Natl. Acad. Sci. Lett. (November–December 2023) 46(6):511–515
https://doi.org/10.1007/s40009-023-01308-w

SHORT COMMUNICATION

# Defluoridation Efficiency of a Novel Fluoride-Resistant *Exiguobacterium indicum* MLN15

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Received: 14 November 2022 / Revised: 18 May 2023 / Accepted: 29 May 2023 / Published online: 21 June 2023


**Abstract** Fluoride contamination of groundwater poses a global threat to humans. The current study focused on the defluoridation efficacy of a fluoride-resistant bacterium, isolated from fluoride-contaminated areas of Birbhum district, West Bengal, India. Strain MLN15 was able to tolerate a maximum fluoride concentration of 3500 mgL$^{-1}$. Based on morphological, biochemical, and 16S rDNA gene sequence, strain MLN15 was identified as *Exiguobacterium indicum* MLN15. Optimal growth conditions were observed at pH 7, temperature 35˚C, and 0.5 molar NaCl. The selected strain also showed maximum defluoridation efficiency of 53.5% at optimal growth conditions of 24 h incubation. In addition, fluoride adsorption on bacterial cell surfaces was confirmed using SEM, SEM–EDX, and TEM analyses. All the results indicate that the bacterial strain *Exiguobacterium indicum* MLN15 could be a potential organism for fluoride-bioremediation.

**Keywords** Adsorption · Bioremediation · Defluoridation · Fluoride-resistant

**Significance Statement** Excessive intake of $F^-$ causes serious health problems in humans, such as dental fluorosis, skeletal fluorosis, osteosclerosis, and osteoporosis. In our study, we introduced bacterial remediation for defluoridation. This environmentally friendly, sustainable, and cost-effective technique could be used in the future to reduce the health hazards caused by $F^-$ contaminated water.

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Fluoride ($F^-$) contamination of groundwater is due to weathering and dissolution of $F^-$ bearing rocks and minerals, discharge of industrial wastes, and excessive use of fluoride-rich phosphate fertilizers and pesticides [1]. Excessive intake of $F^-$ can cause serious health problems such as dental fluorosis, skeletal fluorosis, paralysis, ventricular abnormalities, dyspnea, and gastroenteritis [2]. Bacteria have evolved various mechanisms, such as biosorption, biotransformation, bioaccumulation, biomineralization, and secretion of ionophores to tolerate the toxicity of $F^-$ [3, 4]. Therefore, the present study aims to isolate $F^-$-resistant bacteria from fluoride-containing water and to verify the defluoridation potential of the selected strain *Exiguobacterium indicum* MLN15.

The $F^-$ content in the water samples (18.6 mg L$^{-1}$ to 19.7 mg L$^{-1}$) of Asanjola village (24° 14′ 33″N, 87° 42′ 54″ E) in Birbhum district indicates the high-risk factor for villagers in this area. First, five different bacterial colonies were isolated from water samples using a nutrient medium consisting of peptone 5.0, beef extract 1.0, yeast extract 2.0, sodium chloride 5.0 g L$^{-1}$ at pH 7.0 (HiMedia®), supplemented with 10 mg L$^{-1}$ $F^-$ solution. Of the five different isolates, strain MLN15 was able to tolerate high fluoride concentrations of up to 3500 mg L$^{-1}$ and is selected for the defluoridation study. Several bacteria, such as *Aeromonas* sp. (9200 mg L$^{-1}$), *Brevibacterium* sp. (7200 mg L$^{-1}$), *Paenibacillus* sp. (5200 mg L$^{-1}$) and *Proteus columbae* MLN9 (5600 mg L$^{-1}$), have already been described as the high $F^-$-resistant strains [4, 5]. Strain MLN15 was orange-pigmented, circular, and highly susceptible to most of the antibiotics used in this experiment (ciprofloxacin, levofloxacin, penicillin, mezlocillin, streptomycin, ampicillin, ceftriaxone, amoxicillin, and erythromycin) (Fig. 1). In addition, the strain was able to reach stationary phase after 13 h of incubation, and the growth rate is reduced with $F^-$ treatment

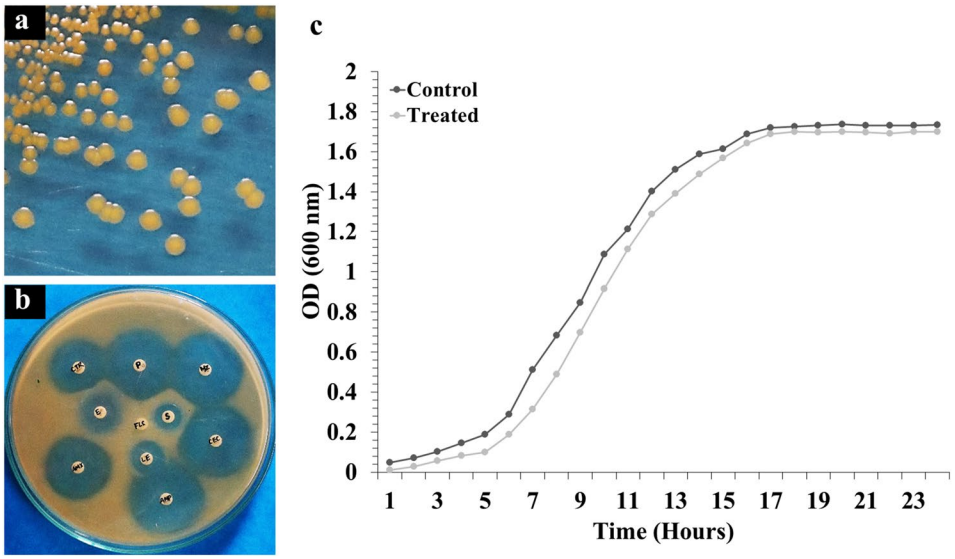


**Fig. 1** Colony morphology (**a**); Antibiotic susceptibility (**b**) and Growth curve under $F^-$-treated and untreated condition (**c**) of strain MLN15

(Fig. 1). All morpho-biochemical characteristics are listed in Table 1.

Phylogenetic analysis based on 16S rDNA gene sequence revealed that strain MLN15 had the maximum similarity (99.57%) to *Exiguobacterium indicum* HHS 31, GenBank accession number AJ846291.1.

To investigate the defluoridation efficiency, the freshly pre-cultured inoculum of the bacterial strain at a concentration of 1% with $3 \times 10^8$ CFU per mL was inoculated into nutrient broth media containing different $F^-$concentration (5, 10,15, and 20 mg $L^{-1}$) and incubated at 37 °C, 140 rpm for 8–48 h [4, 7, 12]. Then, the supernatant was collected by centrifugation at 5000×*g* for 10 min, and the residual $F^-$ content was measured by SPADNS colorimetric method [4]. The absorbance was measured at 570 nm using a UV–Vis spectrophotometer (Cyber lab-UV-100,

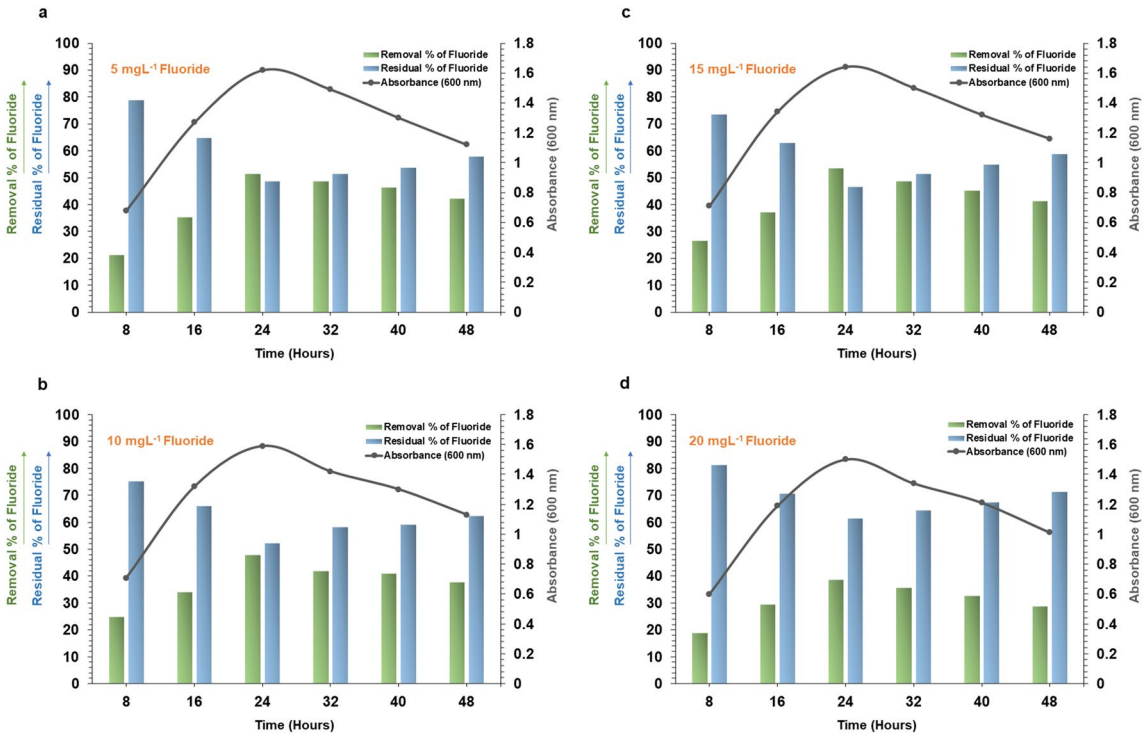


**Fig. 2** Defluoridation efficiency of *E. indicum* MLN15 at different fluoride concentrations **a** 5 mg $L^{-1}$ **b** 10 mg $L^{-1}$ **c** 15 mg $L^{-1}$ and **d** 20 mg $L^{-1}$

**Table 1** Morpho-biochemical characterization of *E. indicum* MLN15

| | |
|---|---|
| *Colony morphology* | |
| Shape | Circular |
| Appearance | Dull |
| Optical property | Translucent |
| Elevation | Convex |
| Texture | Moist |
| Margin | Entire |
| Pigmentation | Orange |
| Citrate utilization | – |
| Cellulose degradation | + |
| Voges-proskauerTest | + |
| IndoleTest | + |
| Ammonia | + |
| Nitrate | + |
| *Extracellular enzyme* | |
| Catalase | + |
| Amylase | + |
| Protease | + |
| Esterase | – |
| Urease | – |
| *Carbohydrate utilization* | |
| Glucose | + |
| Fructose | + |
| Mannitol | – |
| Galactose | + |
| Sucrose | + |
| E (15 mg) | 23 |
| S (10 mg) | 17 |
| CEC (30/10 mg) | 30 |
| MZ (75 mg) | 30 |
| P (10 mg) | 28 |
| FLC (25 mg) | R |
| AMX (10 mg) | 28 |
| AMP (10 mg) | 30 |
| CTR (30 mg) | 26 |
| LE (05 mg) | 22 |

"+" refers to a positive result for the experiment and "–" refers to a negative result

USA). The defluoridation efficiency (%) was calculated using the following equation:

$$\text{Defluoridation efficiency}(\%) = \frac{\text{Ci} - \text{Ce}}{\text{Ci}} \times 100$$

where Ci: initial and Ce: final $F^-$ concentration in solution after treatment. The experimental values for each value were considered three times with significant means ($P < 0.05$), and the data were analyzed using Origin 2018 software.

*E. indicum* MLN15 showed a maximum $F^-$ removal efficiency of 53.5% after 24 h of incubation against $F^-$ concentration of 10 mg $L^{-1}$ (Fig. 2). However, it decreased to 47.8%, and 38.7% $F^-$ concentration of 15 and 20 mg $L^{-1}$, respectively (Fig. 2).

This may indicate that low concentrations of $F^-$ ions (5 and 10 mg $L^{-1}$) can effectively interact with a maximum number of available binding sites on the bacterial cell surface [7]. However, at high concentrations (15 and 20 mg $L^{-1}$), all binding sites saturated [8] and $F^-$ ions were not adsorbed. The result also correlated positively with *Acinetobacter* sp. (GU566361) and *Bacillus flexus* NM25 strains, which have defluoridation ability of 57.3% and 67.45%, respectively [1] and [6]. The effect of contact time plays a crucial role in fluoride adsorption. The results also show that the strain *E. indicum* reached the maximum metabolic rate within 24 h. After that, the bacteria remained in the stationary growth phase, which decreased their biomass and defluoridation capacity (Fig. 2).

The surface morphology of $F^-$ treated and untreated bacterial cells was examined using a scanning electron microscope (Zeiss Gemini; Sigma 300) with EDX according to the standard protocol [9]. For transmission electron microscopy (TEM), both fluoride-treated and untreated bacterial cultures were placed on a carbon-coated copper grid (300 mesh) and observed under a transmission electron microscope (JEOL; JEM 1400plus, 120 keV, Japan).

In the untreated condition, the surface topography of *E. indicum* MLN15 was smooth and cellular integrity was maintained (Fig. 3a). In contrast, under $F^-$-treated conditions, distinct aggregates were deposited on the surface of bacterial cells (Fig. 3b). A similar result was also reported for *Providencia vermicola* KX926492 [7]. The possible reason for the surface roughness of the strain under fluoride stress is the sequestration and precipitation of various functional groups [10]. Nevertheless, strain MLN15 was able to maintain its cellular integrity after $F^-$ treatment. This indicates that the bacterial strain can be tolerated up to a certain concentration of fluoride, which plays a role in cell division and metabolism [11].

The EDX spectrum showed a tiny peak of fluoride ion of 6.04 wt% (Fig. 3) for the bacterial cells involved in $F^-$ adsorption [7]. The localization and intercellular accumulation of $F^-$ in bacterial cells was confirmed in the TEM study (Fig. 3d). Various black aggregates indicated by electron-dense granules were attached to the cell surface of bacterial cells under $F^-$ treated conditions (Fig. 3d), while they were absent in untreated cells (Fig. 3c). This type of intercellular accumulation of fluoride was also observed in *Bacillus licheniformis* [12]. Moreover, microscopic, and spectral characterization revealed that the morpho-structural change with pronounced $F^-$ peak under fluoride stress confirms

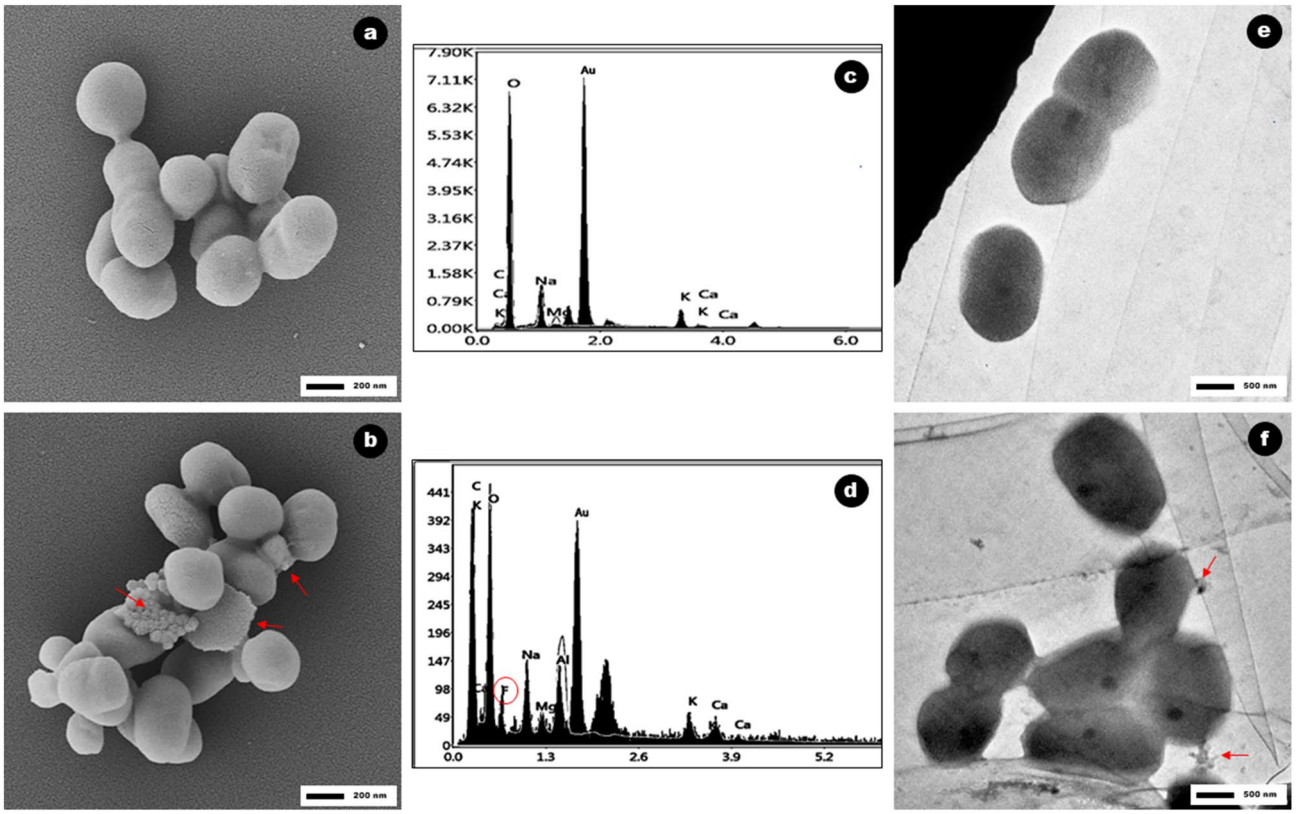


**Fig. 3** SEM micrographs **a** before and **b** after $F^-$ treatment; EDX spectrum **c** before and **b** after $F^-$ treatment; TEM images **e** before and **f** after $F^-$ treatment of *E. indicum* MLN15

$F^-$ adsorption by a bacterium. Therefore, it can be concluded that the fluoride-resistant strain *E. indicum* MLN15 could be used as an effective biosorbent for defluoridation of fluoride-stressed groundwater.

**Acknowledgements** The authors acknowledge UGC-Centre for Advanced Study, Department of Botany, The University of Burdwan, and DST-FIST (No. SR /FST/ LS -1/2018/188 (C), for conducting all research and providing infrastructure. M.L. thanks UGC-SRF (UGC Ref. No.: 737/ CSIR-UGC NET JUNE 2018) for providing the research funds. We thank the University Science Instrumentation Centre (USIC) at The University of Burdwan for TEM, FE-SEM, EDX, and XRD facilities.

**Funding** Not applicable.

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

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