Universitat Autònoma de Barcelona

EXPLORING THE REDUCTIVE DEHALOGENASE DEGRADING 1,2-DICHLOROETHANE IN *DEHALOGENIMONAS ALKENIGIGNENS* STRAIN BRE15M AND ITS PROTEOME GROWING WITH DIFFERENT SUBSTRATES

JUDIT GALMES MELO

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TITLE

Exploring the reductive dehalogenase degrading 1,2-dichloroethane in *Dehalogenimonas alkenigignens* strain BRE15M and its proteome growing with different substrates

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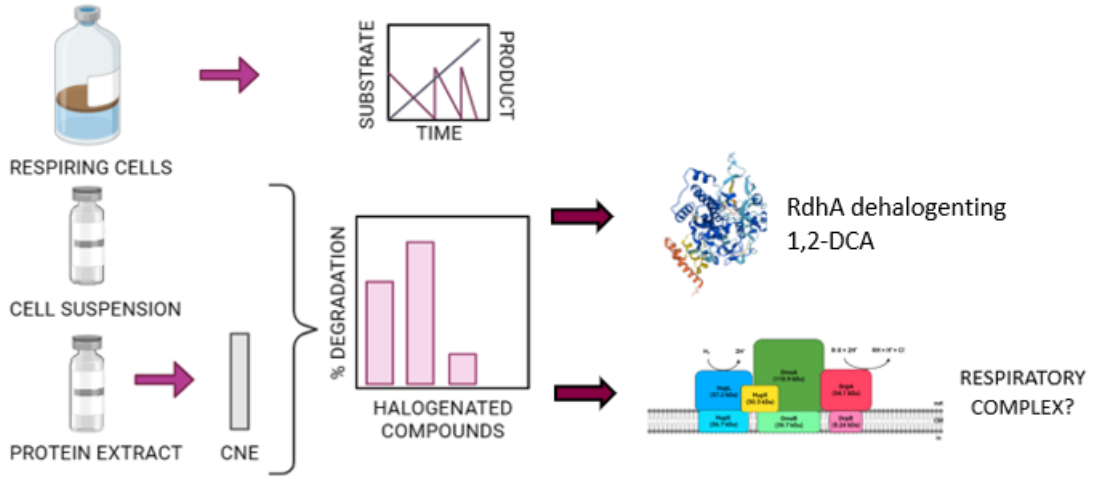
ABSTRACT

The strain *Dehalogenimonas alkenigignens* BRE15M is a strictly anaerobic, slow-growing microorganism that depends on halogenated alkanes as terminal electron acceptors and hydrogen as the electron donor for anaerobic respiration. In the last three decades, there is a growing interest in learning more about organohalide respiring bacteria and its biochemistry, such as the identity and interactions of the respiratory proteins. Here, we demonstrate the ability of strain BRE15M to grow while degrading 1,2-dichloroethane (1,2-DCA) and 1,2-dichloropropane (1,2-DCP). Enzymatic assays with cells from these two degrading cultures transformed 1,2-DCA, 1,2-DCP, 1,2-dibromoethane (1,2-DBA) and 1,1,2-trichloroethane (1,1,2-TCA), although the transformation of 1,2-DCA with cells previously growing with 1,2-DCP is almost negligible. These results suggest that this strain is only capable of respiring vicinal halogenated alkanes via dihaloelimination. To investigate the reductive dehalogenase (RdhA) involved in the degradation of 1,2-DCA we performed a comparative proteomics with strain BRE15M growing with 1,2-DCA, 1,2-DCP and 1,2-DBA. Our results suggests that the RdhAs BRE15M_v1_10075, BRE15M_v1_160015 and BRE15M_v1_20299 could be the responsible for the degradation of 1,2-DCA. Moreover, we observe a change in the expression pattern of the most abundant RdhAs with *Dehalogenimonas* strain BRE15M growing with chlorinated substrates compared to brominated ones. Finally, we performed enzymatic assays with slices of

the Clear Native Gel Electrophoresis of cells grown with 1,2-DCA that shows that the activity was mainly located in the slices >242 kDa. This, indicates that the RdhA responsible of 1,2-DCA degradation can form part of the respiratory complex because the RdhA subunit is a 50-60 kDa protein.

GRAPHICAL ABSTRACT



KEYWORDS

1,2-dichloroethane, 1,2-dichloropropane, 1,2-dibromoethane, Clear Native Electrophoresis, RdhA.

SYNOPSIS

There is an increasing interest in investigating organohalide-respiring bacteria (OHRB) for groundwater bioremediation. This study enhances our understanding of the diverse substrates that a *Dehalogenimonas* strain can degrade and provides valuable insights into the biochemistry for 1,2-DCA degradation.

1. INTRODUCTION

We call organohalide compounds those organic compounds that contain chlorine, fluorine, bromine, or iodine bonded to a carbon atom[1]. These compounds can be originated from both natural and anthropogenic sources, with the latter being the primary contributors. On one hand, natural sources include geothermal activities, volcanic emissions, forest fires, the oxidation of soil organic matter, and the activities of living microorganisms[2]. On the other hand, industrial anthropogenic sources include substances such as solvents, adhesives, plastics, plasticizers, metal degreasing agents, or intermediates for chemical synthesis. Additionally, halogenated compounds are present in pesticides, herbicides, flame retardants, and pharmaceuticals[3]. Once

released into the environment due to their inadequate storage or disposal practices, these substances can present a risk to both human health and the ecosystem[4]. Commonly, these contaminants vertically leach into the groundwater and continue migrating until they reach the water table where they accumulate in the form of dense non-aqueous phase liquid (DNAPL). From the water table onward, they begin to migrate horizontally, forming contamination plumes.

Based on a combination of the toxicity, potential for human exposure, and detection frequency of contaminants in the United States, the Agency for Toxic Substances and Diseases Registry makes a list[5] in which 128 organohalide compounds are registered. Specifically, in this study, we focused on the biodegradation of 1,2-dichloroethane (1,2-DCA), 1,2-dichloropropane (1,2-DCP) and 1,2-dibromoethane (1,2-DBA). 1,2-DCA and 1,2-DBA ranked at 92 and 39 on the list respectively and have gained the interest of the scientific community in the last years[5]. Additionally, the International Agency for Research on Cancer (IARC) catalogue 1,2-DCA and 1,2-DBA as probable human carcinogens. Moreover, there is sufficient evidence to classify 1,2-DCA and 1,2-DBA as carcinogens in experimental animals and there is limited evidence to classify 1,2-DCP as carcinogen in experimental animals[6,7,8,9]. Furthermore, the levels of 1,2-DCA in drinking water (≤ 3 µg/L) are regulated by the Spanish government[10].

Bioremediation has demonstrated to be a suitable and environmentally friendly strategy for degrading organic pollutants in groundwater. In the case of organohalide compounds, they can be degraded by aerobic or anaerobic microorganisms. However, due to the typical reducing conditions detected in aquifers, anaerobic bioremediation is recommended. In this condition, the anaerobic reductive dehalogenation can occur. There are two main pathways of reductive dehalogenation: first, hydrogenolysis, where two electrons are consumed in the process of substituting a halogen atom with an hydrogen atom, and second, dihaloelimination, where two electrons are consumed in the process of substituting simultaneously two adjacent halogenated atoms at the same time forming a carbon-carbon double bond[11].

Several bacteria have demonstrated the ability to link anaerobic reductive dechlorination to energy metabolism, and these are referred to as organohalide-respiring bacteria (OHRB)[11]. The OHRB can be broadly classified into two types: (1) obligate OHRB that can solely use halogenated compounds as electron acceptors and (2) facultative OHRB capable of utilizing non-halogenated compounds as electron acceptors. We identified obligate OHRB from the Chloroflexi phylum, including the genera *Dehalococcoides*, and *Dehalogenimonas,* among others[3]. Also, from the phylum Firmicutes, we found the genera *Dehalobacter* as a relevant

obligate OHRB[12]. For the facultative OHRB we found the genus *Desulfitobacterium, Geobacter*, *Desulfuromonas*, *Anaeromyxobacter* and *Sulfurospirillum*[13].

In this study, we used as a model organism, a OHRB of the genus *Dehalogenimonas*. On the one hand, some strains of *Dehalogenimonas* present a higher substrate specialization because they are only capable of transforming vicinal halogenated alkanes via dihaloelimination[3]. The *Dehalogenimonas* genus have been described to degrade some halogenated compounds such us 1,2,3-trichloropropane, 1,2-DCA, 1,2-DCP and 1,2-DBA via dihaloelimination[14]. On the other hand, few strains can transform these compounds via hydrogenolysis, for example, the *Dehalogenimonas sp.* WBC-2 strain can transform trans-dichloroethylene into vinyl chloride (VC)[15], and *'Candidatus Dehalogenimonas etheniformans*' converts VC to ethane[16].

The biotic reductive dehalogenation of pollutants is catalyzed by enzymes known as reductive dehalogenases, RDase when biochemically characterized and RdhA otherwise. Numerous studies indicate that these enzymes maintain conserved residues in their amino acid sequence, potentially indicating homology[13]. These conserved sequences include a twin-arginine translocation signal, TAT signal, suggesting the exportation of the RdhA protein to integrate into the membrane. Additionally, two iron-sulfur cluster binding motifs, characteristic structures for electron transfer in certain proteins, and cobalamin cofactors attached to the active center are reported. However, motifs for binding corrinoids in the amino acid sequence of RdhAs are rarely observed. Also, it has been described that the protein RdhB is the RdhA membrane-binding protein and within the OHRB genome, the genes *rdhA* and *rdhB* are encoded in the same operon.

The OHRB bacteria commonly accept H_2 as the sole electron donor, oxidizing it to $2H^+$. Considering that two electrons are released in the process and need to reach the contaminant to reduce it, there should be an electron transfer pathway, in other words, a respiratory chain. Several studies have identified elements that might play a role in this respiratory chain, such as dehydrogenase proteins or electron transfer components[4,17]. However, their mechanisms and structure are remain incompletely understood. To begin with, two types of respiratory chain have been described. The first, dependent on quinones, is found in genera such as *Dehalobacter* and *Sulfurospirillum.* The second, is a quinone-independent respiration found in genera such as *Dehalococcoides* or *Dehalogenimonas*[4,17,18]. In the quinone dependent model, the electron transport occurs through a quinol-quinone redox cycle, incorporating and releasing two hydrogens and two protons. In the quinone independent model, which is the one of interest to us, electron transport occurs through a membrane protein complex (Figure 1)[4].

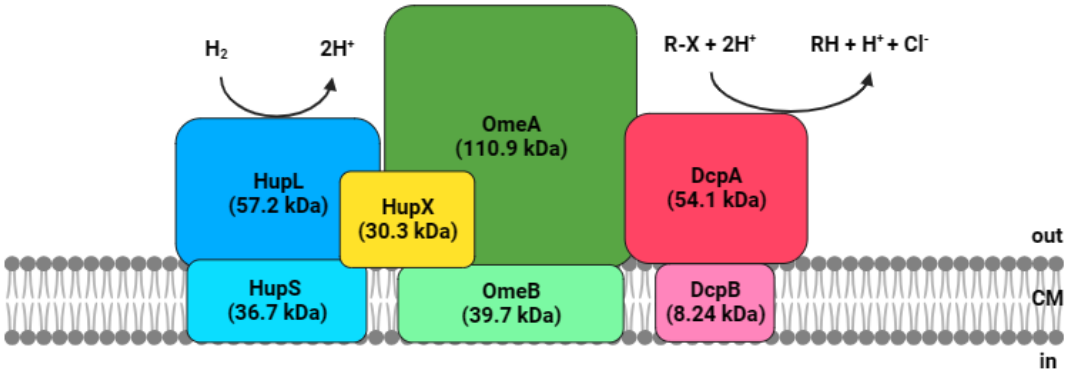


Figure 1. Proposed model for the organohalide respiratory chain independent of quinones in *Dehalogenimonas alkenigignens* BRE15M strain[4]. Taken from[4]. The respiratory chain starts with three hydrogen uptake hydrogenases (HupL, HupS y HupX). Among them, HupL is the hydrogen uptake hydrogenase large subunit, HupS is the hydrogen uptake hydrogenase small subunit, and HupX is an iron-sulfur protein. Furthermore, we find the proteins OmeA, which is an organohalide-respiration-involved molybdenum enzyme, and OmeB, serving as the membrane anchoring subunit of OmeA. Finally, we find the enzymes RdhA (DcpA) and RdhB (DcpB) previously mentioned.

The different RdhA enzymes have different substrate specificities, because of that, in an organism, enrichment culture, or contaminated site, the range of transformed halogenated electron acceptors depends on the type and quantity of reducing dehalogenases. So far, the described RdhA enzymes capable of degrading 1,2-DCA include the DcaA dehalogenase from *Desulfitobacterium dichloroeliminans* DCA1[19] and the VcrA from *Dehalococcoides*[20].

In previous studies in our research group, a culture enriched in bacteria of the genus *Dehalogenimonas* was obtained from sediments of the Besós River (Barcelona, Spain). This culture is capable of dechlorinating alkanes with adjacent chlorines through dihaloelimination[3]. Furthermore, in other previous studies, the genome of this enriched culture was sequenced, revealing a new strain of *Dehalogenimonas* named *Dehalogenimonas alkenigignens* BRE15M. Additionally, a respiratory complex independent of quinones was described for this new strain in a culture growing with 1,2-DCP as a substrate[4]. Taking all this into consideration, for this new study, the following objectives pursued are:

- To monitor the degradation of 1,2-DCA and 1,2-DCP during the growth of *Dehalogenimonas alkenigignens* BRE15M.
- To study the expression of RdhAs in the culture of *Dehalogenimonas alkenigignens* BRE15M growing either with 1,2-DCA, 1,2-DCP or 1,2-DBA.
- To identify the different halogenated substrates that can be degraded by the reductive dehalogenases expressed during the growth of *Dehalogenimonas alkenigignens* BRE15M with 1,2-DCA and 1,2-DCP.
- To ascertain if the RdhA, responsible of 1,2-DCA degradation forms part of the respiratory complex described previously in *Dehalogenimonas alkeniginens* BRE15M.

2. MATERIALS AND METHODS

2.1. Chemicals

Analytical grade reagents, purchased at the highest purity levels (>98%) have been used throughout this project. All the halogenated compounds used were acquired from Sigma Aldrich.

2.2. Preparation of the anaerobic culture media and bacterial cultivation

In previous studies, a sediment-free culture that contained a *Dehalogenimonas* was obtained from sediments of the Besòs river (Barcelona, Spain)[4]. This culture was used as inoculum in this study. The microcosms consisted of 70 mL of a defined medium hermetically closed in 100 mL glass bottles with teflon-coated butyl rubber stoppers and aluminum crimp caps. Briefly, the medium for each microcosm contained 5 mM sodium acetate as a carbon source, a trace metal solution, a mineral solution, and resazurin sodium salt as an oxidation-reduction indicator. Additionally, it included sodium bicarbonate as a buffer (pH=7), sodium citrate as a reducing agent, and a concentrated solution of vitamins. The detailed composition of the medium can be found in Supplementary Information Table S1 and Table S2. Each microcosm was gassed with a mixture of N2/CO2 (4:1 v/v) up to an overpressure of 0.2 bar and with H2 up to an overpressure of 0.4 bar. The halogenated compounds were added through the septum with a syringe (Hamilton) from a stock solution diluted with acetone (Scharlau) to obtain a desired concentration. Hydrogen works as the electron donor and the halogenated compounds as electron acceptors in the respiratory chain of *Dehalogenimonas* BRE15M. The cultures were transferred to fresh medium during the exponential phase of contaminant consumption by inoculating 5 mL of culture from an active culture bottle and subsequently the specific halogenated substrate was added to the desired concentration. All the microcosms were cultivated in darkness, under static conditions, and at a temperature of 25°C.

The concentration of the halogenated compounds and their metabolites were measured by gas chromatography at time zero, and then approximately every 24 hours. When the contaminant was fully consumed, the microcosms were spiked with 50 µM of the corresponding halogenated compound. Three replicates of the experimental bottles were monitored for each contaminant. Abiotic controls, that consisted of microcosms containing media and the halogenated compound but without *Dehalogenimonas*, were performed and monitored in duplicates.

2.3. Preparation of cell concentrate and protein crude extracts

For harvesting the cells, 140 mL of initial culture that had consumed approximately 2100 μM of 1,2-DCA or 1,2-DCP was centrifuged repeatedly under anaerobic conditions and concentrated 10 times fold as described previously[4]. Detailed cell harvesting protocol is described in the Supplementary Information Cell harvesting protocol. These cell concentrates were employed for the dehalogenases activity assays.

To obtain the protein crude extract, approximately 490 mL of a culture growing with 1,2-DCA that had consumed approximately 1250 μM of the substrate, was harvested and concentrated approximately 817 times fold, to obtain a final volume of 600 μL of cell concentrate. After centrifugation, mechanical cellular lysis and solubilization of membrane proteins was performed into an anaerobic glovebox. Firstly, the cell concentrate content (600 μL) was placed into and Eppendorf tube with 0.5 mm silica beads and initiated six cycles of 40 seconds of shaking in a Multi-tube holder, Vortex Genie 2 model G560E (Scientific Industries; New York, USA), alternating with one minute of resting on ice. Secondly, *n*-dodecyl β-D-maltoside (DDM) was added to a concentration of 0.1% w/v, and the mixture was incubated on ice for 1 hour with gentle shaking. Lastly, the sample was centrifugated at 2000 x *g* for 1 minute and transferred to a new Eppendorf tube of 1.5 mL using a pipette tip that discarded the silica beads and centrifuged at 15000 x *g* for 15 minutes to obtain the protein crude extract in the supernatant which was collected in a new tube.

2.4. Dehalogenase activity assays

We tested the ability of the RdhA expressed during growth of *Dehalogenimonas* BRE15M with 1,2-DCA or 1,2-DCP to reduce alternative halogenated compounds using cell concentrates.

Activity assays were prepared in anaerobic conditions inside an anoxic glovebox. Glass vials of 10 mL were utilized, each containing 2 mL of the assay buffer solution which we referred here as 'Master Mix'. The Master Mix contained 200 mM potassium acetate (pH 5.8), 2 mM methyl viologen, artificial electron donor, and 4 mM titanium citrate (4 mM with respect to titanium) for the cell concentrate activity assays. To each experimental vial containing 2 mL of Master Mix we added 200 μL of cell concentrate. Next, each vial was closed with teflon-coated butyl rubber stoppers and aluminum crimp caps. Afterwards, 100 μM of each contaminant was added with a syringe through the septum (Hamilton).

For the cell activity tests, we tested the biodegradability of 1,2-DCA, 1,2-DCP, 1,2-DBA, chloroform (CF), 1,1,1-trichloroethane (1,1,1-TCA), 1,1,2-trichloroethane (1,1,2-TCA),

trichloroethylene (TCE), and tetrachloroethylene (PCE). We also added two controls prepared as the experimental samples: first, the abiotic control, which includes the Master Mix without cell concentrate and spiked with 100 μM of all studied contaminants to observe possible abiotic transformations, volatilization losses and to confirm the biotic transformation observed in the experimental vials. Second, the inoculum control, that was not spiked with any contaminant but contained the cell concentrate, to observe any possible traces of contaminants or products that might have been carried over from the experimental bottles to the activity assay vials. The inoculum control was used to correct the transformation percentage value in each vial if necessary. The degradation percentage of each contaminant was calculated based on the amount of product formed. The experiment was conducted in quadruplicate except for one of the abiotic controls that was conducted in triplicate.

For the Clear Native Gel (CNE) activity assays, a non-stained slice of gel (see section 2.6) was added instead of the cell concentrate in the vials and, the other activity test components were the same. The contaminant evaluated was 1,2-DCA. We also added a few controls: (i) the abiotic control, (ii) the pellet control (iii) the control protein extract or positive control, (iv) the sample control (see section 2.6), (v) the gel slice control, that consists on a clean gel slice without protein, to observe the possible degradation of the contaminant in touch with the gel, (vi) the cell concentrated control, with 15 μL of the cell concentrated, to confirm the activity of the cell concentrated used and (vii) the non-substrate cell concentrated, with 26 μL of the cell concentrated and unfed, to observe any possible traces of contaminants or products that might have been carried over from the experimental bottles. The experiment was conducted in triplicate, except for the controls cell concentrate and non-substrate cell concentrate due to a lack of sample. For these, only one replica was possible.

2.5. Gas chromatography analysis

Halogenated compounds and their degradation products were analyzed from headspace samples using a gas chromatograph (GC) model 6890 N. Half milliliter of headspace gas was injected manually from the 70-mL-microcosms, whereas 1 mL of headspace gas was injected automatically using the autosampler equipment model 7694E (Scientific Industries; New York, USA) from the 10-mL-vials. The autosampler heated the samples to 80°C for 15 min before injection.

The GC was equipped with an DB-624 column (30 m x 0.32 m, with a 0.25 μm film thickness; Agilent Technologies) and a flame ionization detector. Helium was used as the carrier gas (1.5 mL min^{-1})[4]. The detector and oven were both configured to operate at a temperature of 250°C.

Following sample injection (2:1 split ratio), the initial oven temperature (40 °C) gradually increased at 10 °C min^{-1} until reaching 50 °C, and then further elevated at a faster rate of 20°C min^{-1} to 120 °C. Identification and quantification of compounds were done utilizing external chemical standards[4]. The reported concentrations of compounds are expressed as nominal concentration in micromolar (μM) or nanomolar (nM).

2.6. Clear native gel electrophoresis (CNE)

The protein crude extract prepared as described above (section 2.3) was quantified using NanoDrop 1000 spectrophotometer (Thermo Scientific; Massachusetts, USA). A precast 4-16% gradient Bis-Tris gel (NativePAGE Novex, Invitrogen) was loaded with 20 μg (S20) and 40 μg (S40) of protein crude extract to further silver staining. The experimental wells (A, B, C) were loaded with 260 μg of protein crude extract amended with the sample loading buffer (35 μL/well) (Figure 2). The electrophoresis was carried out for 60 minutes at 150 V, followed by an additional 30 minutes at 250 V. All the process is done inside the anaerobic glovebox and keeping all the samples and the electrophoresis case in cold using ice packs.

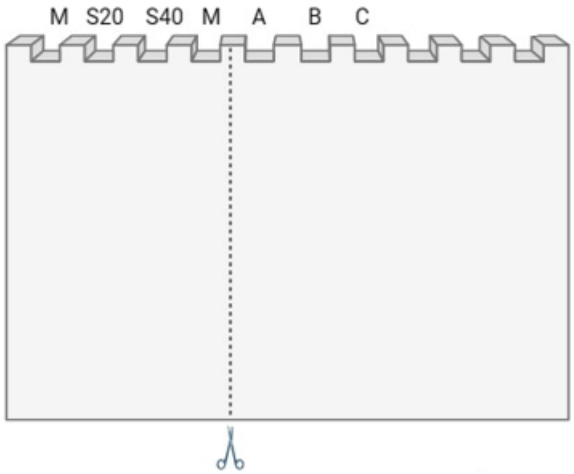


Figure 2. Clear Native Electrophoresis Gel design. Lanes M correspond to the protein ladder, the rest of lanes correspond to the protein extract mixed with the loading buffer solution as described above. A silver staining will be applied to the left part of the gel and the lanes from the right part of the gel will be cut in small slices which further will be used in activity assays.

After running the gel, the left part of the gel was silver stained under the fume hood following the protocol described by[21]. Then, inside the anaerobic glovebox, the stained lanes were placed next to the unstained gel, and the second was cut into slices using a scalpel. The protein ladder was used as a cutting guide, and the non-stained lanes were cut into six pieces. Activity assays were performed with the non-stained gel slices cut following the same procedure mentioned in section 2.4, with the exception that here 2mM of titanium citrate was used in the

Master Mix. The vials were spiked with 100 μM of 1,2-DCA as substrate and prepared in triplicate. Controls were included for the activity assays: (i) abiotic control, consisting of the Master Mix without cell concentrate and spiked with 100 μM of all 1,2-DCA to observe possible abiotic transformations, volatilization losses and to confirm the biotic transformation observed in the experimental vials (ii) the pellet control, with 35 μL of the dissolved pellet, as control of the protein solubilization, (iii) the positive control, with 26 μL of the protein crude extract, (iv) the gel loading control or sample control, with 35 μL of the mixture containing 26 μL protein and 9 μL of sample buffer, which is a positive control that demonstrate that the protein in contact with the sample buffer was active after the gel run.

2.7. Analysis of the RdhA proteins of the *Dehalogenimonas alkenigignens* strain BRE15M

The genome of the *Dehalogenimonas alkenigignens* BRE15M strain cultivated our laboratory was previously sequenced[4], and a shotgun analysis of the expressed proteins during 1,2-DCP degradation was performed[4]. In this study, we compared the proteome obtained from cultures of *Dehalogenimonas alkenigignens* BRE15M growing with either 1,2-DCP, 1,2-DCA or 1,2-DBA.

The data from the proteome (shotgun analysis) were obtained as follows[22]: Extracted peptide samples obtained after in-solution digestion were analysed by nLC-MS/MS on a nanoUPLC system (nanoAc quity, Waters) coupled to an Orbitrap Fusion mass spectrometer (Thermo Scientific) as described previously in the laboratory of Dr. Lorenz Adrian at the UFZ (Leipzig, Germany)[17]. Proteome Discoverer (v2.2, Thermo Fisher Scientific) was used for the identification of proteins, based on a protein database from the draft genome of *Dehalogenimonas alkenigignens* BRE15M strain using SequestHT as a search engine. A false discovery rate threshold of 1% was set for peptide identification using the Target Decoy PSM Validator node. The Minora node in Proteome Discoverer was used to quantify the abundance of proteins using label-free quantification based on intensity values in precursor scans. The relative protein abundance presented in the study is the ratio of a protein abundance in one slice to its overall abundance across all slices of one blue native gel lane. This protocol is extracted from[22].

A manual identification of different key metabolic proteins, with special emphasis on the different reductive dehalogenases, was performed, and their abundances were collected. The shotgun analysis for cells grown with 1,2-DCP was conducted in triplicate, while six replicates were used for 1,2-DCA or 1,2-DBA.

3. RESULTS AND DISCUSSION

3.1. Degradation of chlorinated substrates by *Dehalogenimonas alkenigignens* BRE15M

The degradation of 1,2-DCA to ethene and 1,2-DCP to propene in microcosms containing *D. alkenigignens* BRE15M is shown in Figure 3 and Figure 4, respectively. After the initial addition of 1,2-DCA or 1,2-DCP to the cultures, the contaminants were degraded slowly. Afterwards, with each addition of substrate, the contaminant was consumed faster than the previous one without an observable lag phase, indicating the exponential degradation phase. The increasing degradation rates observed suggest microbial growth coupled to the biodegradation of 1,2-DCA and 1,2-DCP and consequently a metabolic degradation of the pollutants.

One way to obtain information about the metabolic pathway of the degrading culture is to analyze the degradation products. In these experiments, only ethene and propene were obtained from the degradation of 1,2-DCA and 1,2-DCP, respectively, without the formation of any other intermediate product, suggesting a dihaloelimination mechanism. This observation is in line with previous results with *D.alkenigignens* BRE15M as a *Dehalogenimonas* strain capable of dehalogenating alkanes with adjacent halogens via dihaloelimination[3]. At the end of the experiment, the consumption of 1,2-DCA was 161.92 ± 1.26 µM, and the amount of ethene formed was 207.70 ± 5.07 µM. The consumption of 1,2-DCP was 129.55 ± 5.74 µM and the amount of propene formed was 152.76 ± 7.81 µM.

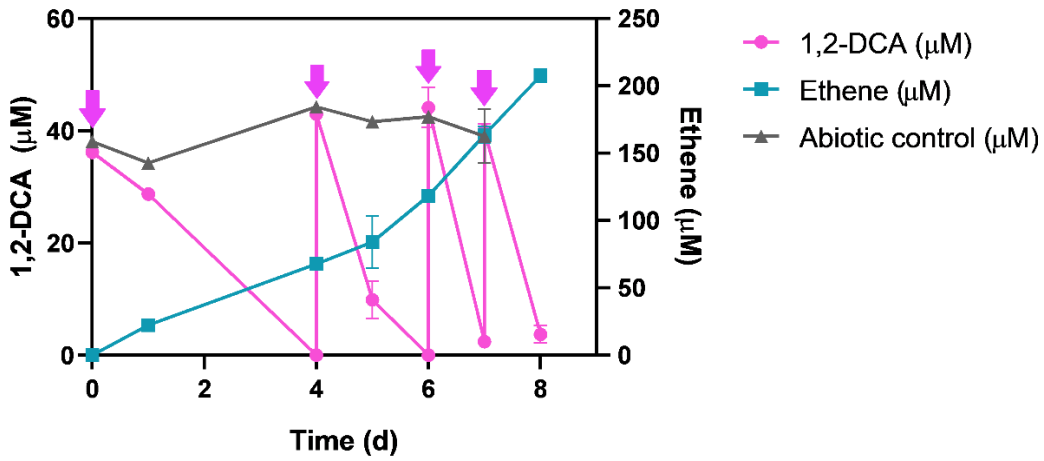


Figure 3. Time-course of 1,2-DCA degradation by a culture containing *Dehalogenimonas alkenigignens* BRE15M. The degradation of 1,2-DCA accumulate ethene as product in the microcosms. Pink circle dots correspond to the amount of 1,2-DCA over time, and the pink arrows represent addition of 1,2-DCA in the culture. The turquoise squares represent the accumulative amount of ethene at each analyzed time. Finally, the grey triangles represent the abiotic control. Bars indicate standard deviation for triplicate bottles.

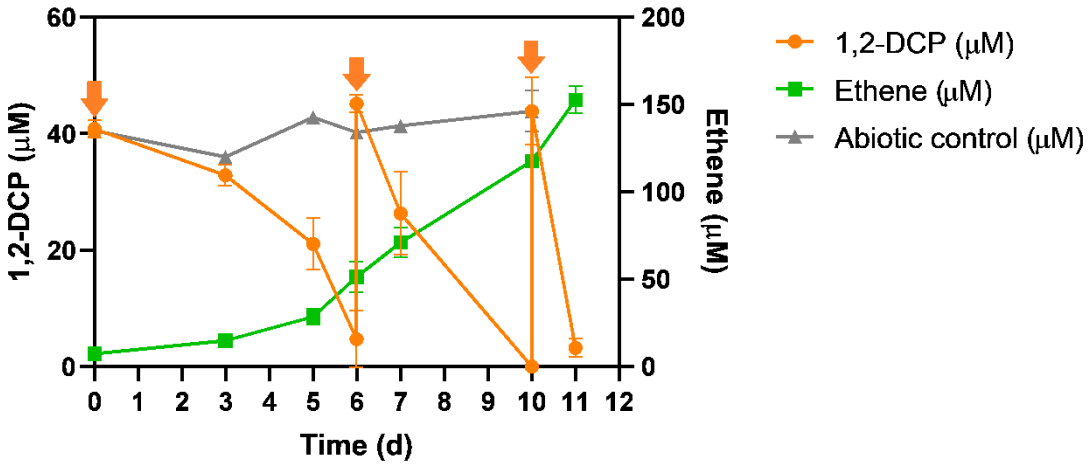


Figure 4. Time-course of 1,2-DCP degradation by a culture containing *Dehalogenimonas alkenigignens* BRE15M. 1,2-DCP degradation accumulate propene as product in the microcosms. The orange circle dots correspond to the amount of 1,2-DCP over time, and the orange arrows represent addition of 1,2-DCP in the culture. The green squares represent the accumulative amount of propene at each time. Finally, the grey triangles represent the abiotic control. Bars indicate standard deviation for triplicate bottles.

3.2. Dehalogenase activity assays

We show the percentage of degradation of the pollutants 1,2-DCA, 1,2-DCP, 1,2-DBA, 1,1,2-TCA, 1,1,1-TCA, CF, PCE, and TCE in enzymatic assays using cells of the strain BRE15M that were growing with 1,2-DCA or 1,2-DCP as the sole electron acceptor (Figure 5). The contaminant added to the activity assay was tested as a probable new final electron acceptor in the respiratory chain of *D. alkenigignens* BRE15M. Therefore, if the cells of *D. alkenigignens* BRE15M can reduce the new pollutant, we could conclude that at least one of the RdhA expressed in the culture growing with 1,2-DCA or 1,2-DCP as a substrate, is capable of transforming the new contaminant.

In the activity assay with the culture growing with 1,2-DCA as the sole electron acceptor source, we observe that only 1,2-DCA, 1,2-DCP, 1,2-DBA, and 1,1,2-TCA were fully transformed to their metabolites ethene, propene, ethene and vinyl chloride (VC), respectively (Figure 5A).

The activity assay performed with cells of *D. alkenigignens* BRE15M growing solely on 1,2-DCP as final electron acceptor source could only completely degrade 1,2-DCP, 1,2-DBA and 1,1,2-TCA. The degradation of 1,2-DCA was also achieved, but at the lower percentage of degradation of 2.21 ± 0.79% (Figure 5B).

All the alkanes transformed in these assays contained two adjacent carbons bounded halogenated atoms and, as observed by the metabolites formed, the reaction mechanism suggests that the expressed RdhA in strain BRE15M is performing exclusively dihaloelimination[3]. However, other strains of *Dehalogenimonas* are capable of dechlorinating other types of

compounds. For instance, *Dehalogenimonas* sp. strain DCF was able to dechlorinate the drug diclofenac through hydrogenolysis[24]. Additionally, "*Candidatus Dehalogenimonas etheniformans* strain GP", was able to dechlorinate trichloroethene (TCE) into all dichloroethene (DCE) isomers and all DCE isomers into VC. However, it was unable to dechlorinate PCE[16,25]. Therefore, what these data show is that each strain of *Dehalogenimonas* has specificity for different substrates, and this depends on the type and quantity of dehalogenases expressed at that time[13].

For the activity assays with cells growing with 1,2-DCA, no degradation was observed in the abiotic vials, which correspond to the negative control, indicating that the RdhA of *D.alkenigignens* BRE15M cells is responsible of the degradation. However, in the activity assay with cells growing with 1,2-DCP we observed ethene production in the abiotic control. This led us to consider two possible hypotheses: firstly, a biotic degradation has occurred because there might have been contamination with *Dehalogenimonas* in the abiotic vials due to their feeding with a needle contaminated with the culture. The second hypothesis was that a possible abiotic degradation of some contaminants occurred due to the reducing effect of a component of the Master Mix. In the past, the research group, observed that the contaminant 1,2-DBA could be reduced abiotically by the effect of titanium citrate, which is the reducing agent that has been used in all experiments to maintain the medium anaerobic, and which the Master Mix contains in relative high amounts (4 mM). To verify which of the two hypotheses was occurring, new abiotic vials were prepared with the old Master Mix used in the activity assay with low reducing power (blue light color that indicates that the titanium citrate (III) is oxidized), and others with fresh Master Mix (dark blue color, that indicates that the titanium citrate (III) is already reduced), both fed only with 1,2-DBA.

The results of the three replicates of the new types of vials showed, for those containing the old Master Mix, no degradation of 1,2-DBA, and for those containing the new Master Mix, 1.48 ± 0.37% degradation of 1,2-DBA. With these results, we conclude that possibly in the abiotic vials of the second activity assay, abiotic degradation of 1,2-DBA was occurring due to the reducing effect of titanium citrate. If this was happening, only 30.24% of degradation in the experimental vial could be attributed to the effect of *Dehalogenimonas*. However, it is still not understood why in the abiotic control of the first activity assay, no abiotic degradation was observed. Therefore, further studies are necessary to confirm and understand what is really happening.

An interesting result of these assays is that the RdhAs expressed in the culture growing with 1,2-DCA degraded completely 1,2-DCP but, on the contrary, the dehalogenases expressed

in the culture growing with 1,2-DCP show very little degradation of 1,2-DCA. These results suggest that the RdhAs responsible for the degradation of 1,2-DCA is not expressed in the cultures growing with 1,2-DCP. In addition, our results indicate that the RdhA expressed during growth with either 1,2-DCP or 1,2-DCA can transform both 1,2-DBA and 1,1,2-TCA.

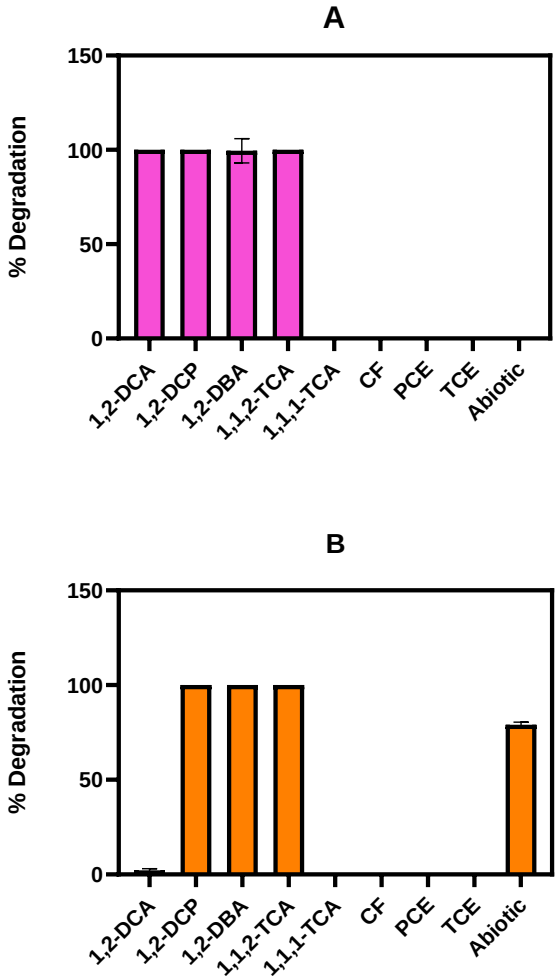


Figure 5. Activity assays of halogenates contaminants (1,2-DCA, 1,2-DCP, 1,2-DBA, 1,1,2-TCA, 1,1,1-TCA, CF, PCE and TCE) with cells of *Dehalogenimonas alkenigignens* BRE15M that were previously growing with (A) 1,2-DCA and (B) 1,2-DCP as substrates. The percentage of degradation has been calculated based on the formation of the product. Standard deviation for quadruplicate samples are presented in bars.

3.3. Expression of RdhA in cultures growing with 1,2-DCP and 1,2-DCA

In this section, we attempted to investigate the pattern of the RdhA expressed with 1,2-DCA, 1,2-DBA and 1,2-DCP in strain BRE15M and, more precisely, the identification of RdhA that could be involved in the transformation of 1,2-DCA. Therefore, the expression of RdhAs in these cultures growing with 1,2-DCA, 1,2-DCP, or 1,2-DBA as the sole electron source were obtained and compared in this study (Figure 6). The comparative analysis of the RdhA expression in cultures

growing with 1,2-DCA and 1,2-DCP as substrates is presented in Figure 6A and Figure 6B. As we can observe, there are some RdhAs that are only expressed in the presence of one of the contaminants. For instance, it is interesting that RdhAs BRE15M_v1_10075, BRE15M_v1_160015, and BRE15M_v1_20299 are exclusively expressed in *Dehalogenimonas alkenigignens* BRE15M growing with 1,2-DCA as the sole electron acceptor.

Connecting these data with the results obtained in the previous section, suggest that the aforementioned RdhAs are possible candidates for degrading 1,2-DCA. This is suggested by lack of 1,2-DCA degradation observed in the assays with cells grown with 1,2-DCP. It would be interesting to conduct further studies where the same activity assay performed for *D. alkenigignens* BRE15M growing with 1,2-DCA and 1,2-DCP as substrates is conducted with the same strain growing with 1,2-DBA as a substrate. If the culture growing with 1,2-DBA can degrade the 1,2-DCA significantly, it could suggest that the RdhA BRE15M_v1_10075 is the dehalogenase involved in the degradation of 1,2-DCA in *D. alkenigignens* BRE15M, since is also expressed in the presence of 1,2-DBA. Nevertheless, if no degradation was observed, then probably BRE15_v1_160015 or BRE15M_v1_20299 could be the RdhA specific for 1,2-DCA dehalogenation. In addition, we do not discard that more than one RdhA can degrade the same substrate.

On the other hand, reductive dehalogenases BRE15M_v1_30093 and BRE15M_v1_150026 are exclusively expressed in the culture of *Dehalogenimonas alkenigignens* BRE15M growing with 1,2-DCP as the substrate. However, drawing definitive conclusions from this difference is challenging, as the culture growing with 1,2-DCA degrades completely 1,2-DCP and 1,2-DBA. Additionally, we can speculate that some of the RdhAs of the 1,2-DCA culture that also shares expression with 1,2-DCP or 1,2-DBA cultures, may be responsible for the degradation of 1,2-DCP and 1,2-DBA. The shared RdhAs expressed are: BRE15M_v1_40117, BRE15M_v1_160008, BRE15M_v1_160007, BRE15M_v1_10074, BRE15M_v1_30086, BRE15M_v1_30016 and BRE15M_v1_170013. Furthermore, the dehalogenase BRE15M_v1_30086 is the second most expressed with 1,2-DCA and 1,2-DBA cultures.

Previous studies reported by our research group showed the results of protein expression in the same culture growing with 1,2-DCP as a substrate and they found that the most expressed RdhAs are BRE15M_v1_160008 and BRE15M_v1_160007[4]. Also, BRE15M_v1_160008 results to be an ortholog of RDase DcpA, which reduces 1,2-DCP to propene in *Dehalococcoides mccartyi* and *Dehalogenimonas*[4,26]. However, the dehalogenases BRE15M_v1_160008 and BRE15M_v1_160007 are also among the most expressed in the culture of *D. alkenigignens*

BRE15M growing with 1,2-DCA as a substrate. In fact, BRE15M_v1_160008 is the most abundant, and BRE15M_v1_16007 is the third most abundant protein. This leads us to think that there might be a constitutive expression of these RdhAs.

When we observed the RdhAs expressed at higher abundance in the culture growing with 1,2-DBA as the sole electron acceptor (Figure 6C), we see that BRE15M_v1_160008 and BRE15M_v1_160007 are not the most abundant RdhAs expressed. In fact, we see that the pattern of the most expressed RdhAs changes, and now the most expressed RdhAs become the BRE15M_v1_40175, BRE15M_v1_30086 and BRE15M_v1_10097. This leads us to think that the expression of the reductive dehalogenases BRE15M_v1_160008 and BRE15M_v1_160007 might be activated more strongly in the presence of a chlorinated substrate than with brominated substrate, and still, its expression could be constitutive. In contrast, brominated substrates would induce the expression of the RdhAs BRE15M_v1_40175, BRE15M_v1_30086, and BRE15M_v1_10097 more strongly. Additionally, the RdhAs BRE15M_v1_40175 and BRE15M_v1_10097 are not expressed with any of the tested chlorinated substrates in this study, therefore, it is likely a change in the expression pattern of dehalogenases with brominated substrate.

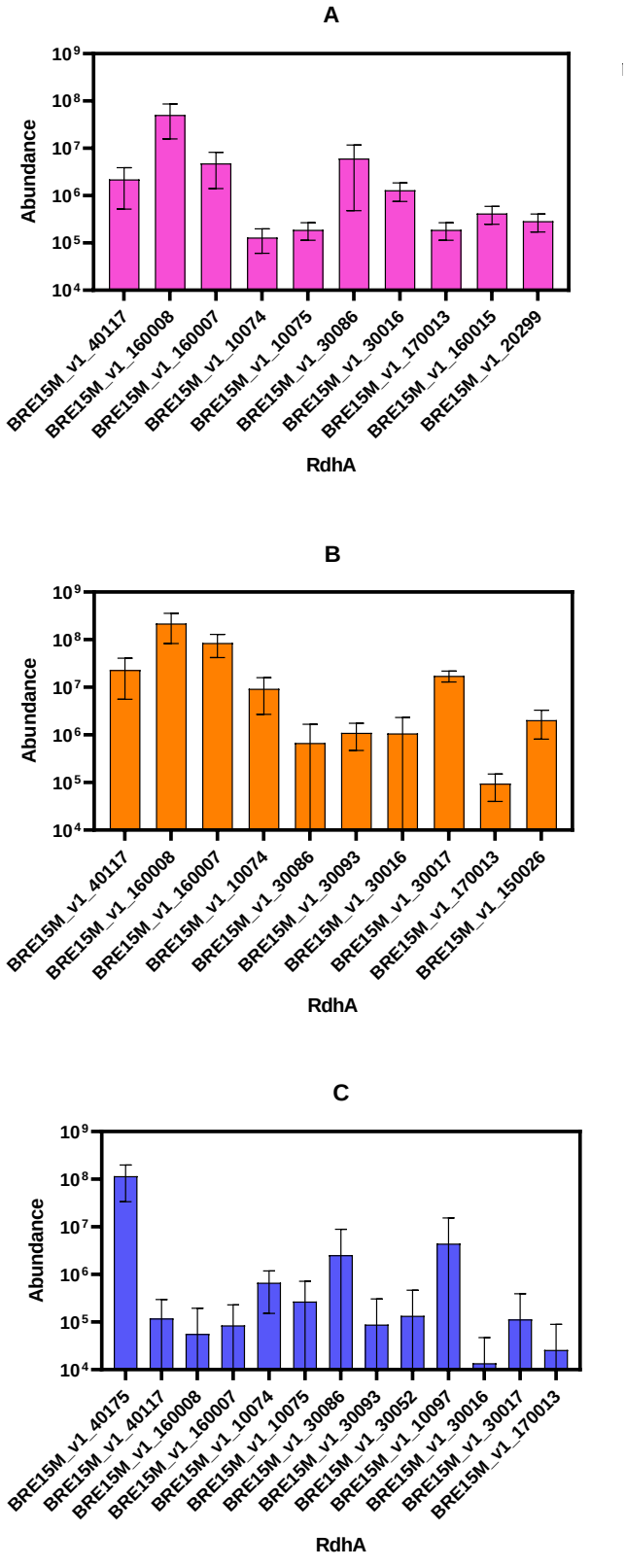


Figure 6. Comparative of the abundance of the RdhAs expressed on *Dehalogenimonas alkenigignens* BRE15M growing with (A) 1,2-DCA (pink bars), (B) 1,2-DCP (orange bars), (C) 1,2-DBA (blue bars) as a substrate. 10 RdhAs were expressed with 1,2-DCA, 10 with 1,2-DCP, and 13 with 1,2-DBA as a substrate. The Y-axis in all three cases is on a logarithmic scale, therefore a small change in the height of the bars implies a large change in the abundance of the expressed proteins. Results are shown as mean of six replicates for 1,2-DCA and 1,2-DBA, and triplicates for 1,2-DCP ± SD.

3.4. Protein activity assay with the reagents of BNE

In previous protein activity assays performed by the research group with gel slices prepared using BNE, there was an observed activity inhibition towards 1,2-DCA, suggesting that probably a compound of the gel reagent inhibited the reaction. To investigate this, an activity assay using 1,2-DCA as electron acceptor was conducted with protein crude extract from *D. alkenigignens* BRE15M previously grown with 1,2-DCA using in the reaction mixture different combinations of the gel reagents. The experimental vials made were: the protein + G250 vial, with 25.6 μL of the protein crude extract and 0.6 μL of the G250 reagent, the protein + buffer + G250 vial, with 25.6 μL of the protein, 0.6 μL of the G250 reagent and 8.8 μL of buffer, the protein + Buffer + G250↓ (with lower amount of G250), with 25.6 μL of the protein with 0.2 μL of the G250 reagent with 8.8 μL of buffer. The controls made were: (i) the abiotic control, to observe possible abiotic transformations (ii) the pellet control, to observe if there was activity remaining in the cellular residues, (iii) the control protein extract or positive control, to confirm the activity of the protein extract used. The experiment was conducted in triplicate, but measurements were only obtained from 1-2 replicas due to errors with the analysis system (section 2.5).

The results are displayed in Figure 7, where it can be observed that the G250 dye inhibits the RdhA activity both at high and low concentrations, the running buffer for the gel does not inhibit its RdhA activity and no degradation was observed in the abiotic control. This led us to the decision to conduct a Clear Native Electrophoresis (CNE) gel, which differs from BNE as it excludes the use of the G250 dye and the light blue cathode buffer.

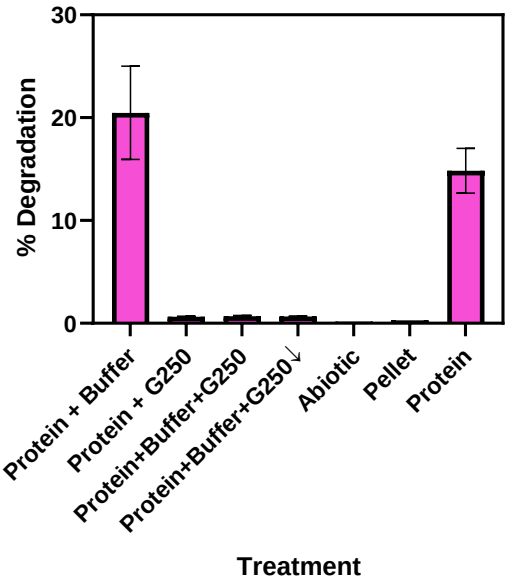


Figure 7. Percentage of degradation of 1,2-DCA to ethene with proteins from *D. alkenigignens* BRE15M transforming the same substrate.

3.5. Clear Native Gel Electrophoresis (CNE) and in gel protein activity assays

Enzymatic activity assays were conducted using different gel slices with proteins obtained through CNE. The objective was to determine whether the RdhA responsible for degrading the contaminant 1,2-DCA forms a complex with other membrane proteins.

After the protein separation with a CNE gel, three gel lanes were cut and gel slices were assayed. Dehalogenase activity was detected in gel slices with a molecular weight ranging from 0 to 66 kDa and from 242 to >720 kDa (Figure 8). The observed amounts of ethene in each gel slice were in the range of nanomoles, indicating very low quantities of ethene. Nevertheless, ethene was still detected.

Elevated dehalogenase activity was detected in the controls of the protein crude extract and the sample control (protein extract + loading buffer), indicating that the protein extraction was successful and that the obtained protein extract contained dehalogenase activity. Furthermore, only 20 nM of ethene were observed in the control pellet compared to the 6201 nM produced in the protein extract and the 7889 nM in the sample control. This suggests that the solubilization of the membrane proteins was effective. Additionally, no ethene production was observed in the abiotic control, indicating that ethene production in the other vials can be attributed to the dehalogenase activity of RdhAs (data not shown). Finally, we observed ethene production of 3 nM in the non-substrate control, which contained the protein extract without feeding suggesting that a small amount of 1,2-DCA or ethene was carried over from the experimental bottles at the end of the process. This quantity was subtracted from all vials in this assay that contain proteins.

The RdhA DcpA in *D. alkenigignens* BRE15M forms part of a complex of 146-242 kDa[4]. Our results were surprising because we expected that the RdhA responsible for 1,2-DCA degradation eluted in the same range of the RdhA (DcpA) responsible for 1,2-DCP (146-242 kDa) as shown in the previous study[4]. Interestingly, we did not observe any activity in this range in any of the lanes in our experiment (Figure 8).

It appears that our observed RdhA activity has shifted towards higher molecular weight bands, with our highest activity in the >720 kDa slice across all three lanes (figure 8) of the gel on average (Figure 8). One explanation for this could be that the cell disruption or solubilization of membrane proteins in our case was not as aggressive, and the reductive complex migrated along with other membrane proteins, resulting in a higher molecular weight complex. We also observe reductive dehalogenase activity in the 0-66 kDa band, in two out of the three gel lanes. Therefore, in this study, it seems that the RdhA alone without the complex (54.1 kDa) could

transform 1,2-DCA. These results suggest that either the responsible RdhA is cytoplasmic (consistent with activity between 0-66 KDa) or it forms a complex that includes other units, causing the activity to be expressed in regions of the gel with higher molecular weight.

Considering that RdhAs work in conjunction with other proteins such as dehydrogenases and an electron transport system, these results lack a clear explanation. Perhaps, the proteins of the complex migrated separately but ended up in the same band. However, this hypothesis might not be entirely plausible, as the OmeA protein weighs 110.9 kDa (Figure 1), so these results do not have a clear explanation.

In all, after observing that the principal activity is shown in the higher molecular weight bands, we could propose that the RdhA that degrades 1,2-DCA could be part of the respiratory complex. Furthermore, we still do not have a clear explanation for the activity shown in the 0-66 kDa band.

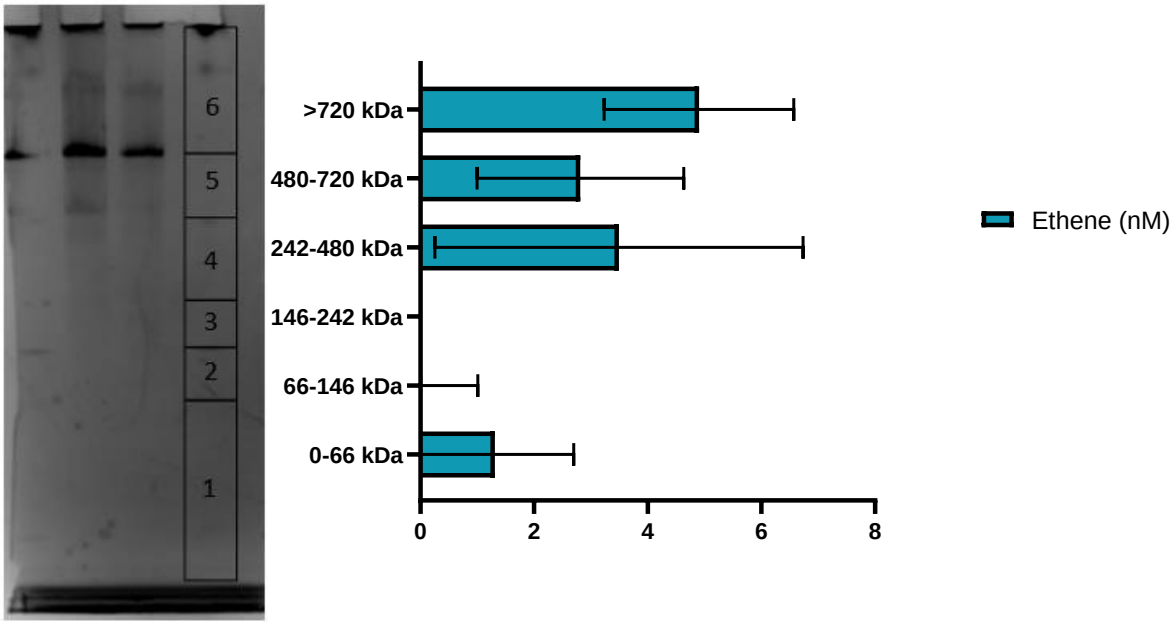


Figure 8. Analysis of dehalogenase activity distribution in CNE using crude extract protein from the BRE15M strain growing with 1,2-DCA. To determine the dehalogenating activity, ethene production was measured after 24h incubation with 1,2-DCA. The nanomoles of ethene shown are the average values obtained from triplicate lanes.

3.6. Study of the aminoacid sequence of the RdhAs of *Dehalogenimonas alkenigignens* BRE15M growing with 1,2-DCA as a substrate

The amino acid sequences of the dehalogenases detected in the cultures of the BRE15M strain growing with the contaminants 1,2-DCA were collected, and their conserved sequences were searched. The sequences searched were TAT signal peptide (RRXFXK), the iron-sulfur binding motifs I (CX2CX2CX3CP) and II (CX10-12CX2CX3CP), and the putative corrinoid binding site

(WXHX2G/L/A/V-Xn-FGYG). The number of dehalogenases identified was 10, and their sequences with the marked conserved parts are shown in Table S3.

Briefly, the conserved sequence corresponding to the TAT signal peptide was detected in RdhAs through manual search and assistance from the TatFind Server tool. It was noted that one of them, BRE15M_v1_160007, the signal peptide for membrane export was not found. Certainly, it has been previously observed that the sequence BRE15M_v1_160007 lacks the TAT signal peptide in previous studies, and a cytoplasmic localization was anticipated for it according to Phobius[4]. This cytoplasmic-predicted RdhA could be capable of degrading 1,2-DCA on its own and not be part of the membrane complex. This could explain the degradation of 1,2-DCA observed in the lower part of the gel (band of 0-66 kDa) where the complex may not reach. Also, for the three dehalogenases candidates to degrade 1,2-DCA (BRE15M_v1_10075, BRE15M_v1_160015 and BRE15M_v1_20299) all have a membrane location prediction, so they could be the responsible for the degradation of the 1,2-DCA in the >242 kDa bands.

The first iron-sulfur binding motif is encountered in these three last-mentioned RdhA sequences and the second is found in BRE15M_v1_160015 and BRE15M_v1_20299 sequences, suggesting a major conservation for the first motif. Finally, the corrinoid bindig site is found in one of the three sequences, although we were not able to find the entire conserved sequence and we only identified the last part of the conserved sequence (-FGYG) suggesting that this sequence is not completely conserved in any of the found reductive dehalogenases of strain BRE15M or that the conserved motif is still not described for all RdhA.

3.7. Conclusion

In this study, we demonstrated that the *Dehalogenimonas alkenigignens* BRE15M strain can metabolically degrade 1,2-DCA and 1,2-DCP through the dihaloelimination mechanism. Furthermore, we showed that the RdhA enzymes expressed in the BRE15M strain when grown with 1,2-DCP are not capable of degrading the contaminant 1,2-DCA. This suggests that when this strain grows with 1,2-DCP, it does not express RdhA enzymes responsible for the degradation of 1,2-DCA. After comparing the dehalogenases expressed in 1,2-DCA and in 1,2-DCP, we suggest that the dehalogenases BRE15M_v1_10075, BRE15M_v1_160015, BRE15M_v1_20299 are candidates for degrading 1,2-DCA. This is because they are not expressed with 1,2-DCP but are expressed with 1,2-DCA. The gel results suggest that these RdhA enzymes could be part of a membrane complex >242 kDa, and there might be a cytoplasmic RdhA that degrades 1,2-DCA. Analyzing the amino acid sequence results of the RdhA enzymes expressed with 1,2-DCA, we

observe that the only cytoplasmic RdhA is BRE15M_v1_160007. Therefore, it could be implicated in the production of ethene in the band of 0-66 kDa.

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5. SUPPLEMENTARY INFORMATION

5.1. Supplementary Tables

Table S1. Composition of the defined medium without vitamins[27]

Chemical formula	Chemical name	Amount per liter
$C_2H_3NaO_2$	Sodium acetate	0.68 g
$CaCl_2{\cdot}2H_2O$	Calcium chloride dihydrate	0.15 g
$CoCl_2{\cdot}6H_2O$	Cobalt (II) chloride hexahydrate	0.19 mg
$CuCl_2{\cdot}2H_2O$	Copper (II) chloride dihydrate	0.19 mg
$FeCl_2{\cdot}4H_2O$	Iron (II) chloride tetrahydrate	20 mg
H_3BO_3	Boric acid	0.06 mg
KCl	Potassium chloride	0.52 g
KH_2PO_4	Potassium dihydrogen phosphate	0.20 g
MgCl2*$6H_2O$	Magnesium chloride hexahydrate	0.41 g
$MnCl_2$*$4H_2O$	Manganese (II) chloride tetrahydrate	0.8 mg
$Na_2MoO_4{\cdot}2H_2O$	Sodium Molybdenum oxide dihydrate	0.36
$NaHCO_3$	Sodium bicarbonate	0.7 g
NaCl	Sodium chloride	1.00 g
NH_4Cl	Ammonium chloride	0.27 g
$NiCl_2{\cdot}6H_2O$	Nickel (II) chloride hexahydrate	0.24 mg
$C_6H_9NO_6$	Nitrilotriacetic acid	128 mg
$C_{12}H_6NNaO_4$	Resazurin sodium salt	0.25 mg
$ZnCl_2$	Zinc chloride	0.7 mg

Table S2. Vitamin composition of the defined medium[27]

Vitamin name	Amount per liter
4-aminobenzoic acid	20 μg
Calcium D-(+)-pantothenate	25 μg
Cyanocobalamin	50 μg
D-(+)-biotin	5 μg
Nicotinic acid	50 μg
Pyridoxine hydrochloride	75 μg
Thiamine hydrochloride	50 μg

Table S3. Amino acid sequences of RdhA proteins are presented with the aim of identifying their conserved domain, this are the ones detected with 1,2-DCA substrate. The sequences are visually marked: TAT signal peptide (RRXFXK) in blue, the iron-sulfur binding motifs I (CX2CX2CX3CP) and II (CX10-12CX2CX3CP) in brown, and the putative corrinoid binding site (WXHX2G/L/A/V-Xn-FGYG) in red. The E values are the values obtained through Conserved Domain Database (CDD) searches that look for homologous domains between the input sequence and another RdhA protein[27]

RdhA of *Dehalogenimonas alkenigignens* BRE15M	e-value	TAT signal peptides	Iron-sulfur binding motifs I and II	Putative corrinoid binding motif	Amino acid sequence
BRE15M_v1_40117	1.68e-84 TIGR02486	Yes	Yes/No	Yes	>BRE15M_v1_40117|ID:57131545| Tetrachloroethene reductive dehalogenase TceA [Dehalogenimonas alkenigignens BRE15M] MSRFHSTVSRREFLKNVGLAGGVGATAALAAPGFHDLDEVAAAKGSAPKRSWWIKEKDEP TVDVDWDLMARHHGFHSTQSDIIQARYYGLEEWKAMTMPSATDRMKAGEPGYSLRDYALA NGANRENSAYGKWEDTFRGANTPAFIGPQRAKTPEQLGVPKWEGTPEENTKMLRAAMKLY GAQDIGVSKLDDHHKKLVGTHGDNISYSYYPPKFNVPTTVTKPMVFADVPTGYVDSATGT FYIPNKDMWEVTFTIPMPKELARTAHSQLFGAANNCRYRMTTVIRPNTQEFLRGLGYQGL ADNPYRGVPSEAGAVFSGLAENSRHTIMAINPEHGSWTGFFHILTDLPLEETKPIDAGIW RFCHSCGTCAKYCPSNSIEPKGGREISYDPYPSAVTPKDPPLPGLGWDKPTPGESEYFKL GRKTYWTDMISCAGYRKSVDACLRCFGSCVFNSADDAMIHDLVRSTSAVTPLFNSFFAEM HEVFGLGLKNDEEKEEWWDMSLPSHGYSTAVTSRHGGYNKG*
BRE15M_v1_160008	2.07e-65 TIGR02486	Yes	Yes/No	No	>BRE15M_v1_160008|ID:57132062| Tetrachloroethene reductive dehalogenase TceA [Dehalogenimonas alkenigignens BRE15M] MKSHSTMNRRDFMKTLGLGATAIGSVGVTAPIFHDLDEMMSISAAETFNSTTSMQKRPWF VKEVDIPTVEIDLKLRTPYAGPTPSAGTLASIYVTKEETAAILDAQKNNAIEGAKNNRPG FTLRDQIGAWASLDRGQTGYLKYPPEGFRTIKVTHETLGVPKWEGSETENAFMIRTFLRQ FGVGAIGYARVDDDSVGPRKPLFNTHVRLDNIADYKYDTNGVFVMPEKCKYAIIMYDRSP RDPNNYRRTVNSPQAFVSNMEKCEYGHKLQNFLWGLGYQSYWFEDGTTSKFTGTPTNVWG ILSGIGEYNRIHNAVSQPEGESGNFASILFTDLPLPSTRPIDFGALEFCKTCGICADVCP AGAIPTVEEYKEPTWNRATGPWSASNDHRGYPNKSIECVKWYFSYAITAYAPSSRPVGVC RRCASHCVFSKDRKAWIHEVVKSVVSTTPVMNSFFTKMDRLSGYSDVISDEGRADYWHQY LPAI*

BRE15M_v1_160007	1.35e-42 TIGR02486	No	Yes/Yes	No	>BRE15M_v1_160007|ID:57132061| Tetrachloroethene reductive dehalogenase TceA [Dehalogenimonas alkenigignens BRE15M] MDKYHSSVSKNDYMKGLRFPRKGEIDSSEFRDLDQMLNSSEHNKPWFIKQVDVPTVEIDL KLHNPYPQSANVYNINSFFRYLNDPIKEAEIVSNSKLREIDGILQQLPGNSLRDISLVKS LISEYYYDLPSPLGGFRSIPSPKDYGVPRWEGTPEENASMILTVARATGCSYVGFIDLSD NKLGPITNLFYSDTAVRFEEGIEDYKLQGLNTICYPSKCRYLIVLVPQMRSIYKRFGNFT TLATAGQDSTDKVLHLAKMMNFLHGIGYLGFEIRSPLPPLEILTGIGEYNRTHGPPITPA FGNECLSGTFSILTDLPLAPSKPIDMGVLKYCEYCLKCAGACPAHAIDKEKLPSWEINTG PWNASNDHKGYKNNSFKCMEFIVESIATGFRPRLMGHCMNCVYSCPFTQSTMLDKYLRPL PNVMPTLSLDREHTRQFWDIDLPEYGMKSIINTPKIGGQNV*
BRE15M_v1_10074	1.44e-69 TIGR02486	Yes	Yes/No	No	>BRE15M_v1_10074|ID:57130418| Tetrachloroethene reductive dehalogenase TceA [Dehalogenimonas alkenigignens BRE15M] VSNKFHSTVSRREFMKVMAMVTGGVGAMAAVNPAFHDVDELISAGAVMQKRPWWVKEREA HNPTTEVDWDVIKRPNPTNTGQQTEMWAYYHGQARADAASAKGAEYAKAKIAAQAPGYTY RGQALKTAVTTSWSAYVSKSWAGASTNGTWTKGGGATYKGVATPADRGEPKWNGTPEENS HMLNAYQKYVGAAISGYGEFSSLDREKLLCTNVKHNAAKKFIIDDTADTAYENSTALAVP AKNQMYHLVHWEHMSHEMSRAAPAMGGRFNGSDFVATALKPSVYNFLRYMGYQMIGDGGD SNYPFIEAAVANLTGVAESSRNNVYSLTPELGPIGRIHSYITDMPVAATHPIDAGMFKFC ADCGKCARACPAECISLAKEPTWEIPDINGKPNLMHNRGTKEFWSDGAACRMVRTELDGC NVCWGNCTFTTNKGAMVHELIRGTISNIQIGPLNNFFFRMGEVFEYGAGTHEGGSPKAEE WWDRSFPVFGMDSTVTSFDGGYKK*
BRE15M_v1_10075	5.48e-73 TIGR02486	Yes	Yes/No	Yes	>BRE15M_v1_10075|ID:57130419| Tetrachloroethene reductive dehalogenase TceA [Dehalogenimonas alkenigignens BRE15M] MSKFHSNISRRDFMKGLGLTGAGMSLAAAGGPLFHDLDELAAGADTHHYRKWWQKERDFE DLTTPIDWDMFRPYDTRKLYMLPLSLVKRQADERNARHVRDVADNTPGGTLRDIALDEAT YDNFNMQHLGWQGNIRTASPADRGLPAWQGAPEDNLQMMRAAAHFYGAPRVGAIEVNEHT KRLFDLGTTIWEDIPAAYIDAAGVYHIPSRCRWILTWLTKQNYPQSLYALRTDDTGPDRN KTFELGQASRNASYSHAPQIRWNVTGFLHGLGYLALQPEVRANVPFGLFSGLAEQGRTAY CCSPDYGLSIRYVDWAITDLPLQPTRPIDAGVTEFCKSCKRCAEICPPGALSLDDDTSWE VAGQWNRGGFKGFHQHWQKCAEWGGPHDCSNCQMTCPFNHPPDASIHNMVRAASAVTPAL NGFFAGMDRAFGYGRQKSDAEREDWWQRDLSKWPYDELKGFGVKDW*
BRE15M_v1_30086	9.39e-78 TIGR02486	Yes	No/No	No	>BRE15M_v1_30086|ID:57131300| Tetrachloroethene reductive dehalogenase TceA [Dehalogenimonas alkenigignens BRE15M] MNKYHSTMSRRDFMKALGVAGGAMAAAPAFADLDEMAAKDGLYTKRPWWIKTREHLEMTT EVDWDEMQRYSENDTMRGTKANGYRLSLYDQTEWDRRSALKKEEETKFLKEDKPGFTLKD

					LAYSSNVGSNQSVSQSFLGAQKATLPEARGVSKWQGSPEEAASLLRTFMRSVGAMSIGFI ELEEGKTKKLIYDFEGGGKIRNVWEDTDKASIRTLANGYQDNVIPNSFKYAIEIINQESI NLFKVNPTLLMSQIRYGRNANTQAATMEFIRSLGYQAVGQYSINTIGIAPALATVSGRGE MGRMNRLITPEHGPIVGAFTMLTNLPLAPDKPIDAGYLNFCKTCMKCAETCGEGAISIEK EPYWETIGGWNNAGHKAWFEDSRKCAAFRALPNACTSGKCLAVCTFSKDHLSGIHEVVQA TLANTSLFNGFFKQMDDVFYHDGLHAPEKFWDIELPTYAIDSTINGRDTHS*
BRE15M_v1_30016	3.15e-71 TIGR02486	Yes	Yes/Yes	No	>BRE15M_v1_30016|ID:57131230| Tetrachloroethene reductive dehalogenase TceA [Dehalogenimonas alkenigignens BRE15M] VSKYHATLSRRDFMKAVGLAGAGVGAAAAMAPGFKDLDDLAATSTNQSHPWWVKDREFND PTNEIDWNTFKPYDNKINAMPTINQAAKDANTIRDNNIQKAAFASKAPGDSLRDYAFGQG SSFIGPDAPWDGPSASLPANAEGKRWEDTPENNLQMMRAALHTYGAVLTGALEVNDKTKK IFDASGFVFDSSDKGYQDEKKVYHIPEKCKYMLTFAVKQNYIQSLYQLRLDDSMPGGYGT KLALGNQAIGHAYSNSSQTGYAAMRMVKTLGYQALKTGVRANVPLGIFSGLGEQGRATSL LTPRYGLMVRYTNYFFTDLPLAPTPPMDAGLITFCKTCVRCGERCPSESISQDNDTTWDT ASTGNRPGFKGFFMNWQSCIDFGSPGACGNCQATCPFNHASDGVIHPIVRAAAATTPVFN SFFATMDRAFDYAKAKSDKELTDWWSRDLDKWQADTTLGSGKNIW*
BRE15M_v1_170013	2.81e-81 TIGR02486	Yes	Yes/No	Yes	>BRE15M_v1_170013|ID:57132100| Tetrachloroethene reductive dehalogenase TceA [Dehalogenimonas alkenigignens BRE15M] MSTFHSTVSRRTFMKALGLSAAGIGAVGAAAPVFHDLDELMSSEGSPKKPWWVKTADKPT VEIDWDVTKRYDRRLNFLNANVKANYYPKEQAEATATGAEFKKANMAANAPGYTNKTRAF SDAFSRMMGTMTASTYSFTGITGTTITQTPAELGVPKWEGTPEENSRLLAAAARIYGAIF TGFTELDNAWRNKLVISHTIGTAGRIVYEDVDLGYSVKDPTGEMAEKWVIPTKPMWGVGF ASAESLFAQKTGPSLTPGNHSFGRTAGRSTYIGLFNLLRTLGYQFLGTPFMTQNLFNSSA LHIMTGNAEASRQNNFSLTPIGGPRFQSGENGVTDLPLAPTKPIDAGMFRFCHSCAKCAD SCPPQAISLDKKPSWEIPLLDGKTDTTHNAGVKSFWLNWASCTIGRSQLAGRCEICWAVC PFNEGRDAVVHDVIKAVTANTGIFGGFFTDMSKAFGYGVREGEAKEAWWNEPQSVFGVES EIFAKYER*
BRE15M_v1_160015	3.70e-39 TIGR02486	Yes	Yes/Yes	No	>BRE15M_v1_160015|ID:57132069| Tetrachloroethene reductive dehalogenase PceA [Dehalogenimonas alkenigignens BRE15M] LAQFHNSISRRDFMKSVMLGVGGTAAAAGVAYAWGVQDGTTPLSFQGHQNRRGFEYEVFD RTPFEVDDVPETSWKVTEQNTTYMRPNQVVNGIHRPDLRWIYNQRFQNRHDPDVNKFKFA IPYSAWPSNGVQALDPFWREYYEMYPDMLERDKEYVYEWLPGFRERYFKTTEMRANFDAQ EGYIANKIVSGDTAVGVSSITTTPDVNDWDKVSTTRAVFESPEKAAENIKWAAYHFGAQY VGIIPVHPEFVWYNHSSNTRGFKVGEEIKIQPWWNYGIFVNAPMEWDSMMADPNYGQSQQ GYNIVSTIAQQIVGYLKALGYPARWNSPNGGYDLTIPPHGALSGYGNIGRTSNCMAPDVG

					GNCRPAVVFTSLPMATDKPIDFNLYAFCKRCMICAENCPTQAISQSPEPDRVEYGMKRWA TNFAVCNDGWAYGAGPTGCRACVAACPWTRRNTWSHRMMRNILARDSTGLVSNVALWAER NMYPKNMLDDLNPPLFKGVFDPPEYLKTANFISGFTPTPMGVK*
BRE15M_v1_20299	3.04e-64 TIGR02486	Yes	Yes/Yes	No	>BRE15M_v1_20299|ID:57131201| Tetrachloroethene reductive dehalogenase TceA [Dehalogenimonas alkenigignens BRE15M] MKGFHSTISRRDFMKSLGIAGAGIGAAAAAGPAFHDLDEMASAGKQSHAWWVKERDYENI TTEVDWQTYKNFDAVAHPATPVSDEVKAVTAARVAKDKADGLAGKVPGADRRGFAWYTAA GRGGTAPAWDGETSFWKPSEGDVTSPYNESPEYNTQVMRAALHSFGTPAVGVVELTPNIK KLFNKDLISWEDRNDAVLDGKVYRFPNKYKYMLVWETQMSATQATYNYKADDSNPSGYGS SVPIGRMDAQGYNYGRVVMAQATSFLKGLGYAAVRPGSSFFLQNSAYGIFAGLSEQARPN YRMSPGYGLCSRQADITITDMPLAPTKPIDFGGHRFCNTCKRCGEVCPSQSIDMRDEPSW DTVVPKNNPGIKAFWMNWDTCAGFGSPVNCGICQPTCPFNHPTEAIIHPVVRATAANTGI FNKFFADMDRVFGYGKVRSAEEMEAWWTRDLATYKGDTILGSGKYIW*

5.2. Supplementary Material and Methods: Cell harvesting protocol for *Dehalogenimonas* BRE15M cultures

Before harvesting cells, contaminant levels of the *microcosms* desired for the study were measured using Gas Chromatography (GC). If the level exceeded 0 µM, the bottles were subjected to an N_2 purge for 8 minutes. Following this, the contents of each bottle were distributed into sterile 50 mL Falcon tubes, 35-40 mL per Falcon, under anaerobic conditions inside an anaerobic glovebox. After that, the falcon tubes were centrifuged anaerobically at 8000 g for 40 minutes at 10ºC, and 10-15 mL of supernatant were removed into the anaerobic glovebox. When necessary, typically after removing the supernatant post the second centrifugation cycle, the concentrated cultures were combined into a smaller number of Falcon tubes, usually half. The process was repeated until obtaining an approximately 10 times concentrated culture for the activity assays and to a final volume of 600 µL for the clear native electrophoresis gel. To achieve the 600 µL of final volume, a last centrifugation step in Eppendorf tubes of 1.5 mL at 9000g and 10 ºC for 20 minutes was added.