attention on bioremediation to remove fluoride. Bioremediation of fluoride is mainly based on biosorption, bioaccumulation, and biomineralization [13], [14], [15]. They are environmentally friendly, relatively sustainable, cost-effective, and mitigate toxicity with high efficiency.

In biomineralization, microbe-derived metabolites such as sulphates, phosphates, silicates, carbonates, and oxides could reduce the mobility and toxicity of contaminants. Microbially induced carbonate precipitation (MICP) is a biomineralization process in which Ca^{2+} reacts with CO_3^{2-} metabolites to form $CaCO_3$ bioprecipitates [16], [17], [18]. It has been used in a variety of applications, including mining waste immobilization [19], heavy metal mineralization [20], [21], biocementation [22], phosphorus and nitrogen remediation [23], soil amendment [24], and waste water treatment [25]. MICP can be regulated by several mechanisms, including extracellular polysaccharide production, urea hydrolysis by urease, and induction of carbonic anhydrase [26]. In the urease-mediated MICP process, urease-active bacteria produce CO_3^{2-} and NH_4^+ via urea hydrolysis, where CO_3^{2-} reacts with Ca^{2+} and metal ions co-precipitated with CO_3^{2-} [25], [27], [28]. Urea can leach out into groundwater in the form of nitrate (NO_3^{2-}) due to agricultural activities. According to Bureau of Indian Standards (BIS), the maximum permissible limit of nitrate in groundwater is 45 mg L^{-1} [28]. It has been found that various bacterial species hydrolyse urea and co-precipitate various heavy metals, including chromium, arsenic, cadmium, lead, and copper, with high efficiency [26], [29]. It can mineralize heavy metals from their ionic state into a stable form, which can reduce their mobility and hazardous effects [18]. During the MICP process, various environmental conditions influence both urease and microbial activity, such as pH, temperature, and initial heavy metal concentration [25], [30].

So far, several bacterial species such as *Pseudomonas* sp. HXF1 [16], [31], *Acinetobacter* sp. H12 [32], [33], [34], and *Cupriavidus* sp. W12 [35] have used extracellular polymeric substances to remove F^- in MICP. But unlike other heavy metals, reports on the F^- biomineralization based on urea hydrolysis have not been explored. Therefore, the current study focuses on the urease-driven MICP of *Proteus columbae* MLN9, which represents a novel microbial resource for F^- biomineralization. In this study, *P. columbae* MLN9, a novel bacterium with F- resistance ability and a high potential for urease enzyme production, was examined in detail for a defluoridation study. Response surface methodology (RSM) was used to optimize the effect of three variables such as pH, urea, and $CaCl_2$ concentration on F^- removal. Genome analysis of strain MLN9 was performed to identify the genes encoding the F^- exporter and urease enzyme possibly responsible for defluoridation. In addition, the mechanism of F^- removal was detected by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), X-ray diffractometer (XRD), and Fourier transform infrared spectroscopy (FTIR).

Section snippets

Sampling and isolation of fluoride resistant bacteria

Fluoride-contaminated water samples were collected from the fluoride-affected village of Madhabpur (24° 22' 76 "N, 87° 70'35 "E) in Birbhum district, West Bengal, India. The fluoride concentration of the water sample was measured using a fluoride ion-selective electrode (Orion, Thermo Scientific, USA) [36], [37]. To isolate fluoride-resistant bacteria, 100μL of the F^- contaminated water samples in nutrient agar (Peptone 10, beef extract 5.0, Yeast extract 2.0, sodium chloride 5.0 g L^{-1}, and…

Isolation, screening, and phenotypic characterization of F^- resistant MICP strain

The present study area was the semi-arid extensive part of Chotanagpur platue, predominantly covered with F^- bearing minerals like fluorite (CaF_2), fluoro-apatite [$Ca_5(PO_4)_3$ F], and cryolite (Na_3AlF_6) [63]. Leaching of F^- rich villiaumite (NaF)-type minerals and semi-arid climatic conditions lead to F^- contamination of groundwater in Madhabpur village of Birbhum district [64], [65]. The fluoride concentration in the collected water sample was found to be 20.3 mg L^{-1}. Initially, twelve different …

Conclusion

The F^- resistant and urease enzymeproducing bacterial strain *Proteus columbae* MLN9 can potentially remove fluoride via MICP. Defluoridation of the bacteria was strongly supported by the homolog of the F^- exporter CrcB and the genes encoding the urease operon already present in the genome. Indeed, chemisorption and co-precipitation were primarily responsible for F^- removal in the MICP process, with calcium, fluoride, and phosphate ions coprecipitated mainly in the form of CaF_2 and $Ca_5(PO_4)_3F$.…

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CRediT authorship contribution statement

Sampling, **RB**, and **ML**; Conceptualization **RB** and Methodology, **ML**, **KM** and **UH**; Experiments, **ML**, **KM**, **UH** and **AD**; Sequencing, **RKV** and **AC**; Software and Visualization, **DS** and **UH**; Writing – review & editing **ML**, **KM**, **UH**, **AD**, **TRC**, **DS** and **RB**....

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper....

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