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Research Article

Characterization of a 2,2-Dichloropropionic Acid (2,2-DCP) Degrading Alkalotorelant *Bacillus megaterium* strain BHS1 Isolated from Blue Lake in Turkey

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ABSTRACT

An acid, 2,2-dichloropropionic acid (2,2-DCP) is an active ingredient in herbicide (Dalapon®). Using 2,2-DCP as a model substrate, an alkalotolerant bacterium was successfully isolated from the Blue Lake, Turkey. This bacterium is a potential bioremediation agent of recalcitrant xenobiotic halogenated compounds. This study aimed to prove the efficacy of the alkalotolerance *Bacillus megaterium* BHS1 in degrading 2,2-DCP as the sole source of carbon. Biolog GEN III system and 16S rRNA analysis were used for the identification of the bacterium. It was discovered that the strain BHS1 *is Bacillus megaterium*, and the bacterium that was observed to thrive in alkaline conditions (pH 7.0−14.0), supplemented with varying concentrations of 2,2-DCP (from 20 to 60 mM). Growth of strain BHS1 was exceptional in 40 mM of 2,2-DCP at pH 9, corresponding to a cell doubling time of 17.7 hour, whereas was fully inhibited at 50 mM 2,2-DCP. Since halogenated pollutants can make their way into highly alkaline environments, therefore, identifying threshold levels of strain BHS1 with respect to alkaline-tolerance and maximum level of 2,2-DCP may prove pertinent. This is to ensure that an optimal environment is created for the bacteria to degrade 2,2-DCP-contaminated water. In addition, this is the first study exploring a *Bacillus* species isolated from an alkaline environment adept in utilizing 2,2-DCP as a sole source of carbon. Hence, the ability of this strain to degrade other types of haloalkanoic acids constitutes a worthy future study.

Keywords: biodegradation, Bacillus, 2,2-dichloropropionic acid, pollutant degradation, alkaline water, alkalotolerance, alkaliphiles

Introduction

Xenobiotic pollutants that enter the environment are usually in limits that far exceed the permitted levels, thereby pose serious effects to the surrounding ecosystem. Such affected environments also include habitats of high temperature, pH, and salinity. Halogenated compounds are among the important class of xenobiotics, found in polluted water, as a result from human activities such petroleum refining and mineral ore mining. Likewise, chemically synthesized pollutants typically

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contain toxic halogen atoms that threaten the environment. Nonetheless, such hazardous substances can be eliminated in an eco-friendly manner, as well as safely through the process of bioremediation. The ideal bioremediation agents to detoxify such compounds often can be found at the very site of contamination. Consequently, information on the genetic, catabolic potential and functional diversity of the microorganisms must be made known so that the bioremediation process can be done effectively [1].

Halogen polluted in extreme environments are more challenging to bioremediate because this would require specially adapted microorganisms to carry out the detoxification reactions. Such environments tend to exist from xenobiotic activities, for instance, calcium carbonate kilns liberate effluents high in NaOH and $Ca(OH)_2$ which turn the surrounding water bodies into artificial hypersaline and alkaline systems (2, 3). There are also naturally occurring water bodies, such as soda lakes that are widely distributed throughout the world. Soda lake environment is an extreme environments with stable elevated pH and most productive aquatic environments on Earth [4]. This is because, over time the microorganisms exposed to such environmental stress began to adapt and give rise to alkali-tolerant organisms with the ability to grow at higher pH of between 9.0−10.0. Their persistence alkaline environments is the result of changes in their metabolism which now produce specific enzymes to help the microbes thrive, of which also include dehalogenases [5]. Dehalogenases are versatile enzymes that specifically cleave the carbon-halogen complex in organohalides contaminants [3, 6, 7].

Likewise, dehalogenase-producing alkaliphilic microorganisms are characterized by their unique metabolic pathways and are therefore, fit to degrade a wide range of toxic industrial pollutants and carbonate/bicarbonates [8]. Alkaliphiles products play a significant role in the degradation of xenobiotics and biogeocycling of inorganic compounds [9]. Alkaliphiles generally refer to microorganisms that grow well at pH values exceeding pH 9, often in the pH of 10–13 range [10], whereas, obligate alkaliphiles grow only at pH values of ~pH 9 and above. Interestingly, facultative alkaliphiles grow optimally under stringent alkaline conditions but do just as well at near neutral pH [11].

Alkaliphilic bacteria are known to be members of Gram-positive group belonging to genera such as *Bacillus, Micrococcus, Staphylococcus, Vagococcus, Arthrobacter, Corynebacterium, Streptomyces, Actinomyces* and *Clostridium* growing at high pH. But there studies have described Gram-negative alkaliphiles from the genera like *Aeromonas, Pseudomonas, Vibrio, Alkaliflexus, Nitrobacter, Nitritalea halalkaliphi-la,* photosynthetic Ectothiorhodospira, sulfur-oxidizing bacteria, methanotrophic bacteria and *Flavobacterium* genera [12, 13]. As a matter of fact, nearly 200 species of *Bacillus* species are reported to be moderately alkaliphilic/alkalitolerant response [14]. Although alkaline lakes contain an unusually high population of alkaliphilic microorganisms based on culture-independent genetic surveys [15], study on their potential to detoxify halogenated substances in extreme environment, remains lacking.

In this milieu, this study isolated a new bacterium from a soda lake called the Blue Lake and assessed the dehalogenation potential of the bacterium to degrade 2,2-dichloropropionic acid (2,2-DCP) as a model halogenated pollutant and the sole carbon source. 2,2-DCP has three carbons, with both chlorine atoms bonded to the α-carbon (C_α), by which when removed forms pyruvate as the product [16-20]. The study aims to objectively establish the biochemical characteristics and the degradation potential of the bacterium under highly alkaline conditions. The bacterium may present a viable source of dehalogenases effectively detoxify toxic substances under alkaline environments.

Material and Methods

Sample collection

Water samples from shallow shores (average depth 0.5 m) of the Blue Lake (Mavi Göl) located at Dereli/Giresun, Turkey (40º38’08”N; 38º22’56”E) were obtained aseptically. Water sample (10 mL) was collected in Late summer (August, 2019) in sterile bottles and stored at 4ºC until further investigations.

Growth media preparation and culture condition

Stock solution was prepared as 10× concentrated of basal salts consisting of $NaH_2PO_4.2H_2O$ (10.0 g/L), K_2HPO_4 (42.5 g/L), and $(NH_4)2SO_4$ (25.0 g/L). The trace metals salts solution was a 10× concentrated that contained nitriloacetic acid $C_6H_9O_6$ (1.0 g/L), $MgSO_4$ (2.0 g/L), $FeSO_4.7H_2O$ (120.0 mg/L), $MnSO_4.4H_2O$ (30.0 mg/L),

$ZnSO_4H_2O$ (30.0 mg/L), $CoCl_2.6H_2O$ (10.0 mg/L), in distilled water [21]. For growing bacteria, the liquid minimal media contain 10 mL of 10× basal salts and 10 mL of 10× trace metal salts per 100 mL of distilled water and were autoclaved (121ºC for 15 min, 15 psi). Bacterial isolates were cultivated aerobically at 30ºC on liquid minimal media containing various concentrations of 2,2-DCP (20 mM, 30 mM, 40 mM, 50 mM and 60 mM), with shaking (200 rpm) over a period of time (an approximately 5-7days).

Bacterial isolates from growth on 2,2-DCP were carefully isolated and a single strain was further investigated for growth in liquid minimal medium at 40 mM 2,2-DCP at different pH (7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0). The pH of each growth solution was adjusted from pH range 7-14 using Na_2CO_3 (1M), HCl (1M, 0.1M) and NaOH (1.0 M, 0.1 M). The turbidity of the growth culture was measured at A_{680nm} using Pye-Unicam SP1750 series spectrophotometer (Pye-Unicam Cambridge, UK).

Chloride ion released in growth medium

Degradation product was measured by determining the release of chloride ion during dehalogenation reaction using a colorimetric method [22]. Sample (1 mL) was added into 100 µL of 0.25M ammonium ferric sulphate in 9 M nitric acid and mixed thoroughly. Mercuric thiocyanate saturated ethanol (100 µL) was then added and the solution was mixed by vortexing. The color was allowed to develop for 10 minutes (min) and chloride ion liberation at A_{460nm}.

Identification of strain BHS1

The isolated strain was identified on the basis of their standard morphological and biochemical characteristics according to the Manual of Determinative Bacteriology [23].

Biolog TM Gen III Micro Plate Identification

Biolog GEN III test was carried out for species identification. The test included 94 phenotypic assays [24]. The test panel contains a phenotypic fingerprint which can be used to classify the level of the species of microorganisms. The isolate was grown on an agar medium, and then suspended at the required cell density in a special gelling inoculating fluid. The cell suspension (100 µL per well) was inoculated into the wells of the GEN III MicroPlate, followed by incubation of the Micro Plate to allow development of a phenotypic fingerprint. All these wells were colorless while they were being inoculated. During incubation the respiration increased in the wells due to the utilization of the carbon for growing. Therefore, reduction of the tetrazolium redox dye due to high respiration produced a purple color while the wells without respiration and the negative control wells remained colorless. The reference values of positive controls are applied for chemical sensitivity assays. The Biolog species library was used for matching the purple wells phenotypic fingerprint.

16S rRNA gene analysis

Genomic DNA was extracted from the isolated bacterium using Wizard Genomic DNA Purification Kit (Promega, USA) harvested during the late exponential phase culture. The polymerase chain reaction has been performed in order to amplify the 16S rRNA of the isolates. The universal primers used were Fd1(27F) (5-AGA GTT TGA TCC TGG CTC AG-3) and rP1(1422R) (5-GGT TAC CTT GTT ACG ACT T) [25]. The 16S rRNA gene amplification was conducted for 30 cycles, each of which was set as a first denaturation phase of 94ºC for 5 minutes, proceeded by denaturation of 94ºC for 1 minute, annealing of 55°C for 1 minute and final extension of 72°C for 10 minutes. For sequencing reactions, the PCR product was purified using the QIAquick PCR purification kit before sequencing. The DNA sequencing reaction was carried out by Apical Scientific (M) Sdn. Bhd.

Basic Local Allignment Search Tool for nucleotides (BLASTn) analysis for genus and species identification

The nucleotide sequence of 16S rRNA of strain BHS1 was analysed further via homology search using BLASTn programme at the website

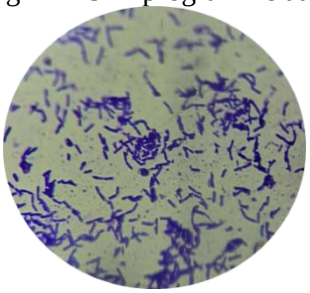

Figure 1. Strain BHS1 showing rod in shape and Gram-positive bacteria at 1000× total magnification.

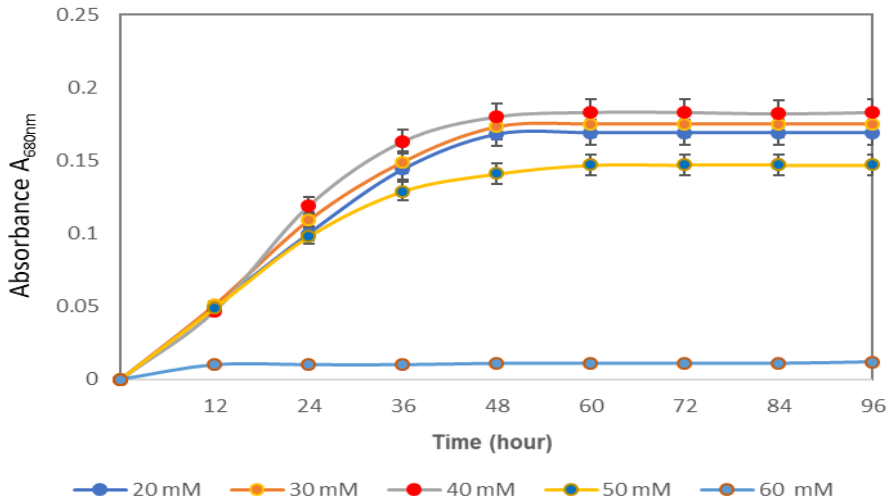

Figure 2. A growth profiles of strain BHS1 in liquid minimal media, containing 20, 30, 40, 50 and 60 mM 2,2-DCP as a sole source of carbon incubated at 30°C. The standard-deviations values were based on triplicates reading for each growth conditions.

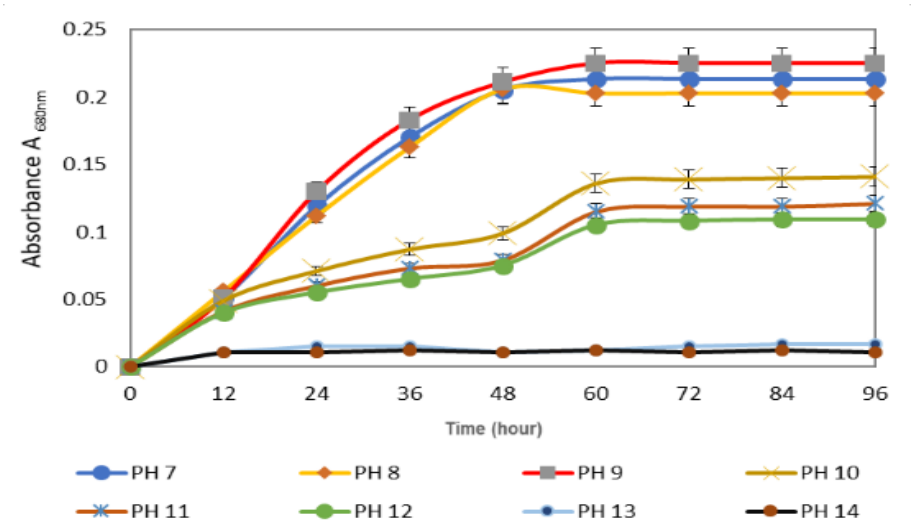

Figure 3. Growth curves showing growth of BHS1 in liquid minimal media at 30ºC supplied with 40 mM of 2,2-DCP as a carbon source. The pH range was set from pH 7 to pH 14.

of the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov). The 16S rRNA sequence was aligned using CLUSTALW.

Results and Discussion

Basic morphological observation and Gram-staining reaction

Morphological characteristics of the isolated bacterium strain BHS1 growing on solid minimal medium supplemented with 40 mM of 2,2-DCP incubated at 30°C after 4 days period was circular and white in colour. Strain BHS1 was Gram-positive, rod in shape (Figure 1) and it was a spore forming bacteria.

Growth of BHS1 in liquid minimal media containing 2,2-DCP as a carbon source

Strain BHS1 was grown in liquid minimal media containing different concentrations of 2,2-DCP ranged between 20-60 mM at 30°C. Strain BHS1 was able to grow in 50 mM 2,2-DCP but it was somewhat slower. The optimal growth conditions were observed at 40 mM 2,2-DCP (Figure 2). However, growth of BHS1 was completely inhibited at 60 mM 2,2-DCP. The calculated cell doubling time was approximately 18 h in the presence of 40 mM 2,2 DCP.

Growth of strain BHS1 at pH range from 7-14

Strain BSH1 was tested to grow at pH range

between pH 7 - pH 14, set at 30°C, in liquid minimal media with 40 mM 2,2-DCP as a carbon source. Figure 3 was a summary of the growth curves in all pH conditions. The cells can tolerate up to pH 12. However, growth at pH 13 and 14 was inhibited. The optimal pH for growth between pH 7 and 9 with cells doubling time of an approximately 17.7 h.

Halide ion assay

Halide ion assay was measured to check the utilization of 2,2-DCP as a sole source of carbon by the released of chloride ion in the growth medium. Growth experiment was set by growing BHS1 in the presence of 40 mM of 2,2-DCP at pH 9 over 96 hours of the growth period. The result showed the highest yield of chloride ion was observed amounted to approximately 0.89 µmol Cl^- /mL as shown in Figure 4. These findings indicated strain BHS1 was able to utilize 2,2-DCP as its sole of carbon and energy source suggesting this bacterium producing dehalogenase enzyme that catalyze 2,2-DCP.

Biolog™ GEN III

This system was applied to identify the properties of strain BHS1 up to the species level identification. Molecular methods such as 16S sequencing provide no information about the properties of the strain. However, Biolog's carbon source utilization technology identifies environmental microorganisms by producing a characteristic pattern or "metabolic fingerprint" using its 4 phenotypic tests with 71 carbon source utilization assays and 23 chemical sensitivity assays. The results for strain BHS1 has been examined using this technique. The strain BHS1 was identified as *Bacillus megaterium* as indicated in Table 1.

PCR amplification of 16S rRNA gene

A standard molecular analysis was carried out in support of the previous analysis using Biolog™ GEN III Microplate method. Using agarose ggel electrophoresis of the amplified DNA revealed a single band pattern at approximately 1.6 kb in size as expected (Figure 5).

The 16S rRNA gene sequence analysis of strain BHS1

The sequencing results were analysed by comparing to the nucleotide sequence with the sequences listed in the National Center for Biotechnology Information (NCBI) (ncbi.nlm.nih.gov). A total 1489 bp of nucleotides were aligned with other significant species using BLASTn application as summarised in Table 2. The results matched with Biolog™ GEN III that strain BHS1 belongs to the genus *Bacillus* and species, *megaterium*. Therefore, the bacterium was designated as *Bacillus megaterium* strain BHS1.

Discussion

An extreme environment demonstrating a great diversity of microbial life could be a good source of unexploited enzymes produced by these alkalophilic bacteria isolated from an alkaline lake. Current studies will focus on characterisation and molecular analysis of an alkaliphilic bacterium isolated from pristine Blue Lake or Mavi Göl in Turkey, that able to utilise 2,2-DCP as the sole source of carbon and energy. A 2,2-DCP is a model subtrate for this investigation in order to search for new kind of dehalogenase(s). 2,2-DCP was previously used as an active consituents in herbicide-DALAPON® to control undesirable weeds in an agriculture activity.

Strain BHS1 has demonstrated its ability to utilise 2,2 DCP as a sole source of carbon. The optimum growth conditions for strain BHS1 was assessed by many variables comprising 2,2 DCP concentrations from 20 to 60 mM and pHs from pH7.0 to pH14.0 at temperature 30°C with agitation rate at 200 rpm. Strain BHS1 grew well at 40 mM 2,2 DCP in agreement with the previous studies by Salim *et al*. (2011) [34] of *Bacillus megaterium* GS1 that was previously isolated from volcanic soil. Inability of strain GS1 to grow at 50 mM 2,2-DCP suggesting that this concentration was toxic to the cells. However, current study demonstrated that growth of strain BHS1 can withstand 2,2-DCP up to 50 mM.

Soda lakes contain high concentrations of sodium carbonate resulting in a stable elevated pH, which provide a unique habitat to a rich diversity of haloalkaliphilic bacteria. Both culture-dependent and -independent methods have aided the identification of key processes and genes in the microbial mediated carbon biogeochemical cycles in soda lakes. In order to survive this extreme environment, haloalkaliphiles have developed various bioenergetic and structural adaptations to maintain pH homeostasis and intracellular osmotic pressure [35]. The isolated BHS1 strain would lead to the isolation of a number of extremozymes, which al-

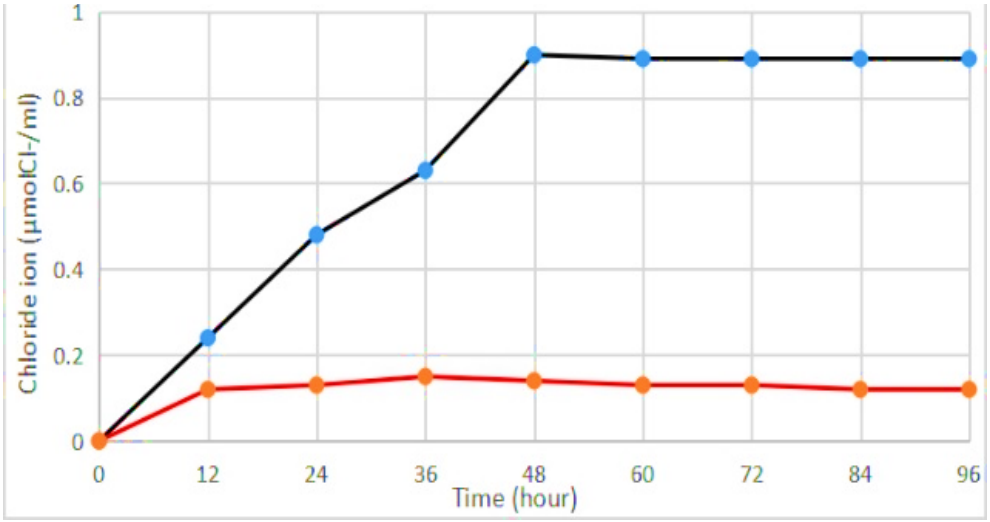

Figure 4. Measurement of maximum chloride ion released in 40 mM 2,2-DCP minimal growth medium. The maximum amount of chloride ion released was observed after 48 h (0.89 µmol Cl^-/mL). Negative control was a minimal medium supplied with 2,2-DCP without strain BHS1.

Table 1. A complete biochemical test for strain BHS1 using Biolog™ GEN III Microplate biochemical analyses.

Properties	Results	Properties	Results
D-trehalose	+	Myo-Inositol	−
D-cellobiose	−	Glycerol	−
Sucrose	+	D-Glucose-6-PO4	−
pH 6	+	D-Fructose-6-PO4	−
α-D-lactose	−	D-Aspartic Acid	−
β-methyl-D-glucoside	−	D-Serine	−
N-acetyl-β-D-mannosamine	−	Gelatin	−
α-D-Glucose	+	Glycyl-L-Proline	−
D-Mannose	-	L-Alanine	−
D-Fructose	+	L-Arginine	−
D-Galactose	-	L-Aspartic Acid	−
3-Methyl Glucose	-	L-Glutamic Acid	−
D-Fucose	-	L-Histidine	−
L-Fucose	-	L-Pyroglutamic Acid	−
L-Rhamnose	-	L-Serine	−
N-acetyl-D-Galactosamine	−	D-Galacturonic Acid	−
N-acetyl Neuraminic Acid	-	L-Galactonic Acid Lactose	−
1% NaCL	+	D-Gluconic Acid	+
4% NaCL	+	D-Glucuronic Acid	−
Inosine	-	Glucuronamide	−
1% Sodium Lactate	+	Mumic Acid	−
D-sorbitol	−	Quinic Acid	−
D-arabitol	−	D-Saccharic Acid	−
Tetrazolium Violet	+	L-lactic acid	−
p-Hydroxy-Phenlacetic Acid	−	Citric acid	−
D-lactic acid Methyl ester	−	α-Keto-Glutaric Acid	−
Bromo-Succinic Acid	−	D-Malic Acid	−
Lithium Chloride	+	Acetoacetic Acid	−
Potassium Tellurite	+	Propionic acid	−
α-hydroxy-D,Lbutyric acid	−	Acetic acid	−
A-Keto-Butyric Acid	−	Formic Acid	−
Sodium butyrate	+		

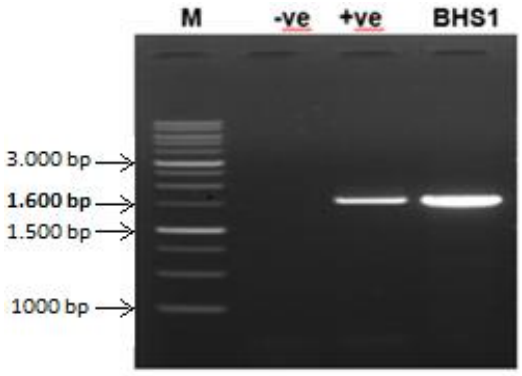

Figure 5. Amplification of the 16S rRNA gene of strain BHS1 showing 1.6 kb DNA fragment on an agarose gel (1%). Lane M: 1 kb DNA Ladder (Promega); Lane (-ve) is negative control, PCR mixture without a primer; Lane (+ve), positive control of an amplification of the 16S rRNA gene of *E. coli* strain JM109, and Lane BHS1, an amplification of the 16S rRNA gene of strain BHS1 (1.6 kb band).

low the cell to perform enzymatic reactions at these extreme conditions. These enzymes potentially contribute to biotechnological applications.

Strains BHS1 is a facultatively alkaliphilic microorganism as demonstrated to grow in both pH 7 and pH 9. The cytoplasmic membranes of alkaliphiles have a higher concentration of membrane lipid. Like other *Bacillus* spp., the alkaliphilic bacilli possess a diverse group of branched chain fatty acids in their membrane lipids and also contain variable amounts of saturated and unsaturated straight chain fatty acids. Almost all alkaliphilic bacilli have squalene and dehydro- or tetrahydro-squalene with substantial quantities of C40 and smaller quantities of C50 isoprenoids in the neutral lipid fraction of cell membrane along with phosphatidyl-ethanolamine and phosphatidyl-glycerol [36]. Facultative alkaliphiles contained much lower content of unsaturated fatty acids and branched chain fatty acids than the obligate alkaliphiles. Studies indicate that fatty acid composition of obligate alkaliphile membrane is a factor pre-cluding growth at low pH values [37]. Facultative alkaliphiles lack desaturase activity and lose their ability to grow at near neutral pH when supplemented with an unsaturated fatty acid [37].

The ability of *Bacillus* to adapt to extreme environmental conditions may be revealed by genetic studies such as full genome sequence. The genome studies will reveal numerous adaptations that contribute to alkaline pH homeostasis for instance to balance transporters and enzymes that promote proton capture and retention on to survive in an alkaline environment and to maintain an intracellular neutral pH and osmotic pressure. Studies by Akcay and Kaya [17] revealed *B. megaterium* CTBmeg1 from a hypersaline environment of Lake Tuz, with optimum condition pH 8.0, NaCl 20% able to survive in the presence of 10 mM 2,2-DCP. While other reported strains of the same genus were able to utilise dichloromethane as a source of carbon for growth [38] and degradation of monochloroacetic acid [MCA] by *Bacillus* sp. TW1 [39] with low concentration of MCA. Strain BHS1 showed cell doubling was approximately 18 h in the presence of 40 mM 2,2 DCP. While in pH 7 and 9 at 40 mM 2,2 DCP strain BHS1 cells doubling time was an approximately 17.7 h. Growth of BHS1 with shorter doubling time at pH 7-9 reflects the ability of strain BHS1 with adaptation for their alkalinity environment compared to the previous studies [17] with the cells doubling time of 26.41 h at pH 8 with 10 mM of 2,2-DCP using *Bacillus megaterium* strain CTBmeg1 isolated from a hypersaline environment.

The results of this study highlighted important findings of BHS1 strain that can be cultivated in the laboratory at pH 9-12 in the presence of 2,2-DCP as a carbon source and releasing halide ions in the growth medium. This gives the possibility in the future, to exploit this isolate in the biodegradation and intrinsically may serve as a cost-effective means to clean up the environment. In addition, their role in biotechnological and commercial are significant. Most alkaliphiles have optimal growth at pH 10, which is the most significant difference from well-investigated neutrophilic microorganisms. These alkaliphilic microorganisms can grow in such extreme environments because their internal pH is maintained at 7.5–8.5, despite a high external pH of 8–13. Therefore, one of the key features in alkaliphily is associated with the cell surface, which discriminates and maintains the intracellular neutral environment separate from the extracellular alkaline environment. Most alkaliphiles have two mechanisms of cytoplasmic pH regulation [40]. To allow this, the cell wall structure of this bacteria may contain acidic polymers that function as a negatively charged matrix and may reduce the pH value at the cell surface [41]. The surface of the cytoplasmic membrane

Table 2. BLASTn analysis showing possible identity of strain BHS1. The identity of strain BHS1 matched with *Bacillus megaterium* NBRC1530

Identical genus and species to strain BHS1	Accession No.	Identity (%)	Reference
Bacillus megaterium NBRC15308	NR112636.1	100.0	[26]
Bacillus aryabhattai B8W22	NR115953.1	99.80	[27]
Bacillus simplex NBRC12720	NR115603.1	98.70	[28]
Bacillus qingshengii strain G19	NR133978.1	98.04	[29]
Bacillus flexus strain IFO15715	NR135732.1	98.93	[30]
Bacillus iocasae strain S36	NR158045.1	95.00	[31]
Bacillus pocheonensis strain Gsoil 420	NR041377.1	95.00	[32]
Bacillus circulans strain NBRC13626	NR112632.1	95.00	[33]

must presumably be kept below pH 9, because the cytoplasmic membrane is very unstable at alkaline pH values [pH 8.5–9.0] much below the pH optimum for growth [40]. The second strategy to maintain pH represented with homeostasis consists of the use of the Na^+/H^+ membrane antiporter system [Dc dependent and DpH dependent], the K^+/H^+ antiporter, and ATPase driven H^+ expulsion [40].

The results of chloride ion released shown in Figure 4 indicated that strain BHS1 can grow at 40 mM 2,2-DCP, suggesting the bacterium has the ability to produce dehalogenase enzyme. The rate of chloride ion released was proportional to the growth of strain BHS1 in the 2,2-DCP minimal media. Similar observation was seen in other studies as reported earlier [42,43]. Chloride ions released into the growth medium was due to the hydrolysis of 2,2-DCP inside the cells resulted in chloride ion released outside the cells [42, 43] [44,45]. The product of 2,2-DCP dehalogenation resulted in pyruvate, that was essential for cell growth. Pyruvate was further catabolized to supply carbon and energy required by cells to grow. Therefore, the high concentration of released chloride ion may indicate the high demand of pyruvate as required for a rapid growth [45]. The tolerance to the high concentration of 2,2-DCP may be related to frequent exposure of the organic halide at the sampling location especially in the presence of naturally occurring halogenated compound [45, 46]. Strain BHS1 can utilise 2,2-DCP due to the fact that such organism should produce dehalogenase in response to the presence of halogenated substances. This substance must be non-toxic to the organism at normal intracellular concentrations. The substance can enter the cell passively or by active transport to reach the site of dehalogenase activity. The product of dehalogenation reaction should simply be converted to an intermediate of the organism's central metabolic pathways [47].

The 16S rRNA genes considered the most predominantly used molecular marker for bacterial identification [48]. The 16S rRNA results of the current study was in agreement with Biolog GEN III Micro Station System and confirmed the identity of strain BHS1 as *Bacillus megaterium* [GeneBank accession number NR_112636.1]. BLASTn results revealed that strain BHS1 is closely related to *Bacillus alcalophilus, Bacillus cohnii, Bacillus gordonae, Bacillus sphaericus, Bacillus garadherens, Bacillus subtilis* and *Bacillus clarkia*, all of which were isolated from soda lakes [49]. This supports our study, during which we were also able to isolate *Bacillus* genus from the same environment. The previously reported halotolerant halogen-degrading *Bacillus* sp., *Bacillus zhanjiangensis, Bacillus cohnii*, and *Bacillus halmapalus* were isolated from Indian Lonar Soda Lake which showed an optimum growth at pH 7.5 and 37°C [50].

Conclusion

An important outcome discovered by this study is the ability of the isolate to utilize 2,2DCP at high concentration of 40 mM at pH 9. This might be useful in the future to exploitation of the bacterium for *in situ* efforts to detoxify halogen-contaminated highly alkaline environments. Pertinently, the findings of this study further add to the limited body of knowledge with regards to the halogen-degrading halotolerant bacteria.

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