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POSSIBLE MECHANISM OF DEGRADATION OF PERFLUORINATED COMPOUNDS BY THE BACTERIAL STRAIN *Bacillus* sp. MX3 ISOLATED FROM SOIL COLLECTED AT A FIREFIGHTING AGENTS TESTING SITE

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ABSTRACT

The study evaluated the destruction of perfluorinated compounds, specifically perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), using the microorganism *Bacillus* sp. MX3, isolated from soil that was collected at a firefighting agents testing site. The results showed that in the initial stages, the degradation of PFOS in an aqueous environment occurred more slowly than that of PFOA; by the third day, the concentration of PFOS had decreased by 50%, while PFOA decreased by 55%. From the fourth day onward, the degradation rate of PFOA slowed down, whereas PFOS was degraded by 80%; by the end of the experiment (7 days), however, both perfluorinated compounds were degraded by more than 95% (initial contamination was 200 mg/L). Analysis of fluoride ion release did not show a direct correlation with substrate depletion. The products of biodestruction were studied using chromatographic mass spectrometry, revealing active hydrolysis of PFOS and PFOA, with the accumulation of degradation products. In a soil model experiment on day 150, with augmentation using strain MX3, the level of PFOS decreased by 77%, and PFOA by 74%, with an initial contamination of 10 mg/kg. Genome sequencing confirmed the presence of genes responsible for xenobiotic metabolism and fluoride protection, namely: 1) genes encoding haloacetate dehalogenase class enzymes (*dehH1*), 2) genes coding for F^--specific transporter proteins (CrcB), which mitigate the negative impact of fluoride ions generated during the enzymatic breakdown of PFS, and 3) the alkanesulfonate monooxygenase gene (SsuE) – facilitating the desulfonation of substrates under sulfur-limiting conditions. Based on the obtained data, a metabolic pathway for the degradation of PFOS and PFOA was proposed.The results of the study highlight, on the one hand, the potential of *Bacillus* sp. MX3 for effective bioremediation of perfluorinated compounds; on the other hand, this potential requires confirmation under real environmental contamination conditions.

Keywords: perfluorocarboxylic acids, perfluorooctanoic acid, perfluorooctane sulfonate, biodegradation, transformation; genome

INTRODUCTION

The problem of environmental pollution by xenobiotics is a pressing issue. Particular attention should be paid to chemical pollutants that possess the ability to accumulate, migrate, and are compounds that are difficult to degrade under natural conditions. The growing demand for materials with new properties leads to an increase in their use in industry. Perfluorinated compounds (PFCs) are traditionally used in industry as additives to plastics, in firefighting compositions **(Guillette T.C. *et al.*, 2020)**, and in everyday life (for example, in the production of non-stick coatings for cookware). In 2009, perfluorooctanesulfonate (PFOS) and its salts were included in the Stockholm Convention on Persistent Organic Pollutants (POPs), and it is undeniable that the list of substances posing a danger to the biosphere will only continue to grow with representatives from the group of fluorinated compounds, as they are resistant to high temperatures, alkalis, acids, and oxidizers **(Khurana P. *et al.*, 2022)**. In addition to reducing the use of POPs, it is necessary to address the remediation of already contaminated ecosystems, as PFCs are referred to as "forever chemicals," and their stability under extreme conditions is well known **(Haupt, 2021)** and the issue of remediation will remain relevant for a long time **(Cheng J. *et al.*, 2008)**.

In addition to PFOS, perfluorocarboxylic acids (PFCAs) have been found in products contaminated with PFCs. The vast majority of pollution with perfluorinated organic compounds is concentrated in Western Europe, China, Korea, and Japan **(Muir D. & Miaz L.T., 2021)**, but they have even been detected even in Antarctica **(Kim D. H. *et al.*, 2024)**. The problem is closer to each of us than it seems – PFCAs have even been found in household dust **(Zhu L. *et al.*, 2023)** and in items that come into direct contact with food **(Loureiro P. V. *et al.*, 2024)**. Addressing pollution removal requires a comprehensive approach, considering not only the ecological but also the economic aspects. Existing chemical and physical purification methods are very expensive and technically challenging to implement outside of laboratories. These include chemical transformation, high-temperature oxidation **(Cheng J. *et al.*, 2008; Wang F. *et al.*, 2013)**, photocatalytic oxidation, plasma treatment, the use of adsorbents, and membrane filtration **(Hou J. *et al.*, 2022; Saleem M. *et al.*, 2020)**. At the same time, the use of technologies based on bacteria is very promising due to their cost-effectiveness and technological feasibility **(Marquínez-Marquínez A.N. *et al.*, 2023)**. Despite the extremely strong C-F bond, there are already reports in the scientific literature of successful applications of microorganisms for the degradation of PFCs **(Khurana P. *et al.*, 2025)**. Several strains of pseudomonads and closely related bacterial genera capable of degrading perfluorinated organic compounds are known: *Pseudomonas aeruginosa* HJ4 **(Kwon B. *et al.*, 2014)**, *P. parafulva* YAB-1 **(Yi L. *et al.*, 2015)**, *Delftia acidovorans* D4B **(Harris J. D. *et al.*, 2021)**, and the strain *Acidimicrobium* sp. A6 **(Jaffé P. R. *et al.*, 2024)**. The mechanism of PFAS cleavage with the release of free fluoride ions is largely similar to the mechanisms of chlorinated organic compound degradation. Dehalogenases associated with the decomposition of PFCAs have been identified in the strain *D. acidovorans* D4B, namely DeHa2 (haloacid dehalogenase) and DeHa4 (fluoroacetate dehalogenase) **(Farajollahi S. *et al.*, 2024; Harris J. D. *et al.*, 2022)**, while for the strain *Acidimicrobium* sp. A6, the gene *rdhA* has been identified **(Jaffé P. R. *et al.*, 2024)**.

When PFCs are degraded, the formation of F^- is inevitable, which has toxic effects **(Qin J. *et al.*, 2006)**. The fluoride ion can replace phosphate ions through the formation of AlF_4^- and BeF_3* H_2O **(Marquis R. E. *et al.*, 2003)**, leading to the suppression of metabolic reactions involving phosphate ions **(Zuo H. *et al.*, 2018)**. Additionally, by strongly binding with metals, F^- can inhibit the activity of metalloproteins and induce oxidative stress **(Agalakova N. & Gusev G., 2011)**.

Due to the high toxicity of the fluoride ion, microorganisms have developed various ways to mitigate its negative effects. **Baker *et al.* (Baker J. L. *et al.*, 2012)** reported on riboswitches that control the expression of genes involved in alleviating oxidative stress, acidification of the intracellular environment, repair mechanisms, and genes responsible for the transport of fluoride ions. Moreover, such mechanisms are not only present in fluorinated compound destructors; they have also been found in *Neurospora crassa*, *Saccharomyces cerevisiae*, and *Candida albicans* **(Li S. *et al.*, 2013)**, highlighting the importance of proteins that facilitate F^- transport for the proper functioning of microorganisms. We previously identified similar genes in the strain *Pseudomonas mosselii* 5(3), isolated from pesticide-contaminated soil, and proposed a mechanism for the destruction of C_7-C_{10} perfluorocarboxylic acids **(Chetverikov S. *et al.*, 2023)**. The discovery of new potential destructors of perfluorocarboxylic acids and understanding the mechanisms of biodegradation is a relevant task from both practical and scientific perspectives.

MATERIAL AND METHODS

Strain

The strain MX3 was isolated from soil at a fire extinguishing agents testing site in Ufa, Republic of Bashkortostan, using the enrichment culture method and was deposited in the microorganism collection of the Ufa Institute of Biology under the number UIB-252 and in the All-Russian Collection of Microorganisms (the deposition certificate VKM V-3775D).

Preparation of inoculum

Liquid cultures was prepared using Raymond's medium with the following composition (grams per liter of distilled water): NH_4NO_3—2.0; $MgSO_4 \times 7H_2O$—0.2; KH_2PO_4—2.0; Na_2HPO_4—3.0; $CaCl_2 \times 6H_2O$—0.01; and Na_2CO_3—0.1 **(Raymond R. L., 1961)**, with the addition of peptone at a concentration of 1 g/L. The inoculum of strain MX3 was prepared by centrifuging the culture medium and subsequently diluting it with sterile tap water to an OD_{600} of 0.1.

Assessment of the destruction of C_8 perfluorinated compounds in a liquid medium

The assessment of the destruction of C_8 perfluoroalkyl substances was conducted by adding PFOA or PFOS (200 mg/L) as the sole source of carbon and energy in Raymond's medium. Incubation was carried out on an orbital thermostatic shaker ES-20/60 (SIA BIOSAN, Riga, Latvia) at 180 rpm. To assess the effectiveness of PFCs degradation, strain MX3 was grown in a liquid mineral medium with individual C_8 perfluorinated compounds at 28 °C for 7 days.

Analysis of the fluoride ion concentration

The fluoride balance was assessed through ion chromatography utilizing an LC-20 Prominence HPLC system equipped with a CDD-10Avp conductometric detector from Shimadzu (Japan). Fluoride ions were separated using a Shodex column (Shodex, New York, NY, USA) at a flow rate of 1 mL/min. An eluent composed of an aqueous solution of sodium carbonate and sodium bicarbonate was prepared, containing 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$. For post-column eluent suppression, a Xenoic® XAMS suppressor was used in conjunction with an ASUREX-A100 from Diduco AB (Umeå, Sweden).

Model experiment on the bioaugmentation of strain MX3 in artificially perfluorinated compound-contaminated conditions.

For the model experiment, we used chernozem soil collected from an organic farming enterprise (Republic of Bashkortostan, Russia) that was not contaminated with chemical organic compounds. The experiment included four lines, each treated with PFOA or PFOS to a final concentration of 10 mg/kg of dry soil: 1) soil contaminated with PFOS; 2) soil contaminated with PFOA; 3) soil contaminated with PFOS and augmented with the bacterial culture of strain MX3 (final culture concentration 2×10^6 CFU/g of soil; 3) soil contaminated with PFOA and augmented with the bacterial culture of strain MX3 (final culture concentration 2×10^6 CFU/g of soil). The cultivation was conducted at 28±1°C, and the soil moisture was maintained at 40% of the soil's moisture capacity. The duration of the experiment was 150 days. Extraction of PFCs from soil samples was carried out using a modified method **(Rankin K. *et al*, 2016)**. The prepared sample (3.5 g of dry soil) was transferred into polypropylene centrifuge tubes, previously washed with methanol. For PFCs extraction, a mixture of acetonitrile and water in a 9:1 ratio (8.5 ml) was used, with the addition of 400 µl of a 2M NaOH solution. After adding the reagents, the samples were subjected to ultrasonic treatment in an ice bath for 15 minutes, followed by shaking on a shaker for 30 minutes and centrifugation for 10 minutes at 12,000 rpm on benchtop centrifuge TG16 (Changsha, China). The resulting supernatants from the three extraction steps were combined in glass tubes. For sample preparation for analysis, the combined supernatant was evaporated to dryness under a stream of air. The dry residue was dissolved in two consecutive washes of acetonitrile and water in a 2:3 ratio, each with 0.5 ml. All experiments were conducted in three independent biological replicates.

The efficiency of PFCs destruction was calculated using the formula:

$D(\%) = 100 - ((C_t \times 100)/C_0)$,

where D is the destruction efficiency (%), C_t is the concentration of PFCs after a certain period of time, and C_0 is the concentration of PFCs at the initial moment in time.

Determination of the amount of perfluorocarbon compounds and their degradation products

The levels of PFCs in the medium were assessed, and the byproducts of their biotransformation were analyzed using a liquid tandem chromatography-mass spectrometer LCMS-IT-TOF (Shimadzu, Kyoto, Japan) at the AGIDEL UFSC RAS Equipment Collective Use Center. This analysis was performed on ultrafiltrates (≤3 kDa) of culture liquids that were obtained through ultrafiltration using Vivaflow 50 (Sartorius AG, Göttingen, Germany), as previously outlined **(Chetverikov S. *et al.*, 2017)**.

To determine the degradation products of perfluorocarboxylic compounds, HPLC analysis was performed, followed by the determination of mass spectra. For the HPLC analysis, a Shim-pack XR-ODS column (75 mm × 2.0 mm id, 2.2 µm) from Shimadzu (Kyoto, Japan) was utilized in a gradient elution setup comprising 5 mM ammonium acetate in acetonitrile (A) and 0.1% acetic acid (v/v) in water (B). The gradient elution followed these parameters: from 0 to 10 minutes, the composition changed from 60% to 30% (B); then from 10 to 20 minutes, it reversed back from 30% to 60% (B). Chromatographic separation was carried out at a constant flow rate of 0.25 mL/min with an injection volume of 5 mL. Mass spectrometry data were collected using an electrospray ionization (ESI) source operated in negative ion mode. The conditions for mass spectrometry included a probe voltage of −3.5 kV, a curve desolvation line (CDL) and heat block temperature set at 200 °C, a detector voltage of 1.57 kV, and a nebulizing gas (N_2) flow rate of 1.5 L/min. Collision gas and cooling gas pressures were maintained at 50 kPa and 105 kPa, respectively. Parent ion m/z data were recorded within the range of 150–700, with an ion accumulation time of 100 ms (event time of 432 ms, repeated three times).

Sequencing, assembly, and annotation of the genome

The genomic DNA of strain MX3 was extracted from biomass using the phenol-chloroform method **(Sambrook J. & Russell D. W., 2006)**. Sequencing was performed on DNBSEQ-G50 equipment (MGI, China), and paired-end reads of 100 bp were obtained.

Quality control of the reads, annotation, analysis of target gene sequences, construction of the minimum evolutionary tree, the average nucleotide identity was conducted as described earlier **(Chetverikov S. *et al.*, 2023)**.

Metabolic pathway chart for perfluorocarboxylic acids

Metabolic pathways were created utilizing ChemSketch version 2023.1.1.

Statistics

All experiments were conducted in triplicate. Standard methods of parametric statistics were used to describe the research results: the arithmetic mean and standard deviation were calculated. The comparison of two groups was carried out using a two-tailed Student's t-test. The critical level of significance was set at 0.05 for this study.

RESULTS AND DISCUSSION

The experiment on the decomposition of perfluorinated compounds in a liquid medium by the Raymond strain MX3 showed that during the destruction of C_8 perfluorinated compounds of various classes, the biodegradation patterns of PFOS (shown in the Figure 1A) and PFOA (shown in the Figure 1B) differed. In the initial stages, the destruction of PFOS occurred more slowly than that of PFOA, but by the third day, the amount of PFOS had decreased by 50%, while the decrease in PFOA was 55%. However, from the fourth day until the end of the experiment, the decrease in PFOA slowed down, while the degree of destruction of PFOS reached 80% by the fourth day, compared to 63% for PFOA, and this trend persisted until the end of the experiment; by the end of the experiment (7 days), degradation had reached 98% for PFOS and 96% for PFOA. Moreover, the release of F^- and the decrease in substrate followed a linear pattern, and the amount of released fluoride ion was inversely proportional to the substrate concentration. When analyzing the distribution of biodegradation products using chromatography-mass spectrometry methods which are shown in the figure 2, it becomes clear that in the case of PFOS, enzymatic hydrolysis occurs more actively, and the accumulation of deeper degradation products begins specifically from the third day. In contrast, for PFOA, a breakdown into larger components is observed. It is evident that the diversity of biological decomposition products under the influence of the MX3 strain's enzymes correlates not only with a decrease in the amounts of PFOS and PFOA in the medium but also with the amount of fluoride ions released during this process. Thus, based on the data presented in figure 1 and figure 2, respectively, one can hypothesize that for the enzymatic destruction of PFOS and PFOA, the MX3 strain employs different substrate decomposition strategies. This, in turn, affects the qualitative and quantitative characteristics of the degradation products of the initial substances. The biological degradation of PFCs presents a challenging task for bacteria; scientific literature reports that strains such as *Pseudomonas* sp. YAB-1 can utilize up to 48.1% of PFOA within 96 hours at an initial concentration of 500 mg/L **(Yi L. *et al.*, 2016)**, while the strain *Acidimicrobium* sp. A6 can degrade up to 77% of PFOA at a concentration of 100 mg/L over 18 days **(Ruiz-Urigüen *et al.*, 2022)**. Xie *et al.* isolated a consortium capable of utilizing up to 38% of PFOA at an initial concentration of up to 3 mg/L, with the experiment lasting 8 days **(Xie H. *et al.*, 2022)**. The strain most similar in its ability to degrade PFOA is *Pseudomonas* sp. YAB-1. Data on PFOA and PFOS destructor strains are summarized in Table 1. The search for new strains that can degrade perfluorinated compounds, as previously mentioned,

remains a relevant task both for fundamental science, aiming to uncover new aspects of the metabolism of "forever chemicals," and for ecological purposes, particularly for remediation efforts.

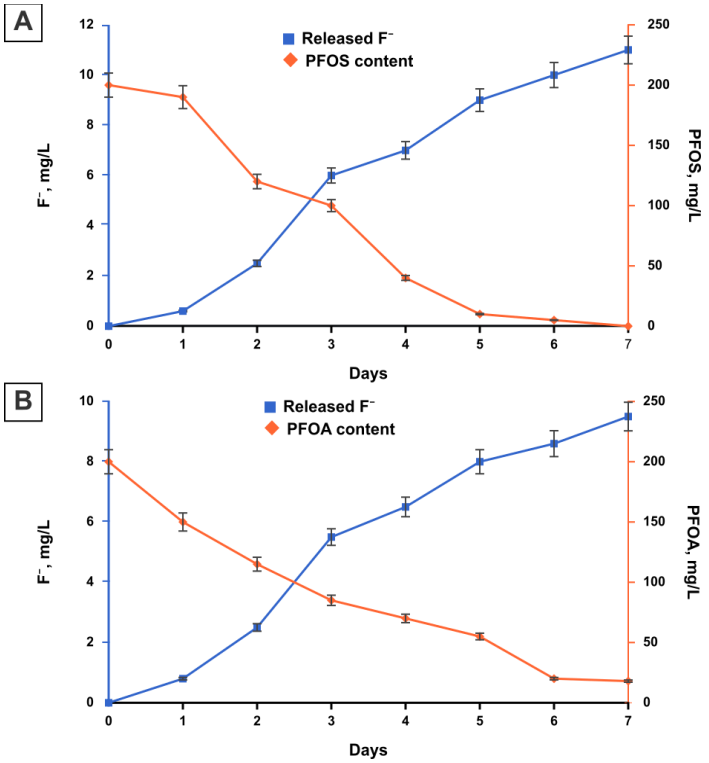


Figure 1 Dynamics of fluoride ion accumulation and concentration changes during the cultivation of the MX3 strain in Raymond's liquid medium on substrates: PFOS (A) and PFOA (B). Data are presented as mean ± standard deviation (SD), n = 3. Different letters indicate significant differences ($p < 0.05$).

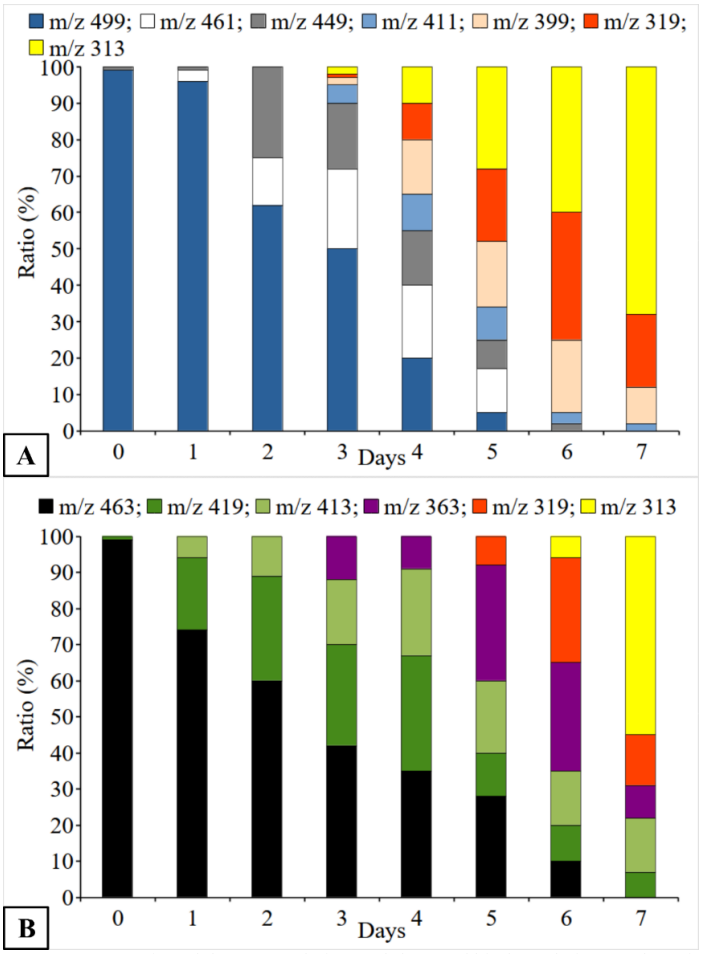


Figure 2 Dynamics of the accumulation and decay of biodegradation products in a liquid medium during the cultivation of the MX3 strain in Raymond's medium over 7 days with PFOS (A) and PFOA (B) as the sole carbon and energy source. Data are presented as mean values, n = 3.

Table 1 Bacterial strains of PFOA and PFOS biodegradation

Strain	Concentration (mg/L)	Conditions of incubation	Degradation effenciency (%)	References
Pseudomonas aeruginosa HJ4	1,8	Aerobic, 28°C, 2 days	67	**Kwon *et al.*, 2014**
Pseudomonas parafulva YAB1	500	30°C, pH 7, 2 % inoculum, 96 h, (with 1 g/L glucose as co-substrate)	32,4 (48,1)	**Yi *et al.*, 2015**
Delftia acidovorans D4B	100	Aerobic, 28 C, 60 days	49	**Harris *et al.*, 2021**
Acidimicrobium sp. A6	0,1 and 100	Anaerobic, 30°C, 100 days	63; 50	**Huang *et al.*, 2019**
Acidimicrobium sp. A6	100	Anaerobic, 30°C, 18 days	77	**Ruiz-Urigüen *et al.*, 2022**
Pseudomonas mosselii 5(3)	100	Aerobic, 28 C, 7 days	100	**Chetverikov *et al.*, 2023**

In the model experiment on the biodegradation of PFOS and PFOA in soil, the augmentation of the MX3 strain contributed to a reduction in the amount of the perfluorinated compound. For the PFOS variant, after 150 days of the experiment, the decrease in the pollutant amount from the initial level was 77%, while for PFOA, it was 74%. The dynamics of the substrate reduction curves were also very similar, Results are shown in the figure 3. In the variants without the augmentation of the MX3 strain, the level of PFCs remained unchanged, meaning it was not subject to degradation by physical-chemical factors or by the influence of the indigenous soil microflora. The distribution of the initial substance and the metabolites of PFOS and PFOA at the end of the model experiment on the degradation of perfluorinated compounds in soil (150 days) is presented in Table 1. In the case of the perfluorinated carboxylic acid, the concentration of the initial substance was higher than that during the degradation of PFOS. This is likely related to the more complex multi-stage enzymatic cleavage of the PFOA molecule, similar to the biodegradation of the studied substances in a liquid medium as shown in figure 2.

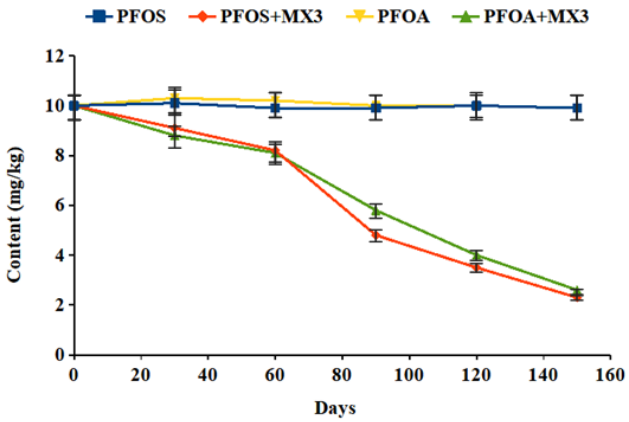


Figure 3 Dynamics of changes in the concentration of PFOS and PFOA in soil (model experiment, 150 days of exposure) with the augmentation of the MX3 strain. Data are presented as mean ± standard deviation (SD), $n = 3$. Different letters indicate significant differences ($p < 0.05$).

It should be noted that PFPeA with m/z 263 was found in the soil, whereas in the experiment on the degradation of PFOS and PFOA in a liquid medium, we did not detect it. This phenomenon may be related to the immobilization of cells on particles present in the soi, for example. It is known that cell immobilization can enhance the enzymatic activity of microorganisms under stress conditions caused by various factors: substrate toxicity, unfavorable temperature, pH, etc. **(Hou J. *et al.*, 2024)**. Other hypotheses suggest that in the soil, pollutant molecules are localized and cannot migrate quickly, and/or the duration of the experiment in soil, which significantly exceeds the duration of the experiment in a liquid medium—150 days versus 7 days, respectively—affects the depth of substrate destruction. On the other hand, the amount of metabolized substrate by the MX3 strain is higher in the liquid medium. The reason may lie in the higher aeration during cultivation on an orbital shaker compared to soil. It is worth noting that in the study by Huang *et al.*, for the strain *Acidimicrobium* sp. A6, microaerophilic conditions were required for more profound degradation of perfluorinated compounds **(Huang S. & P.R. Jaffe, 2019)**, and this is likely partly true for the MX3 strain as well. However, for strain A6 under such conditions, the degree of biodegradation did not exceed 63% at an initial concentration of 100 mg/L over 100 days.

Table 2 Percentage ratio of PFOS and PFOA and their degradation products in soil with the augmentation of strain MX3 at the end of the model experiment (150 days). Data are presented as mean values, $n = 3$.

	PFOA m/z 413	PFHpA m/z 363	PFHxA m/z 313	PFPeA m/z 263	
PFOA	28	10	40	22	

	PFOS m/z 499	PFHpS	PFHxS	PFHx	PFHxA m/z 313
PFOS	6	8	12	16	58

From other studies, it can be noted **(Beškoski V. P. *et al.*, 2018**; **Tang Z. *et al.*, 2024)**, in which demonstrated the removal of PFOA and PFOS over 28 days by microbial consortia with chemo-organotrophic bacteria at 16%, and by a consortium of yeast and mold at 36%, resulting in the formation of monofluorinated fatty acids according to LC/MS analysis and the degradation of various perfluoroorganic compounds by a consortium of microorganisms after 10 months of incubation was observed, with the detection of minor quantities of PFHxA and PFHpA as metabolites, respectively.

The genome sequencing of the active strain MX3, a destructor of perfluorocarboxylic acids (PFCA), has been conducted. The data is publicly available in GenBank under BioProject number PRJNA1145257. The MX3 strain has been identified as belonging to the species group *B. amyloliquefaciens/velezensis/methylotrophicus/siamensis*. The bacilli of these species constitute a closely related species group, among which it is impossible to obtain reliable differences. A comparative analysis of the nucleotide sequence of the gene encoding 16S rRNA with known structures from GenBank (http://www.ncbi.nlm.nih.gov/genbank) allows us to assert with a high degree of certainty that the microorganism belongs to the species *Bacillus siamensis* (with 99.93% similarity found with the type strain of *Bacillus siamensis* KCTC 13613^T).

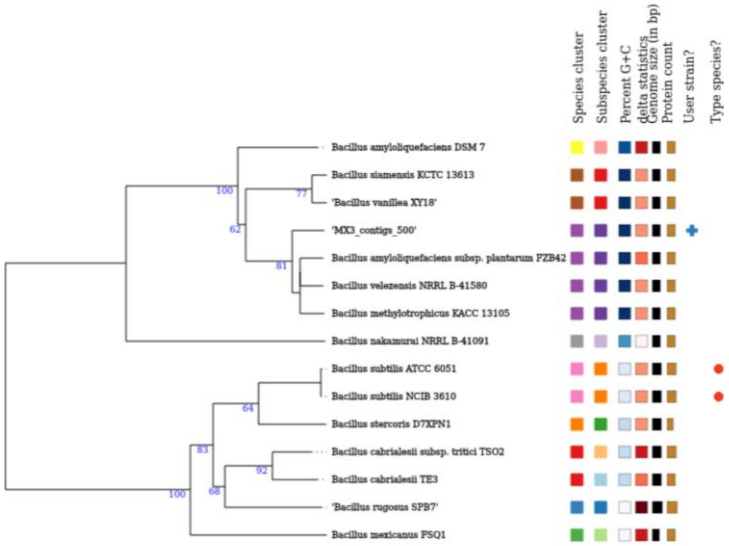


Figure 4 Whole-genome phylogenetic tree of strain MX3.

Figure 5 presents a pie chart of the genome of the *Bacillus* sp. MX3 strain. The data shown in the graph indicate that the studied strain contains a group of genes responsible for xenobiotic metabolism. The most interesting genes in terms of defluorination of perfluorinated compounds are listed in Table 2. The genome of strain MX3 contains a complex: haloacetate dehalogenase H-1 gene (*dehH1*) – genes responsible for encoding enzymes of the haloacetate dehalogenase class **(Sota M. *et al.*, 2002)**, fluoride ion transporter gene (CrcB) – a sequence encoding F--specific transporter proteins **(Calero P. *et al.*, 2022)**, for mitigating of the negative impact of fluoride ions generated during the enzymatic breakdown of PFCs, and alkanesulfonate monooxygenase gene (SsuE) – which, under sulfur limitation conditions is able to facilitate desulfonation **(Chulwoo P. *et al*, 2020)** of substrates. We hypothesize that the presence of these genes enables the *Bacillus* sp. MX3 strain to not only exhibit catabolic activity toward perfluorinated organic compounds (including sulfonated ones) but also to resist the accumulation of F^- within cells, the negative impact of which has a complex toxic effect **(Baker J. L. *et al.*, 2012)**. Genes for various dehalogenases with similar functions have been found in some microorganisms capable of degrading perfluorinated compounds. For instance, *dehH1* and haloalkane dehalogenase (*dhaA*) were identified in *Delftia acidovorans* D4B, which is capable of degrading PFCA **(Farajollahi S. *et al.*, 2024)**. In the bacterium *Acidimicrobium* sp. A6, genes responsible for the synthesis of reductive dehalogenase (RdhA) and fluoroacetate dehalogenase (FceA) were identified. Additionally, two putative genes for halogenated acid dehalogenases (*dhl_1* and *dhl_2*) were found in its genome **(Jaffé P. R. *et al.*, 2024)**.

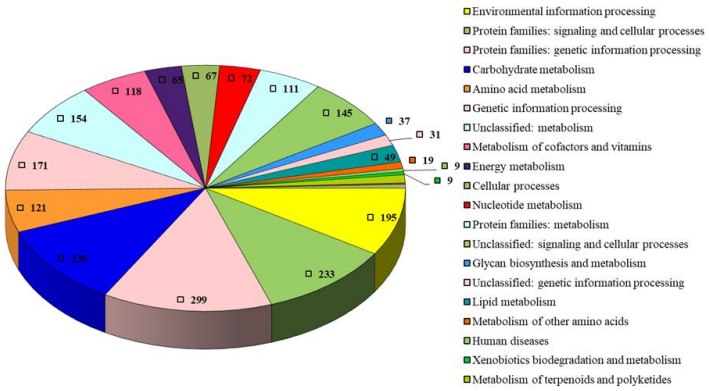


Figure 5 Distribution of genes across functional categories in the MX3 genome according to KEGG classification

Table 3 Gene complex of strain MX3 putatively responsible for the degradation of perfluorinated compounds.

Traits	Gene	Gene function
dehH1	haloacetate dehalogenase H-1 gene	haloacetate dehalogenase class enzymes"
CrcB	fluoride ion transporter gene	F^--specific transport proteins
SsuE	alkanesulfonate monooxygenase gene	Desulfonation of substrates

Based on the above, a proposed hypothetical pathway for the destruction of C_8 perfluorinated compounds has been outlined; the results are shown in Figure 6, and it can be described as follows. For PFOA (m/z 413), the first step involves decarboxylase, which cleaves the carboxyl group to yield perfluoroheptane (m/z 369). Subsequently, defluorination occurs via haloalkane dehalogenase, resulting in the formation of perfluoroheptanoic acid (m/z 363), followed by the cleavage of the carboxyl group to produce the final product, perfluorohexane (m/z 319). For PFOS (m/z 499), the initial two stages involve the participation of haloacetate dehalogenase DehH1, releasing CF_2 at each step. Initially, perfluoroheptane sulfonate (m/z 449) is formed, followed by perfluorohexane sulfonate (m/z 399). As a result, alkane sulfonate monooxygenase detaches the SO_3 group, and the resulting product (m/z 319) is analogous to that of PFOA in the final stage.

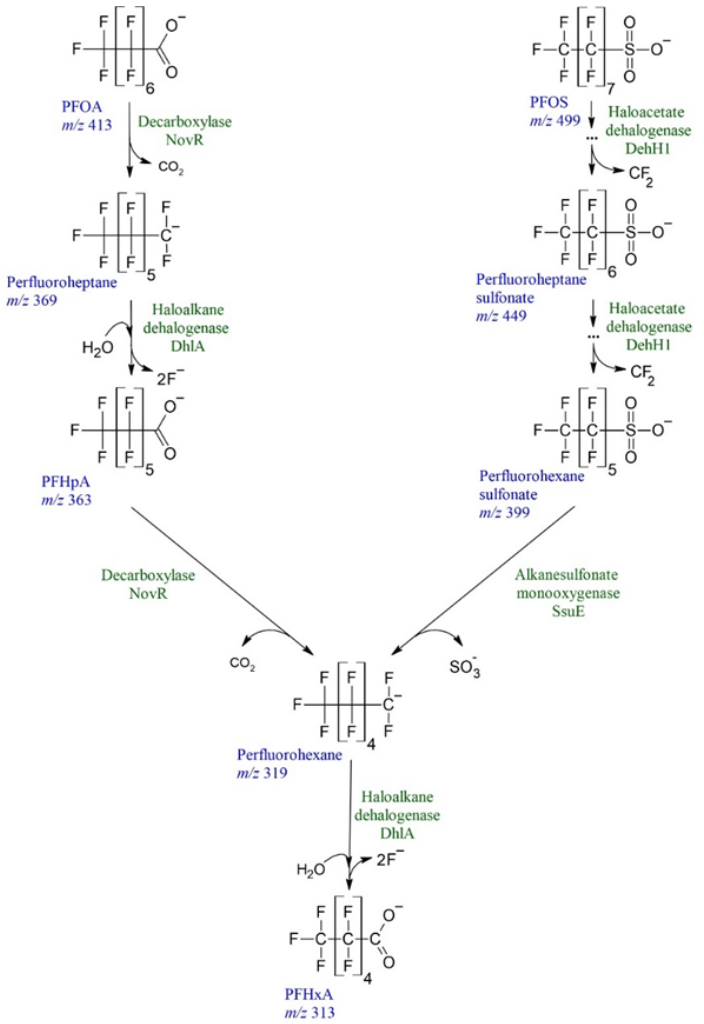


Figure 6 Hypothetical pathway for the enzymatic destruction of PFOS and PFOA by the *Bacillus* sp. MX3 strain.

However, in the case of PFOS, there is another stage of defluorination by haloalkane dehalogenase, and the end product in this case is perfluorohexanoic acid (m/z 313). The more extensive destruction of PFOS can be explained by the formation of F^- only at the final stage; at least this explains why PFOS is better metabolized by the MX3 strain than PFOA. Results are shown in the figure 2 and table 3. Nevertheless, even with the presence of transporters from *Bacillus* sp. MX3, one should not underestimate the toxic 'pressure' of accumulated fluoride ions. Understanding the mechanism of biodegradation of perfluorinated compounds, as well as the possible refinement of the proposed hypothetical scheme shown in Figure 6, requires a more detailed and in-depth study of this topic and is the subject of a separate article.

CONCLUSION

A whole-genome analysis of the studied strain MX3 was conducted, which was identified as belonging to the species group *B. amyloliquefaciens/velezensis/methylotrophicus/siamensis* based on average nucleotide identity (ANI) between strains and related strains. The genome analysis of the MX3 strain revealed the presence of genes that may be responsible for coding haloacetate dehalogenase (*dehH1*), fluoride-specific transporter proteins (CrcB), and alkanesulfonate monooxygenase (SsuE), which facilitates the desulfonation of substrates under sulfur-limiting conditions. The *Bacillus* sp. MX3 strain is capable of utilizing up to 98% PFOS and up to 96% PFOA in liquid media within 7 days at an initial concentration of 200 mg/L. For soil (at a contamination level of 10 mg/kg), these figures were 77% and 74% within 150 days, respectively. LC-MS analysis of the metabolism products showed that the number of substances with different m/z values was higher during the biodegradation of PFOS than for PFOA. Additionally, in the soil augmentation variant with the *Bacillus* sp. MX3 strain, perfluoropentanoic acid with m/z 263 was detected in the experiment with perfluorooctanesulfonate. While during the biodegradation of perfluorooctanoic acid, cleavage only resulted in perfluorohexane with m/z 319. Full genome analysis showed that the *Bacillus* sp. MX3 strain possesses genes such as the haloacetate dehalogenase H-1 gene (*dehH1*), fluoride ion transporter (CrcB), and alkanesulfonate monooxygenase (SsuE), which presumably play a key role in the destruction of perfluorinated compounds. By analyzing and comparing LC-MS data with the aforementioned genes, a hypothetical scheme for the biodegradation of C_8 perfluorinated compounds by strain MX3 was proposed. The results obtained in this study will help expand the understanding of fundamental issues related to the biological degradation of perfluorinated compounds, and, after extended testing of their destruction under real-world conditions, may provide impetus for the development of microbial preparations for the remediation of waters and soils contaminated with perfluorinated compounds.

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