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Dehalogenase-Producing Alkaliphilic Bacteria and their Bioremediation Potential

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ABSTRACT: Pollutants, especially halogenated ones, pose a serious threat to humans, animals, and plants because most are pervasive in the environment due to their resistance to degradation by microorganisms or through exposure to environmental elements. One sustainable way to remove this group of pollutants from the environment is through bioremediation assisted by suitable microorganisms, as fungi and bacteria. Bioremediation using bacteria as dehalogenase-producing alkaliphilic bacteria has been shown useful in remediating organohalogen-based pollutants. Most alkaliphilic bacteria have been isolated from harsh ecosystems such as soda lakes, characterized by high pH and occasionally high salinity, the first resulted from excess sodium compared to calcium in basaltic rocks, forming carbonate-rich, alkaline aquatic habitats. Bioprospecting works, isolating dehalogenase-producing alkaliphilic bacteria from soda lakes, has successfully yielded halogen-degrading enzymes, which successfully nullify the toxicity of the compounds. In this review, we describe microorganisms in soda lakes; and the distribution of alkaliphiles in these soda lakes along with the utility of alkaliphiles in bioremediation. Bioremediation of polluted alkaline water habitats has emerged as the most pressing issue of biotechnological relevance nowadays.

Keywords: Alkaliphiles, 2,2-dichloropropionate, dehalogenase, xenobiotics, pollution, Soda Lake, Bioremediation.

1. Introducation

Historically, the significance of *Bacillus Alcalophilus* was the first alkaliphilic bacterium to be isolated (1). Unverified data suggests Alkalobacteria was isolated in 1889 (2). A bacterium belonging to the genus *Bacillus* named *Bacillus alkaliphiles* is isolated. It thrives at an alkaline pH of 10 to 11.5 (1). Alkalobacterium is a resilient bacterium that thrives in alkaline ecosystems, for example, saline-alkaline and non-saline soda lakes (3), involving various eubacteria and archaeobacteria that have evolved to overcome environmental challenges like high/low temperatures, high sodium concentrations, high pressure, and pH (4). Alkaliphilic eubacteria are part of the Gram-positive and negative groups, including *Bogoriella, Micrococcus, Pseudomonas, Halomonas*, and *Alkalibacillus* (5), *Cyanobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Verrumicrobia, Deinococcus-Thermus, Spirochaetes*, and *Chloroflexi* (6). Spirochaetes, and the magnetotactic bacteria. This includes cyanobacteria to Archaeobacteria. Other research about Gram-negative alkaliphiles has investigated genera such as Aeromonas (4). Many of the prominent bacteria and Archaea are isolated frequently from the various soda lakes of Africa, North America, Europe, and Asia (7, 8). Notably, alkaline soda lakes have similar fundamentals, although separated by great distances between Asia and North America. Bacteroidetes, Alphaproteobacteria, Gammaproteobacteria, and Euryarchaeota were the most prevalent species in different alkaline soda lakes, despite varying salinity between 170 to 400 g/L. Based on the growth of alkaliphiles in the alkaline environment, they are classified and grouped by Wahhab (2021) in Fig. 1. Alkaliphilic bacteria are found in alkaline and non-alkaline environments, with higher alkaliphile counts in alkaline environments as per (10).

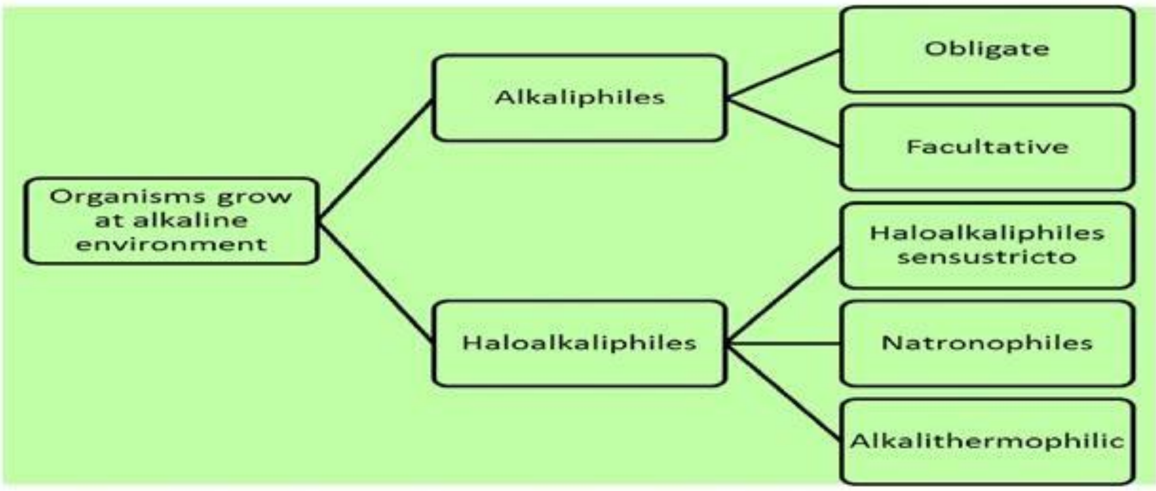

Figure 1: Classification of organisms grow in an alkaline environment (adapted from Wahhab, 2021).

2. Physicochemical characteristics of the Soda lakes:

Physico-chemical characteristics of various soda lakes fluctuate based on position, season, and year. Except for salinity, all variables fluctuate significantly across seasons ($p < 0.05$). Physicochemical changes increase during dry seasons due to low rainfall and climate changes, which lead to higher evapotranspiration levels (11). These changes in the lake's physical, chemical, and biological features may result in a decline in some microorganisms and the extinction of other organisms (12). Microbial blooms are the reason for the distinct coloration of the soda lakes (13). The color changes in soda lakes are attributed to chemolithoautotrophic denitrifying bacteria responsible for the decomposition of nitrogen and sulfur in these lakes (14). Salinity in sodium lakes ranging from (35 to 250) g/L is excellent for the thriving of halo-alkaliphilic, which use their ability to cycle (carbon C, nitrogen N_2, and sulfur S) as energy forms (15). At salinity ranges between 50 and 250g/L. Energy that involves carbon and nitrogen cycling is inhibited partly. At a salinity of >250g/L, nitrogen cycling is completely inhibited (16).

3. Alkalophiles management for the Physicochemical characteristics of Soda lakes:

Alkalophiles thrive in neutral environments and even in acidic soil. Alkalophiles fall into two categories. The first requires an alkaline pH of 9 or more for growth, with an optimal pH of around 10 (17). The second category, haloalkalophiles, needs an alkaline pH of 9 with a high percentage of NaCl (more than 33%) for growth (10). According to geochemical studies of brines based on saturation levels, the soda saline type is an intermediate stage between the soda and saline types. It is inaccurate to distribute alkaline soda lakes based solely on pH levels. So, large-scale studies were conducted to assess chemical and pH readings of soda brine in wide geographical areas and across the wide salinity ranges in Eurasian inland saline surface waterways. Two kinds of soda lakes were identified and represented (soda and soda-saline). Soda lakes arise when (sodium Na^{+}) and the sum of (bicarbonate ions ‘HCO_3^{-}‘, and dissolved carbon dioxide ‘CO_3^{2-}‘) are the first dominant ions (> 25e%), while soda-saline lakes develop when sodium (Na^{+}) is the first dominant cation and the sum of (HCO_3^{-} and CO_3^{2}) concentrations is above 25e%. Still, it is not the first dominant anion (18). Salinity in Ethiopia's Chittu's Lake and Shala was recorded at 38.41 g/l^{-1} and 16.78 g/l^{-1}, respectively (11). In contrast, haloalkaliphiles are microorganisms typically found in extremely alkaline saline environments like the Rift Valley lakes in East Africa and western soda lakes in the US (17). Natronophiles grow in high-pH environments with carbonates, while haloalkaliphiles require high pH and salt concentrations (19). They adapt to alkaline and saline ecosystems (osmotic control), which maintains pH homeostasis utilizing mediating neutral cytoplasmic processes (20). Alkaline niche Halobacteria, soda lakes, and chloride-rich alkaline habitats with low concentrations of magnesium and calcium (21).

Sodium salts-based applied in salt-alkali and salinized soils become the primary osmolyte for Haloalkaliphilic archaea and Haloalkalithermophilic bacteria (21, 22). The soda lake has high sunlight exposure, plenty of energy concentrations in in-organic carbon forms, and upcycled items. These pheno-type traits with soda lakes' characteristics to encourage rich phototrophy, autochthony, high production, and ecological balance might be correlated across lakes. As a result, microbial richness between different lakes is likely similar (23).

Microbes' adaptation in the soda lakes is based on their biological function. when multi-cation and proton antiporters (sodium Na^+, potassium K^+, calcium Ca_2^+, and hydrogen H^+) in alkaline soda lakes raise the capacities of the membranes, endowing energy (fused to ATP generation) ample to permit cell growth in harsh pH conditions. The structure of the cell wall polymer, in turn, consists of teichuronic acid and teichuronopeptide, which significantly contribute to maintaining pH homeostasis by negatively charging the matrix and reducing the pH value at the alkaliphilic cell surface (24). The inhabitant microorganisms in the soda lake are based on the energy sources. A group of haloalkaliphilic obligatory chemolithoautotrophic (sulfur-oxidizing bacteria) obtain their energy by the sulfur substances' aerobic oxidation at high sodium concentrations and alkaline pH (23). The study analyzed the microbial composition and metabolic potential of lakes containing chloride sediments, carbonate, and sulfate brines. Microorganisms from major phylogenomic branches thrive in extreme saline and alkaline conditions, utilizing sulfur oxidation and reduction, as well as polysaccharide phosphorylation. Microbes that use sulfur respiration are more likely to thrive in high pH environments due to thermodynamic advantages, which explains their prevalence in such environments (25). The influence of physicochemical factors on the composition of sulfide-oxidizing bacteria and sulfate-reducing bacteria in soda lakes remains unclear (20).

4. Alkaliphilic with bioremediate xenobiotics

Pollution is a global issue causing health issues and death, affecting millions. Cities face higher contamination rates, while rural areas are also affected. Chemicals and pesticides are found on the Antarctic ice sheet, and the Great Pacific Ocean contains a massive accumulation of tiny plastic particles (26). Human activity has introduced xenobiotics into the world, causing environmental damage and negative effects on the natural environment. However, some microorganisms can break down man-made xenobiotics, making them ideal models for research studies, as they are deteriorating bacterial strains that employ xenobiotics to produce carbon and energy (27). Heavy metals in agricultural wastewater pose a significant threat to the ecosystem and human health due to their toxic effects (28). Heavy metals are recalcitrant and difficult to biologically transform, making their removal from polluted ecosystems challenging. Alkaliphilic organisms or enzymes that precipitate metal ions are the best choice to bioremediates metal contamination. *Alkaliphilus metalliredigens* strain QYMF has been shown to successfully reduce iron in an anaerobic alkaline ecosystem, indicating the potential for employing such alkaliphiles to remove metal pollutants from alkaline effluents. Alkaliphile-induced metal precipitation restricts metal movement, preventing pollution spread (29). Alkalophile metal removal can immobilize radioactive substances in a variety of circumstances, even in extremely alkaline environments (30). The issue of alkaline waste in chemical industries is a growing concern due to economic growth and the rise in pollutants. Alkaliphiles, which have stable enzymes and can be used for biotechnological applications, are being considered as an eco-friendly solution (31). Manufacturing processes like chrome metal generation, iron and steel polishing, and alumina processing contribute to highly alkaline waste, which is polluted with radioactive trace metals (32). Bauxite remnants from extracting alumina lead to increased alkaline tailings concentrations (19, 33). Handling those pollutants at alumina plants is challenging due to their ecosystem risk and financial costs (32). *Bacillus* species, particularly the Firmicutes phylum, have shown potential in treating alkaline contaminants. They play a significant role in bauxite recycling and have been isolated from the red clay, indicating their promise in a biological remediation strategy (32). Strictly anaerobic alkaliphilic bacteria, like *Alkaliphilus transvaalensis* isolated from a deep gold mine in South Africa, were used in the bioremediation of red clay, a strong alkalinity waste primarily made of iron oxide from the alumina refining process (29). Alkaliphilic bacteria like *Bacillus krulwichiae, Mycobacterium* sp., and *Bacillus badius can* degrade and remove each of the benzoates and pyrenes, highlighting their potential in bioremediation (29). Xenobiotic contamination in surface waterways, including marine waters, is a significant concern due to the increased demand for azo and synthetic dyes (24, 34). These dyes, commonly utilized in the rubber and plastics industries, contributed to an overabundance of colored water. Azo dyes are a significant category of commercially available dyes. Contamination occurs when colored wastewater containing organic dyes is released into ecosystems, causing dangerous pollution issues. Alkaliphilic bacteria like *Bacillus flexus* VITSP6 and *Nocardiopsis alba* have been shown able to bioremediate the toxic dye at pH (11), while Z-7937T strain *Natroniella acetigena* can break down components into acetate

in alkaline ecosystems in a pH level ranging from (8.1-10.7) (33, 35).
Corynebacterium humireducens MFC-5(T) was able to create lactic acid via the simulated alkaline wastewater pH (10) (33). Chemolithoautotrophic denitrification bacteria treat wastewater containing nitrogen or sulfur to oxidize reduced forms of inorganic sulfur substances and nitrate (7). Alkaliphiles flourish at pH nine or ten. These species have valuable enzymes, including hydrolases utilized in pulp and paper modification, detergent formulation, and bioconversion of lipids, protein, and carbohydrates (26). Table 1 shows the bacterial species that aid in biological remediation, chemical degradation, waste management, and biofuels development. *Anoxybacillus*, a genus of alkalitolerant thermophiles, can grow at 37°C based on alkali-growth, despite being extremophilic (26, 57).
Microorganisms, such as hexavalent chromium bioremediation, are considered an economical and environmentally friendly method for metal pollution removal from industrial effluent and the environment (51). Their features, such as heavy metal elimination, absorption, and biodegradation, provide a low-cost alternative approach to metal pollution removal (52).

Table 1: Some important alkaliphilic bacteria are responsible for degrade the pollutants.

Microorganism	Optimal pH	Pollutant Degraded	Source	Reference
Thioalkalivibrio	9.5	Sulfidic	Russian soda lake sediment	(36)
Pseudomonas stutzeri, *Staphylococcus arlettae* (SDT1), *Staphylococcus* species (SDT2)	10.5	Phenol	Lonar Lake	(37)
Bacillus cohnii MTCC 3616	9.0	Azo dye	Obtained from Institute of Microbial Technology, Chandigarh, India	(38)
Bacillus licheniformis MTCC 5514	12	Anthracene	Marine samples	(39)
Bacillus badius D1	9	Benzidine	Bacillus badius D1 was used	(40)
Halomonas pacifica strain NK2, *Halomonas campanieinsis* strain NRS-01	10	Aniline	Marine ecosystems of Goa, India	(41)
Nesterenkonia lacusekhoensis EMLA3	8-11.5	Azo dye	Highly alkaline textile effluent sample (pH 13.0) collected from local textile industry, National Capital Region, Delhi, India	(33)
Bacillus cohnii BRA1, *Bacillus pseudofirmus* BRA3, *Bacillus clarkia* BRA5	10.5	Bauxite Residue	Bauxite residue in the Southern region of Minas Gerais, Brazil	(43)
Mycobacterium sp. strain MHP-1	9	Petroleum hydrocarbons	Soil sample from Chiba Prefecture	(44)
Bacillus thuringiensis EEEL02	10	Bauxite residue	Bauxite residue deposit area, Guangxi Province, China	(42)
Pseudomonas pseudoalcaligenes CECT5344	9.5	Cyanide	Guadalquivir River downstream from the city of Córdoba	(45)

Nesterenkonia lacusekhoensis EMLA3	11.5	Azo dye (violet dye)	Textile effluent was used for decolorizing the dye	(46)
Pseudomonas aeruginosa sanai	9.8	Petroleum compounds	Industrial alkaline mineral metal-cutting oil	(47)
Acinetobacter baumannii strain VITVB	9	Reactive Blue 221 and Reactive Black 5 dyes	Local textile industry at Tirupur, Tamil Nadu, India	(48)
Pseudomonas mendocina	8.5	Various azo dyes	Textile effluent contaminated soil samples	(49)
Bacillus licheniformis CDBB B11	11	Free-cyanide	Soil samples exposed to industrial wastewater, agrochemicals, hydrocarbons, heavy metals, gold, and silver mining industry tailings	(50)

5. Alkaliphilic with bioremediate of Halogenated compounds

Halogenated compounds are a significant class of xenobiotics contributing to pollution (53) and environmental contaminants. They are produced in large amounts and are utilized in various enzyme-catalyzed dehalogenation and degradation paths. Dehalogenase catalyzes the halogen cleavage from halogenated compounds, including haloalkanes, haloaromatics, and haloalkanoic acids (54, 55). Organohalides are significant chemical pollutants that pollute the ground and waterways (56). The use and misuse of these halogenated organic components in agriculture and industrial production result in widespread pollution (56).

Pollution with halogenated compounds poses serious health and environmental problems. Strategies involving sequence and effectivity-based screening predictors of the discovery of novel dehalogenases with enhanced activities. Experiments have been conducted to enrich growth states with the carbon required for screening of dehalogenase-producing bacteria, as shown in Table 2. *Pseudomonas putida* strains and *Alcaligenes xylosoxidans* grow in enrichment media with 2,2DCPA as ‘’ the only source of carbon and energy’’ (54). To isolate dehalogenase-producing *Anthrobacter* spp. strains, minimal salts were used, including a/ ß-halocarboxylic acids (58). Novel bacteria from Proteobacteria, *Bacillus*, and *Enterococcus* genera capable of a-halocarboxylic acid decomposition were isolated with dalapon (54). Chlorinated aliphatic acid culture media were used for dehalogenase production in *Rhizobium* sp. (54).

Alkaliphiles are extreme bacteria that can survive in alkaline ecosystems and are crucial in biotechnology and bioremediation. This includes dehalogenases, the different forms of this enzyme, which can be harmful (59) if not properly disposed of. There have also been several reports on the characterization of dehalogenases at different pH ranges, where *Pseudomonas putida* strain AJ1/23 D-2-haloacid dehalogenase displays maximum enzyme activity at around pH 9.5 and 50 °C (60), at the same time another group found that Pseudomonas also displayed activity on the “D,L-2-chloropropionate (D,L-2CP) and 2-chloroacrylate (2-CAA))” with the maximum efficiency of D-specific dehalogenase enzyme occurring at pH (61).

Table 2: Dehalogenase-producing bacteria and their source.

Bacteria	Dehalogenase/substrate	Source	Reference
Aminobacter sp. SA1	UN / D,L-2-chloropropionic acid	Soil	(62)
Labrys sp. Wy1	UN / 2,2-dichloropropionate	Rubber estate	(63)
Bacillus megaterium strain GS1	*UN* / L-2-haloalkanoic acids	Volcanic Soil	(64)
Serratia marcescens sp. SE1	UN / 2,2-dichloropropionate	Lake water	(65)

Arthrobacter sp. S1	UN / 2,2-dichloropropionic acid, D, L-2-chloropropionic acid, 3-chloropropionic acid	Soil	(58)
Enterobacter cloacae MN1	UN / 2,2-dichloropropionate	Seaside	(66)
Pseudomonas stutzeri DEH130	DehI/ D-2-chloropropropionate	Marine	(67)
Paracoccus sp. DEH99	Deh99 / DL-2-chloropropionate	Marine sponge	(68)
Arthrobacter sp. strain D2	UN / 2,2-dichloropropionate, d,l-2-chloropropionate	University Technology Malaysia Agricultural Area	(69)
Alcanivorax dieselolei B-5	DadB / 1,2-dichloropropane	Marine	(70)
Bacillus sp. strain EK1	UN / 2,2-Dichloropropionate, 2,3-dichloropropionate, d,l-2-chloropropionate	Marine Sediment of Danga Bay and East Coast of Singapore Island	(71)
Raoutella ornithilolytica	UN / 2,2-Dichloropropionate	Wastewater of Tioman Island	(72)
Bacillus cereus strain SN1	UN / 2,2-dichloropropionic acid	Ruminant animal waste	(73)
Ancylobacter aquaticus strain UV5	l-2-DhlB / 1,2-dichloroethane	Soil	(74)
Pseudomonas aeruginosa MX1	2,2-dichloropropionic	Seawater samples of the Malaysian coast Desaru	(75)
Pseudomonas halophila HX	2,2-dichloropropionic	Water sample of hypersaline Turkish Lake Tuz	(76)
Acinetobacter calcoaceticus BB9.2, *Pseudomonas plecoglossicida* BC14.3	2,2-dichloropropionic	Agricultural soil in Malang, Indonesia	(77)
Bacillus megaterium strain CTBmeg1	2,2-dichloropropionic	Water sample of hypersaline Turkish Lake Tuz	(51)
Bacillus subtilis strain H, *Bacillus thuringiensis* strain H2	2,2-dichloropropionic acid, D,L-2-chloropropionic acid, 3-chloropropionic acid	Water sample of hypersaline Turkish Lake Tuz	(59)
Bacillus megaterium BHS1	2,2-dichloropropionic	Water of Turkish Mavi Lake	(78)

DatA, a new haloalkane dehalogenase, was identified by Hasan *et al.* (2011). Its effectiveness was measured using 1, 3-Dibromopropane. The enzyme showed the highest activity at pH 9.8 (79). Fluoroacetate-degrading *Pseudomonas fluorescens* DSM (8341) and its dehalogenase enzyme were isolated and characterized (80). 2-haloacid dehalogenase (Deh99) from the marine bacteria *Paracoccus* sp. DEH99, predominates under high pH but is isolated from nonalkaline ecosystems (81). These microorganisms produce dehalogenases in an elevated pH environment and play a crucial role in the bioremediation of halogenated compounds in polluted environments. *Pseudomonas halophila* strain HX and *Bacillus megaterium* CTBmeg1 were found to degrade 2,2-dichloropropionic acid (20 mM) and (10 mM), respectively, under optimal conditions in Turkish Lake Tuz (pH 8.0) (76, 51). *Bacillus subtilis* strain H1 from Lake Tuz, Turkey, demonstrated its optimal ability to degrade 20 mM of 2,2-dichloropropionic acid at pH 8.0 (59), suggesting that enzymes generated by bacteria at extreme pH levels can be beneficial in highly alkaline conditions (82). The biodegradation of halogenated compounds by alkaliphilic bacteria is limited, but alkalotolerance *Bacillus megaterium* strain (BHS1) from Turkish (Mavi Lake) showed tolerance well to (40mM) 2,2-DCP at (pH 10) (78). These bacteria are environmentally friendly as they aid in removing and detoxifying pollutants from the ecosystem through dehalogenation (54). This review briefly describes the potential role of alkaliphiles in removing various environmental pollutants.

6. CONCLUSION

Alkaliphilic bacteria make a major contribution, some of which are at the cutting edge of modern applications of alkaliphilic products or their cellular capacity to decontaminate unique extreme environments. Due to the ability of alkaliphilic bacteria to catalyze the enzyme, they eliminate the halogen atom through the dehalogenation process. The presence of dehalogenase-producing microorganisms in soda lakes is important to be environmentally friendly to reduce pollutants represented by the halogenated compounds. Therefore, alkaliphiles are one of the microorganisms that are indispensable in bioremediation.

References

1. Aino K, Hirota K, Okamoto T, Tu Z, Matsuyama H, Yumoto I. Microbial communities associated with indigo fermentation that thrive in anaerobic alkaline environments. Front Microbiol. 2018; 9:2196.
2. Wiegel J. Anaerobic alkaliphiles and alkaliphilic poly-extremophiles. In: Horikoshi K, editor. Extremophiles Handbook. Tokyo: Springer; 2011.
3. Wang M, Zhang X, Shu Z, Wang Z, Tao Y, Lv C, et al. Bacterial and archaeal communities within the alkaline soda Langaco Lake in the Qinghai-Tibet Plateau. Ann Microbiol. 2022;72(1):33.
4. Preiss L, Hicks DB, Suzuki S, Meier T, Krulwich TA. Alkaliphilic bacteria with impact on industrial applications, concepts of early life forms, and bioenergetics of ATP synthesis. Front Bioeng Biotechnol. 2015; 3:75.
5. Kulkarni S, Dhakar K, Joshi A. Alkaliphiles: diversity and bioprospection. In: Das S, Dash HR, editors. Microbial diversity in the genomic era. London: Academic Press; 2019; 239-263.
6. Kipnyargis A, Kenya E, Khamis F, Mwirichia R. Spatiotemporal structure and composition of the microbial communities in hypersaline Lake Magadi, Kenya. F1000Research. 2024; 13:11.
7. Andreote AP, Dini-Andreote F, Rigonat J, Machineski GS, Souza BC, Barbiero L, *et al*. Contrasting the genetic patterns of microbial communities in soda lakes with and without cyanobacterial bloom. Front Microbiol. 2018; 9:319029.
8. Jeilu O, Gessesse A, Simachew A, Johansson E, Alexandersson E. Prokaryotic and eukaryotic microbial diversity from three soda lakes in the East African Rift Valley determined by amplicon sequencing. Front Microbiol. 2022;13:999876.
9. Wahhab BHA. Functional genomic analysis and molecular modeling of a novel alkalotolerant dehalogenase enzyme from Bacillus megaterium DehLBHSl [dissertation]. Malaysia: University of Technology Malaysia; 2021.
10. Uma G, Babu MM, Prakash VSG, Nisha SJ, Citarasu T. Nature and bioprospecting of haloalkaliphilics: a review. World J Microbiol Biotechnol. 2020; 36:1-13.
11. Melese H, Debella HJ. Comparative study on seasonal variations in physico-chemical characteristics of four soda lakes of Ethiopia (Arenguade, Beseka, Chitu and Shala). Heliyon. 2023; 9(5):e15693.
12. Wagaw S, Mengistou S, Getahun A. Review of anthropogenic threats and biodiversity assessment of an Ethiopian soda lake, Lake Abijata. Afr J Aquat Sci. 2019; 44(2):103-111.
13. Pellegrinetti TA, Cotta SR, Sarmento H, Costa JS, Delbaje E, Montes CR, *et al*. Bacterial communities along environmental gradients in tropical soda lakes. Microb Ecol. 2023; 85(3):892-903.
14. Borsodi AK, Szirányi B, Krett G, Márialigeti K, Janurik E, Pekár F. Changes in the water quality and bacterial community composition of an alkaline and saline oxbow lake used for temporary reservoir of geothermal waters. Environ Sci Pollut Res. 2016; 23:17676-17688.

15. Banciu HL, Sorokin DY. Adaptation in haloalkaliphiles and natronophilic bacteria. In: Seckbach J, Oren A, Stan-Lotter H, editors. Polyextremophiles: life under multiple forms of stress. Dordrecht: Springer; 2013. p. 121-178.
16. Sorokin DY, Berben T, Melton ED, Overmars L, Vavourakis CD, Muyzer G. Microbial diversity and biogeochemical cycling in soda lakes. Extremophiles. 2014;18:791-809.
17. ZeinEldin RA, Ahmed MM, Hassanein WS, Elshafey N, Sofy AR, Hamedo HA, *et al*. Diversity and distribution characteristics of viruses from soda lakes. Genes. 2023;14(2):323.
18. Boros E, Kolpakova M. A review of the defining chemical properties of soda lakes and pans: an assessment on a large geographic scale of Eurasian inland saline surface waters. PLoS One. 2018; 13(8): e0202205.
19. Xue Q, Zhao D, Zhang S, Zhou H, Zuo Z, Zhou J, *et al*. Highly integrated adaptive mechanisms in Spiribacter halalkaliphilus, a bacterium abundant in Chinese soda-saline lakes. Environ Microbiol. 2021; 23(11):6463-6482.
20. Li X, Yang M, Mu T, Miao D, Liu J, Xing J. Composition and key-influencing factors of bacterial communities active in sulfur cycling of soda lake sediments. Arch Microbiol. 2022; 204(6):317.
21. DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. The prokaryotes: other major lineages of bacteria and the archaea. Berlin: Springer; 2014.
22. Banciu HL, Muntyan MS. Adaptive strategies in the double-extremophilic prokaryotes inhabiting soda lakes. Curr Opin Microbiol. 2015;25:73-79.
23. Zorz JK, Sharp C, Kleiner M, Gordon PM, Pon RT, Dong X, *et al*. A shared core microbiome in soda lakes separated by large distances. Nat Commun. 2019;10(1):4230.
24. Pham VHT, Kim J, Chang S, Bang D. Investigating bio-inspired degradation of toxic dyes using potential multi-enzyme producing extremophiles. Microorganisms. 2023;11(5):1273.
25. Zhao D, Zhang S, Xue Q, Chen J, Zhou J, Cheng F, *et al*. Abundant taxa and favorable pathways in the microbiome of soda-saline lakes in Inner Mongolia. Front Microbiol. 2020; 11:1740.
26. Lebreton L, Slat B, Ferrari F, Sainte-Rose B, Aitken J, Marthouse R, *et al*. Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. Sci Rep. 2018; 8(1):1-15.
27. Nagata Y. Microbial degradation of xenobiotics. Microorganisms. 2020;8(4):487.
28. Alalwan HA, Kadhom MA, Alminshid AH. Removal of heavy metals from wastewater using agricultural byproducts. J Water Supply. 2020; 69(2):99-112.
29. Mamo G, Mattiasson B. Alkaliphiles: The versatile tools in biotechnology. In: Alkaliphiles in Biotechnology. Singapore: Springer; 2020. p. 1-51.
30. Kanekar PP, Joshi AA, Kulkarni SO, Borgave SB, Sarnaik SS, Nilegaonkar SS, *et al*. Biotechnological potential of alkaliphilic microorganisms. In: Biotechnology and Bioinformatics, Advances and Applications for Bioenergy, Bioremediation, and Biopharmaceutical Research. Oakville: Apple Academic Press; 2014. p. 249-280.
31. Singh S, Bajaj BK. Potential application spectrum of microbial proteases for clean and green industrial production. Energy Ecol Environ. 2017; 2:370-386.
32. Zampieri BDB, Nogueira EW, Quaglio OA, Brucha G. Overview of known alkaliphilic bacteria from bauxite residue. J Min Mech Eng. 2019; 1:41-50.
33. Wu H, Liao J, Zhu F, Millar G, Courtney R, Xue S. Isolation of an acid producing Bacillus sp. EEEL02: Potential for bauxite residue neutralization. J Cent South Univ. 2019; 26(2):343-352.
34. Piwowarska D, Kiedrzyńska E. Xenobiotics as a contemporary threat to surface waters. Ecohydrol Hydrobiol. 2022; 22(2):337-354.
35. Saha P, Rao K. Biotransformation of Reactive Orange 16 by alkaliphilic bacterium Bacillus flexus VITSP6 and toxicity assessment of biotransformed metabolites. Int J Environ Sci Technol. 2020; 17:99-114.
36. De Graaff M, Bijmans MF, Abbas B, Euverink GJ, Muyzer G, Janssen AJ. Biological treatment of refinery spent caustics under halo-alkaline conditions. Bioresour Technol. 2011;102(15):7257-7264.

37. Tambekar D, Dhundale V. Studies on the physiological and cultural diversity of bacilli characterized from Lonar lake (MS) India. Biosci Discov. 2012; 3(1):34-39.
38. Prasad AA, Rao KB. Aerobic biodegradation of Azo dye by Bacillus cohnii MTCC 3616; an obligately alkaliphilic bacterium and toxicity evaluation of metabolites by different bioassay systems. Appl Microbiol Biotechnol. 2013; 97:7469-7481.
39. Swaathy S, Kavitha V, Pravin AS, Mandal AB, Gnanamani A. Microbial surfactant mediated degradation of anthracene in aqueous phase by marine Bacillus licheniformis MTCC 5514. Biotechnol Rep. 2014;4:161-170.
40. Sarwade V. Biodegradation of Benzidibe by Alkaliphilic Strain Bacillus badius D1. IOSR J Environ Sci Toxicol Food Technol. 2014; 8(12):48-51.
41. Krishnamurthy NK. Response of Alkaliphilic Bacteria to Aromatic Amines. In: Bioprospects of Coastal Eubacteria: Ecosystems of Goa. Switzerland: Springer; 2015. p. 107-128.
42. Xue S, Kong X, Zhu F, Hartley W, Li X, Li Y. Proposal for management and alkalinity transformation of bauxite residue in China. Environ Sci Pollut Res. 2016; 23:12822-12834.
43. Nogueira EW, Hayash EA, Alves E, Lima CA, Adorno MT, Brucha G. Characterization of alkaliphilic bacteria isolated from bauxite residue in the southern region of Minas Gerais, Brazil. Braz Arch Biol Technol. 2017; 60:e17160215.
44. Rajkumari J, Bhuyan B, Das N, Pandey P. Environmental applications of microbial extremophiles in the degradation of petroleum hydrocarbons in extreme environments. Environ Sustain. 2019; 2:311-328.
45. Sáez LP, Cabello P, Ibáñez MI, Luque-Almagro VM, Roldán MD, Moreno-Vivián C. Cyanate assimilation by the alkaliphilic cyanide-degrading bacterium Pseudomonas pseudoalcaligenes CECT5344: mutational analysis of the cyn gene cluster. Int J Mol Sci. 2019; 20(12):3008.
46. Prabhakar Y, Gupta A, Kaushik A. Enhanced decolorization of reactive violet dye 1 by halo-alkaliphilic Nesterenkonia strain: Process optimization, short acclimatization and reusability analysis in batch cycles. Process Saf Environ Prot. 2019;131:116-126.
47. Medić A, Lješević M, Inui H, Beškoski V, Kojić I, Stojanović K, *et al*. Efficient biodegradation of petroleum n-alkanes and polycyclic aromatic hydrocarbons by polyextremophilic Pseudomonas aeruginosa san ai with multidegradative capacity. RSC Adv. 2020;10(24):14060-14070.
48. Sreedharan V, Saha P, Rao KV. Dye degradation potential of Acinetobacter baumannii strain VITVB against commercial azo dyes. Bioremediat J. 2021;25(4):347-68.
49. Sridharan R, Krishnaswamy VG, Archana KM, Rajagopal R, Kumar DT, Doss CGP. Integrated approach on azo dyes degradation using laccase enzyme and Cul nanoparticle. SN Appl Sci. 2021; 3:370.
50. Uribe-Ramírez D, Cristiani-Urbina E, Morales-Barrera L. Biodegradation of Free Cyanide by a new isolated alkaliphilic Bacillus licheniformis strain. Microbiol Res. 2023;15(1):33-49.
51. Akcay K, Kaya Y. Isolation, characterization and molecular identification of a halotolerant Bacillus megaterium CTBmeg1 able to grow on halogenated compounds. Biotechnol Biotechnol Equip. 2019; 33(1):945-953.
52. Sheth Y, Dharaskar S, Khalid M, Sonawane S. An environment friendly approach for heavy metal removal from industrial wastewater using chitosan based biosorbent: A review. Sustain Energy Technol Assess. 2021; 43:100951.
53. Siti Nurul F, Arman Shah AS, Mohamed Faraj Edbeib MF, Aliyu Adamu AA, Firdausi Aliyu FA, Roswanira Abdul Wahab RA, *et al*. Dehalogenase producing bacteria from extreme environment: a review. Malays J Microbiol. 2018;14(5):424-434.
54. Ang TF, Maiangwa J, Salleh A, Normi YM, Leow TC. Dehalogenases: from improved performance to potential microbial dehalogenation applications. Molecules. 2018; 23(5):1100.
55. Wang Y, Xiang Q, Zhou Q, Xu J, Pei D. Mini review: advances in 2-haloacid dehalogenases. Front Microbiol. 2021;12:758886.
56. Bhandari S, Poudel DK, Marahatha R, Dawadi S, Khadayat K, Phuyal S, *et al.* Microbial enzymes used in bioremediation. J Chem. 2021; 2021:8849512.

57. Goh KM, Kahar UM, Chai YY, Chong CS, Chai KP, Ranjani V, *et al.* Recent discoveries and applications of Anoxybacillus. Appl Microbiol Biotechnol. 2013; 97:1475-1488.
58. Bagherbaigi S, Gicana RG, Lamis RJ, Nemati M, Huyop F. Characterisation of Arthrobacter sp. S1 that can degrade α and β-haloalkanoic acids isolated from contaminated soil. Ann Microbiol. 2013; 63:1363-1369.
59. Oyewusi HA, Wahab RA, Kaya Y, Edbeib MF, Huyop F. Alternative bioremediation agents against haloacids, haloacetates and chlorpyrifos using novel halogen-degrading bacterial isolates from the hypersaline lake Tuz. Catalysts. 2020;10(6):651.
60. Smith J, Harrison K, Colby J, Taylor S. Determination of D-2-halopropionate dehalogenase activity from Pseudomonas putida strain AJ1/23 by ion chromatography. FEMS Microbiol Lett. 1989; 57(1):71-74.
61. Thasif S, Hamdan S, Huyop F. Degradation of D, L-2-chloropropionic acid by bacterial dehalogenases that shows stereospecificity and its partial enzymatic characteristics. Biotechnology. 2009; 8(2):264-269.
62. Amini S, Zulkifly A, Wen-Yong W, Huyop F. Molecular identification and characterization of a bacterium that has potential to degrade low concentration of haloalkanoic acid. Res J Microbiol. 2011; 6(6):552-559.
63. Wong WY, Huyop F. Characterization of a Labrys sp. strain Wy1 able to utilize 2, 2-dichloropropionate (2, 2-DCP) as sole source of carbon. Afr J Microbiol Res. 2011; 5(20):3282-3288.
64. Mashitah MS, Roslan D, & Huyop F**.** Molecular analysis of dehalogenase gene in genomic DNA of Bacillus megaterium strain GS1 isolated from volcanic area Gunung Sibayak. *Journal of Biological Sciences* 2011;11(5): 394-398.
65. Abel E, Ibrahim N, Huyop F. Identification of Serratia marcescens SE1 and determination of its herbicide 2, 2-dichloropropionate (2, 2-DCP) degradation potential. Malays J Microbiol. 2012; 8(4):259-265.
66. Nemati M, Abdulghader MF, Gicana RG, Lamis RJS, Ibrahim N, Hamid AAA, *et al.* Identification of putative Cof-like hydrolase associated with dehalogenase in Enterobacter cloacae MN1 isolated from the contaminated sea-side area of the Philippines. Malays J Microbiol. 2013; 9:253-259.
67. Zhang F, She YH, Banat IM, Chai LJ, Huang LQ, Yi SJ, Wang ZL, Dong HL and Hou DJ. Genomovar assignment of Pseudomonas stutzeri populations inhabiting produced oil reservoirs. *MicrobiologyOpen*, 2014; 3(4):446-456.
68. Zhang J, Xin Y, Cao X, Xue S, Zhang W. Purification and characterization of 2-haloacid dehalogenase from marine bacterium Paracoccus sp. DEH99, isolated from marine sponge Hymeniacidon perlevis. J Ocean Univ China. 2014; 13:91-96.
69. Alomar D, Abdul Hamid AA, Khosrowabadi E, Gicana RG, Lamis RJ, Huyop F, *et al.* Molecular characterization of monochloroacetate-degrading Arthrobacter sp. strain D2 isolated from Universiti Teknologi Malaysia agricultural area. Bioremediat J. 2014;18(1):12-19.
70. Li A, Shao Z. Biochemical characterization of a haloalkane dehalogenase DadB from Alcanivorax dieselolei B-5. PLoS One. 2014; 9(2): e89144.
71. Khosrowabadi E, Huyop F. Screening and characterization of several 2, 2-dicholoropropionic acid–degrading bacteria isolated from marine sediment of Danga Bay and east coast of Singapore island. Bioremediat J. 2014;18(1):20-27.
72. Maryam R, Fahrul H, Roswanira A. Identification and characterization of Raoutella ornithilolytica and determination of its herbicide 2, 2-dichloropropionate (2, 2-DCP) degradation potential. Malays J Microbiol. 2014; 10:249-254.
73. Ismail SNF, Wahab RA. and Huyop F. Isolation and identification of bacteria isolated from ruminant animal waste that able to degrade 2, 2-dichloropropionic acid (2, 2-DCP). *Malaysian Journal of Microbiology*, 2016; 155-163.
74. Kumar A, Pillay B, Olaniran AO. L-2-Haloacid dehalogenase from ancylobacter aquaticus UV5: sequence determination and structure prediction. Int J Biol Macromol. 2016; 83:216-225.

75. Abdulgader Edbeib MF, Wahab RA, Huyop F. Characterization of an α-haloalkanoic acid–degrading Pseudomonas aeruginosa MX1 isolated from contaminated seawater. Bioremediat J. 2016; 20(2):89-97.
76. Edbeib MFA. Characterization of 2, 2-DCP degrading ability of halotolerant bacteria and its dehalogenase structural analysis [dissertation]. Malaysia: University of Technology Malaysia; 2017.
77. Mulyawati AI. Analysis of 2, 2-DCP degrading bacteria isolated from a paddy field at a rural area in Malang, Indonesia. Malays J Microbiol. 2018; 14:674-679.
78. Wahhab BHA, Anuar NFSK, Wahab RA, Al-Nimer MS, Samsulrizal NH, Hamid AAA, *et al.* Characterization of a 2, 2-Dichloropropionic Acid (2, 2-DCP) Degrading Alkalotorelant Bacillus megaterium strain BHS1 Isolated from Blue Lake in Turkey. J Trop Life Sci. 2020;10(3):245-252.
79. Hasan K, Fortova A, Koudelakova T, Chaloupkova R, Ishitsuka M, Nagata Y, *et al.* Biochemical characteristics of the novel haloalkane dehalogenase DatA, isolated from the plant pathogen Agrobacterium tumefaciens C58. Appl Environ Microbiol. 2011;77(5):1881-1884.
80. Donnelly C, Murphy CD. Purification and properties of fluoroacetate dehalogenase from Pseudomonas fluorescens DSM 8341. Biotechnol Lett. 2009; 31:245-250.
81. Mamo G, Mattiasson B. Alkaliphilic microorganisms in biotechnology. In: Biotechnology of Extremophiles: Advances and Challenges. Cham: Springer; 2016. p. 243-272.
82. Mesbah NM. Industrial biotechnology based on enzymes from extreme environments. Front Bioeng Biotechnol. 2022; 10:8700