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Dehalogenase producing bacteria from extreme environment: A review

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ABSTRACT

Halogenated compounds create the most important class of xenobiotic which commonly lead to pollution. Some of these compounds are very toxic and cause enormous problems to human health and to the environment. Many of these toxic chemicals have been shown to occur in various extreme habitats. Pollutant-degrading microorganisms, adapted to grow in various environments, play an important role in the biological treatment of polluted extreme habitats. The presence of dehalogenase producing microorganisms in extreme habitat in particular is necessary since the enzyme can catalyze the removal of a halogen atom from a substrate. Therefore, it can reduce the toxicity of the halogenated compound and some are of interest for study in industrial application. Thermophiles, psychrophiles, acidophiles, alkaliphiles and halophiles are types of extremophiles. Knowledge of the biodegradation of toxic chemicals in extreme environment is limited. Here, examples of dehalogenase producing bacteria isolated from various extreme conditions and its special characteristics/features will be discussed in this review.

Keywords: halogenated compound, extremophiles, dehalogenase, environment

INTRODUCTION

Halogenated hydrocarbons are commonly found in environment due to their extensive use in industry and agriculture as insecticides, herbicides, solvents and intermediates for chemical syntheses. Halogenated compounds are toxic and persistence thus cause environmental pollution and human health problems (Chaudhry and Chapalamadugu, 1991). Dehalogenase are enzymes that catalyse the removal of a halogen atom from halogenated compounds. Microbial dehalogenases detoxify harmful halogenated compounds by cleaving carbon-halogen bonds in such compounds (Copley, 1998; Kurihara *et al.*, 2000). These enzymes are important for bioremediation of environmental pollution caused by halogenated hydrocarbon pollution, which are synthetically produced as herbicides and growth regulators (Allpress and Gowland, 1998).

Extremophiles are organisms that tolerate extreme environmental conditions such as high temperature, pressure, salt concentrations as well as low temperature, pH, nutrient concentrations and water availability. High level of radiation, harmful heavy metals and toxic compounds also are categorized as extreme environment. The normal microbial conditions for growth and reproduction include temperature of between 20 °C to 40 °C, pH value is near neutral, air pressure of about 1 atm and sufficient amount of water of activity (Satyanarayana *et al.*, 2005). Because microorganisms have broader tolerable range of environmental conditions, extremophiles are mostly found among microbes. Some microorganisms can grow and reproduce at between −12 °C to > 100 °C, pH 0 to 13, saturated brine and pressure of up to 1400 atm. Extremophilic organisms do not only tolerate but also require extreme conditions to survive. It can develop its whole life cycle in these conditions. Extremophilic organisms are mainly prokaryotes (archaea and bacteria), with few eukaryotes (López-García, 2005).

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Depending on the extreme conditions, extremophiles are of various types, which include: thermophiles (extreme heat), psychrophiles (extreme cold), alkaliphiles (high pH levels), acidophiles (low pH levels) and halophiles (extreme saltiness).

It is important to investigate dehalogenase from extreme environment. Usually, mesophilic organisms have been used to obtain enzymes of interest due to its advantages. However, the application of these enzymes are restricted by their stability in mesophic environments (Kumar *et al.*, 2011). Potential applicatons of such enzymes are not restricted to mesophic condition. Therefore, investigation of dehalogenase from extreme environment is very important, so as to allow their application beyound normal environmental conditions. For example, the enzymes isolated from thermophilic organisms regularly show stability to several extrinsic factors such as temperature and organic solvents. These properties make them particularly useful for application as industrial biocatalyst (Rye *et al.*, 2009).

Extremophiles are source of enzymes (extremozymes) with extreme stability; and the application of these enzymes as biocatalysts is attractive because they are stable and active under conditions that were previously regarded as incompatible with biological materials. Furthermore, some extremophiles, particularly those from the Archaea, have novel metabolic pathways and thus can serve as a source of enzymes with novel activities and applications (Kumar *et al.*, 2011). Over the last few decades, extremophiles have attracted the attention of researcher in the search for new bioactive substances, such as enzymes and biocides to be used in major sectors of the world economy, including the agricultural, chemical, food, textile, pharmaceutical, bioenergy and cosmetic industries (Dalmaso *et al.*, 2015). Current review will focus on how these micoorganisms can survive at extreme environments and some of them produce dehalogenase enzymes that have the ability to remove the halogenated compound from severe environments.

DEHALOGENASE PRODUCING BACTERIA ISOLATED FROM EXTREME ENVIRONMENT

Thermophiles

Thermophiles can be divided into 3 groups such as moderate thermophile (growth optimum 50-60 °C), extreme thermophile (growth optimum 60-80 °C) and hyperthermophile (growth optimum 80-110 °C) (Kumar *et al.*, 2011). Many investigations focused on their potential as sources of highly active enzymes ‘extremozymes’ (Aanniz *et al.*, 2015). Thermophilic microorganisms can be isolated from virtually any environment that receives intermittent heat, such as soil and compost. Hyperthermophiles thrive only in a very hot and constantly hot environments, including hot springs, both terrestrial and undersea (hydrothermal vents), and active sea mounts, where volcanic lava is emitted directly onto the sea floor (Raven and Johnson, 2001). Thermophilic bacteria grow at very high temperatures and have a variety of properties and specialisations that allow their cells and molecules to remain functional at high temperatures. For an organism to grow at high temperatures, all cellular components, including proteins, nucleic acids, and lipids, must be heat stable. The structural features that dictate thermal stability in proteins are not well understood but a small number of noncovalent features seem characteristic of thermostable proteins. These include a highly a polar core, which undoubtedly makes the inside of the protein "sticky" and thus more resistant to unfolding, a small surface-to-volume ratio, which confers a compact form on the protein, a reduction in glycine content that tends to remove options for flexibility and thus introduce rigidity to the molecule, and extensive ionic bonding across the protein's surface that helps the compacted protein resist unfolding at high temperature. In addition to these intrinsic stability factors special proteins called chaperonins are synthesized by hyperthermophiles. Chaperonins functions to bind heat denatured proteins and refold them into their active form (Raven and Johnson, 2001).

Dalmaso *et al.* (2015), described that thermophiles have several mechanisms to support extreme temperatures. It is believed that the thermostability of cellular components such as ATP, amino acids, and peptides may exceed 250 °C, suggesting that the maximum temperature for life goes beyond the temperatures that have been observed until now. The proteins of organisms adapted to extreme temperatures generally have similar three-dimensional structures of mesophilic organisms but the amino acid content is different from ordinary proteins and the number of charged residues on their surfaces is much greater than non-adapted organisms. In addition, such proteins often have shorter loops, thus preventing the occurrence of nonspecific interactions due to their increased flexibility at high temperatures. Extreme thermophilic bacteria produce thermostable proteins that can be readily crystallized to obtain stable enzymes for structural and functional studies. Proteins from hyper/thermophiles require sufficient structural rigidity to resist unfolding. This is an important feature to characterize antidrug targets. A classical instance is the bacteria *Thermus thermophilus* that was originally isolated from a thermal vent within a hot spring in Izu, Japan, and is frequently used in genetic manipulation studies. The DNA gyrase from this extremophile has been used as an antidrug target model. DNA gyrase is a type IIA topoisomerase that introduces negative supercoils into closed circular bacterial DNA using ATP hydrolysis. It is an important antibacterial target that is sensitive to the widely-used fluoroquinolone drugs (Dalmaso *et al.*, 2015).

Moreover, the thermal hypothesis determines that a G:C pair and its contents are related to thermostability. This is observed for several thermophilic bacteria. *Geobacillus thermoleovorans* CCB US3UF5 is a thermophilic bacterium that was isolated from a hot spring in Malaysia and is a source for thermostable enzymes. The bacteria contains a circular chromosome of 3,596,620 bp with a mean G:C content of 52.3% (Sakaff *et al.*,

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2012). However, a study reported thecomparative analyses of G:C composition and optimal growth temperature with 100 prokaryote genomes (Archaea and Bacteria domains) that failed to demonstrate this correlation (G:C/thermostability). Moreover, the G:C content of structural RNA (16S and 23S) is strongly correlated with optimal temperature and it is higher at high temperatures (Hurst and Merchant, 2001). An increased number of disulfide bonds improves stability within thermophilic proteins and play a role in preventing the alteration of the quaternary structure (Reed *et al.*, 2013).

Another specialization of thermophiles is membrane stability. Heat can also affect membrane stability. In organisms living at moderate temperatures cell membranes are constructed along the typical "lipid bilayer" model: hydrophobic residues (fatty acids) inside oppose each other and retain an affinity for one another while hydrophilic residues (glycerol phosphate) lie at the surface of the environment and the cytoplasm, respectively, maintaining contact with the aqueous phase. If one applies sufficient heat to such membrane architecture the two leaflets of the membrane will pull apart, leading to membrane damage and cytoplasmic leakage. To prevent this from occurring at very high temperatures, hyperthermophiles have evolved a novel membrane structure. Instead of forming a membrane as a lipid bilayer, hyperthermophiles chemically bond the opposing hydrophobic residues from each layer of the membrane together. This forms a lipid monolayer instead of a bilayer, and prevents the membrane from melting at high temperature. Although the precise chemistry of lipid monolayer membranes can vary somewhat from species to species, they are universal among hyperthermophiles and are undoubtedly an evolutionary response to life at high temperature (Raven and Johnson, 2001).

Table 1 shows the thermophilic organism that can produce dehalogenase. Smith *et al.* (1989) extracted HadD enzyme from *Pseudomonas putida* strain AJ1/23. They found that the highest temperature in which HadD enzyme can maintain it activity was 50 °C. Nonetheless, it was inactivated at higher temperatures. Diez *et al.* (1996) studied L-2-haloacid dehalogenase from *Azotabacter* sp. strain RC26. The temperatures ranged from 30 to 60 °C for active enzyme activities. Jesenská *et al.* (2002) isolated *Mycobacterium avium* subsp. *Avium* N85 from swine mesenteric lymph nodes.The dehalogenase gene found in *Mycobacterium avium* subsp. *Avium* N85 was called *dhmA*. Temperature from 20 to 50 °C exhibited the increasing of dehalogenase activity. The highest activity was detected at 50 °C.

Bachas-Daunert *et al.* (2009) identified a putative dehalogenase, $_{L}$-HAD$_{ST}$, derived from the thermophile *Sulfolobus tokodaii*. $_{L}$-HAD$_{ST}$ was incubated for 4 h at 70 °C showed fully active and stands at extreme pH conditions ranging from 4 to 10. Rye *et al.* (2009) studied L-2-haloacid dehalogenase from the thermophilic archaeon *Sulfolobus tokodaii.* The enzyme shows haloacid dehalogenase activity towards carboxylic acids with the halide attached at the carbon 2 (C2) position with the maximum activity towards chloropropionic acid. At 60 °C the enzyme displays maximum activity but the enzyme had a half-life over 1 h at 70 °C.

Thasif *et al.* (2009), isolated a thermostable L-specific dehalogenase (DehL) from cells of *Pseudomonas* sp. strain S3. DehL still shows enzyme stability even at 55 °C. Godinho and de Sá-Nogueira (2011) studied a member of the ubiquitous haloalkanoate dehalogenase superfamily that is AraL from *Bacillus subtilis*. The temperatures used for AraL's activity, ranging from 25 to 70 °C.

Novak *et al.* (2013a) isolated L-haloacid dehalogenase from the marine bacterium *Psychromonas ingrahamii*. It displays activity towards monobromoacetic (100%), monochloroacetic acid (62%), *S*-chloropropionic acid (42%), *S*-bromopropionic acid (31%), dichloroacetic acid (28%) and 2-chlorobutyric acid (10%). The substrates with shorter carbon chain lengths, exhibited the highest activity of L-haloacid dehalogenase. The optimal temperature for activity was at 45°C and retains above 70% of its activity after incubated at 65°C for 90 min. Novak *et al.* (2013b) established the putative L-haloacid dehalogenase gene (DehRhb) from a marine *Rhodobacteraceae*. The DehRhb protein was revealed to be an L-haloacid dehalogenase with highest activity on brominated substrates. The optimal temperature for enzyme activity was 55 °C with a melting temperature of 67 °C. After incubation at 50 °C for 90 min, the enzyme showed 85% of its activity.

Li and Shao (2014) reported that *Alcanivorax dieseloleistrain* B-5 strain from marine environment was capable of degrading halogenated alkanes. The putative haloalkane dehalogenase (HLD) produced by *Alcanivorax dieseloleistrain* B-5 named DadB. The optimum temperature of this strain was 50 °C but severely lost activity at 60 °C.

In addition, there are some examples of dehalogenase producing bacteria isolated from thermophilic area in which the isolates were not reported to survive within a temperature range of 50 to 110 °C. As an example, Hamid *et al.* (2010), isolated unknown strain AZZ2 from extreme environment that was from volcanic area Gunung Sibayak, Indonesia. This unknown strain AZZ2 was cultured in 2,2-dichloropropionic acid as sole source of carbon and energy at 30 °C. Based on molecular analysis, strains AZZ2 has the highest sequence similarity to *Citrobacter* sp. JC73/SL7. Hence, it was designated as *Citrobacter* sp. strain AZZ2. Moreover, Roslan *et al.* (2011), isolated *Bacillus megaterium* GS1 from thermophilic area that is from volcanic area Gunung Sibayak. They aimed to investigate 2,2-dichloropropionic acid (2,2-DCP) degrading bacteria that can grow at higher temperature rather than at normal 25 to 30 °C temperature. Therefore, the culture was incubated for 2 days at 30, 40 and 60 °C incubator shaker aerobically. Thus, it was resulted that the isolated bacterium grew best at 40 °C but failed to grow at 60 °C. Salim *et al.* (2011) also isolated bacteria from volcanic area Gunung Sibayak, *Bacillus megaterium* GS1. It could grow on various concentrations of 2,2-DCP up to 40 mM. A putative partial dehalogenase DehGS1 amino acid sequence was proposed from *Bacillus megaterium* GS1 catalyzed 2,2-DCP.

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Table 1: Dehalogenase producing organisms isolated from thermophilic environment.

Environmental Factor	Organisms	Temperature	Reference
High Temperature (Thermophile)	*Pseudomonas putida* strain AJ1/23	50 °C (moderate thermophile)	Smith *et al.* (1989)
	Azotabacter sp. strain RC26	60 °C (hyperthermophile)	Diez *et al.* (1996)
	Mycobacterium avium	50 °C (moderate thermophile)	Jesenská *et al.* (2002)
	Sulfolobus tokodaii	70 °C (hyperthermophile)	Bachas-Daunert *et al.* (2009)
	Sulfolobus tokodaii	70 °C (hyperthermophile)	Rye *et al.* (2009)
	Pseudomonas sp. strain S3	50 to 55 °C (moderate thermophile)	Thasif *et al.* (2009)
	Bacillus subtilis	65 °C (hyperthermophile)	Godinho and de Sá-Nogueira (2011)
	Psychromonas ingrahamii	65 °C (hyperthermophile)	Novak *et al.* (2013a)
	Rhodobacteraceae.	55 °C (moderate thermophile)	Novak *et al.* (2013b)
	Alcanivorax dieseloleistrain B-5	50 °C (moderate thermophile)	Li and Shao (2014)

Table 2: Dehalogenase producing organisms isolated from psychrophilic environment.

Environmental Factor	Organisms	Temperature	Reference
Low temperature (Psychrophile)	*Psychrobacter cryohalolentis* K5	-10 °C	Bakermans *et al.* (2003)
	Psychrobacter cryopegella	-10 to 28 °C	Bakermans and Nealson (2004)
	Psychrobacter cryohalolentis K5	5 °C	Drienovska *et al.* (2012)

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Psychrophiles

Psychrophiles (cryophiles) also known as cold-loving are extremophiles organisms that can grow and reproduce in cold temperatures of less than 15 °C (van den Burg, 2003). Psychrophilic microorganisms have successfully colonised all permanently cold environments from the deep sea to the mountain and polar regions (Morita, 1975). This unique property implies that psychrophiles have successfully overcome two main challenges such as low temperature, because any decrease in temperature exponentially affects the rate of biochemical reactions and the viscosity of aqueous environments, which increases by a factor higher than two between 37 °C and 0 °C (Xu *et al.*, 2003).

Among the bacteria that have been detected, the most commonly reported microorganisms are the Gram-negative α-, β- and γ-proteobacteria (*Pseudomonas* spp. and *Vibrio* spp.) and the *Cytophaga–Flavobacterium–Bacteriodes* phylum. Coryneforms, *Arthrobacter* sp. and *Micrococcus* sp. are the most frequently found Gram-positive bacteria. Bacteria generally dominate in some areas such as deepsea waters are *Methanogenium* and *Methanococcus* being the most cited genera (Pandey *et al.*, 2004).

There are several properties of psycrophiles able to thrive at extreme conditions such as membrane fluidity, transcription and translation, antifreeze proteins and cryoprotectants and cold-adapted enzymes. Decreasing temperatures have an adverse effect on the physical properties and functions of membranes, typically leading to a reduction in membrane fluidity, the onset of a gel-phase transition and, ultimately, a loss of function. The lipid composition governs the physical properties of membranes and hence it is not surprising that this varies with the thermal habitat of the microorganism. In general, lower growth temperatures produce a higher content of unsaturated, polyunsaturated and methyl-branched fatty acids, and/or a shorter acyl-chain length, with studies reporting a high proportion of *cis*-unsaturated double-bonds and antesio-branched fatty acids (Chintalapati *et al.*, 2004; Russell, 1997). This altered composition is thought to have a key role in increasing membrane fluidity by introducing steric constraints that change the packing order or reduce the number of interactions in the membrane. Further adaptations that have been suggested to increase membrane fluidity include an increased content of large lipid head groups, proteins and non-polar carotenoid pigments (Chintalapati *et al.*, 2004).

Some of the main barriers to protein synthesis at low temperatures include: reduced activity of transcriptional and translational enzymes; reduced protein folding, owing primarily to a reduced rate of prolyl isomerization; and a stabilization of DNA and RNA secondary structures. In psychrophiles, enzymes involved in these processes have adapted to be optimally active at low temperatures. For example, a ribosomal extract, RNA polymerase, elongation factor and peptidyl–prolyl *cis–trans* isomerase have all been shown to retain activity near 0 °C in several psychrophilic microorganisms. Indeed, these latter enzyme catalyses *cis–trans* prolyl isomerizations, and its high activity and overexpression at low temperatures might be important for maintaining protein-folding rates at low temperatures. Furthermore, nucleic-acid-binding proteins for example, *Escherichia coli* CspA-related proteins and RNA helicases that might be important for the destabilization of DNA and RNA secondary structures are also overexpressed at low temperatures in psychrophiles (Berger *et al.*, 1996; Lim *et al.*, 2000).

Antifreeze proteins (AFPs) can bind to ice crystals through a large complementary surface and thereby create thermal hysteresis and lower the temperature at which an organism can grow (Jia and Davies, 2002). AFPs have been recently demonstrated in Antarctic lake bacteria (Gilbert *et al.*, 2004), one of which, from *Marinomonas primoryensis*, is Ca^{2+}-dependent and hyperactive (Gilbert *et al.*, 2005). The AFP from the Arctic plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 shows both antifreeze and ice-nucleating activities (Muryoi *et al.*, 2004). Trehalose and exopolysaccharides (EPSs) might also have an important role in cryoprotection in psychrophiles. Trehalose is thought to have a colligative effect, but probably also helps in preventing protein denaturation and aggregation (Phadtare, 2004). The modification of physico-chemical environment of bacterial cells, participate in cell adhesion to surfaces and retention of water, favour the sequestration and concentration of nutrients, retain and protect extracellular enzymes against cold denaturation and act as cyoprotectants (Nichols *et al.*, 2005). Riley *et al.* (2008) studied whether the sizes of proteins of a psychrophile differ from those of a mesophile. The sequences of all proteins of *Psychromonas ingrahamii* were compared to sequences of all proteins of three other bacteria such as *Shewanella oneidensis* MR-1, *Vibrio cholerae* and *Escherichia coli* K-12 MG1655 and 916 protein sequences were conserved among all four bacteria. Most of the conserved amino acids were enzymes *P. ingrahamii* contains proteins with relatively high asparagine content, and low content of amino acids potentially sensitive to the higher concentration of oxygen present in cold waters. These properties would seem to be appropriate for an extreme psychrophiles.

Psychrophiles produce cold-adapted enzymes that have high specific activities at low temperatures, often up to an order of magnitude higher than those observed for their mesophilic counterparts. Psychrophilic enzymes increase the flexibility of their structure to compensate for the 'freezing effect' of cold habitats (Johns and Somero, 2004).

There are some examples of dehalogenase psychrophilic organisms and the low temperature which they can thrive (Table 2). Bakermans *et al.* (2003), discovered microorganism from saline-water lenses found from 40,000-year-old Siberian permafrost named *Psychrobacter cryohalolentis* K5. *P. cryohalolentis* was nominated for genome sequencing for its capability to reproduce at −10 °C with a generation time of 39 days and rapid growth at low temperature. Bakermans and Nealson (2004) identified *Psychrobacter cryopegella* isolated from

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a briny water lens inside Siberian permafrost, where the temperature is −12 °C. *P. cryopegella* could reproduce at temperature from −10 °C to 28 °C, with its maximum growth rate at 22 °C.

Drienovska *et al.* (2012) studied a haloalkane dehalogenase, DpcA, from *Psychrobacter cryohalolentis* K5. It represents a novel psychrophilic member of the haloalkane dehalogenase family. The temperature ranges from 5 °C to 35 °C were experimented for temperature profile of DpcA. The highest activity of the enzyme was detected at 25 °C and retained almost 27% of its maximal activity at 5 °C.

The studies on the isolation of bacteria from psychrophilic environment that is marine sponge *Hymeniacidon perlevis* were done by Huang *et al.* (2010) and Huang *et al.* (2011). Sponge was a good source for the dehalogenase producing bacteria isolation as reported by Huang *et al.* (2010). The sponge *H. perlevis* inhabits the estuarine intertidal area of the Chinese Yellow Sea. The optimal growth temperature of the marine sponge in its natural habitat is ranging 8-18 °C. Huang *et al.* (2011) reported that bacteria isolated from marine sponge could degrade 2-chloropropionic acid (2-CPA). However, they did not study the ability of bacteria from marine sponge to survive at psycrophilic temperature.

Alkaliphiles / Acidophiles

The term alkaliphilic microorganisms or “alkaliphiles,” generally refers to microorganisms that grow well at pH values exceeding pH 9 or between pH 10 to 13. Alkaliphiles can be found at neutral environments, acidic soil and feces (Horikoshi, 1999). Enzymes produced by microorganisms from extreme pH will be useful for applications under highly acidic or highly alkaline, for example in the production of detergents (van den Burg, 2003). Microorganisms growing in highly acidic environments (pH values below 3) are found in all three domains of life such as Archaea, Bacteria and Eucarya (López-García, 2005). Acidophiles can withstand and even thrive in acidic environments at pH below 5. Acidophiles can be found in acidic environments, including sulfuric ponds, geysers, or polluted acid mine drainage, and our own stomach. Acidophilic prokaryotes involved in the industrial leaching of copper and other metals from ores.

Alkaliphiles and acidophiles have unique properties that make them survive in extreme pH. One of the striking properties of alkaliphilic and acidophilic microorganisms is their ability to maintain a neutral pH internally, and so the intracellular enzymes from these microorganisms do not need to be adapted to extreme growth conditions. If cells are to survive in an alkaline environment, they must make their cytoplasm more acidic to buffer the alkalinity. Acidophilic and alkaliphilic microorganisms use their proton pumps to maintain a neutral pH internally and so the intracellular enzymes from these microorganisms do not need to be adapted to extreme growth conditions. However, the extracellular enzymes of acidophiles have to function at low pH whereas those of alkaliphiles function at alkaline pH (Kumar *et al.*, 2011). For the cells to survive in the aggressive conditions of pH, alkaliphiles and acidophiles develop other systems as well. Alkaliphiles have negatively charged cell wall polymers apart from peptidoglycan which may reduce the charge density at the cell surface and help to stabilize the cell membrane. Cellular fatty acids in alkaliphilic bacterial strains contain predominantly saturated and mono-unsaturated straight-chain fatty acids. For acidophiles employ a range of mechanisms to withstand low pH such as a positively charged membrane surface a high internal buffer capacity, over-expression of H^+ exporting enzymes and unique transport systems (Horikoshi, 1999).

A major contribution of alkaliphiles to enzymes used in industry is the diversity of enzymes with activity optima shifted to the alkaline pH region. Examples of alkaliphile enzymes and their uses include alkaline proteases, which are used as detergent additives and for removing hair from hides; starch-degrading amylases with elevated pH optima are also suitable for laundry use and debranching enzymes, together with amylase, play a role in stain removal (Preiss *et al.*, 2015). Enzymes produced by acidophiles such as amylases, glucoamylases, proteases, cellulases and oxidases are used for starch processing, feed component and desulfurization of coal (van den Burg, 2003).

Alkaliphiles and acidophiles also produced dehalogenase enzyme. Table 3 summarized the pH of alkaliphilic dehalogenase and acidophilic organisms that they can survive in both conditions. *Pseudomonas putida* strain AJ1/23 produces HadD enzyme and able to withstand pH 9.5 (Smith *et al.*, 1989). Smith *et al.* (1990) isolated D-2-haloacid dehalogenase from *Pseudomonas putida* strain AJ1/23. The maximum enzyme activity ariseat pH 9.5 and 50 °C. Significant enzyme activity only happened in alkaline conditions which is dependable with the nature of the reaction mechanism. The enzyme activity lost below pH 5. The enzyme was stable at pH range from pH 6-9, and became fewer in strongly alkaline conditions. Hasan *et al.* (1994) studied the activity of *Pseudomonas* on D,L-2-chloropropionate (D,L-2CP) and 2-chloroacrylate (2-CAA). At pH 9.5 the maximum activity of D, L-2CP and pH 10 showed the maximum activities of the 2-CAA-induced enzymes.

A bacterium *Rhodococcus* sp. capable of utilizing 3-chloropropionic acid as a sole carbon source was isolated from Universiti Teknologi Malaysia agricultural soil area. Activity of the enzyme was measured by determining the release of chloride indicated by a colorimetric method employing mercuric thiocyanate. The dehalogenase enzyme was preceded rapidly at pH 6.0 and 9.0 (Jing and Huyop, 2007).

A *Pseudomonas* sp. strain S3, which can utilise a halogenated compound of D,L-2CP as sole carbon and energy source. The maximum activity of D-specific dehalogenase (DehD) enzyme on D-2CP was found at pH 9.5 at 35 °C. *Pseudomonas* sp. strain S3, which can utilize a halogenated compound of D, L-2CP as a carbon was also isolated. The maximum activity of D-specific dehalogenase enzyme was established at pH 9.5 (Thasif

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Table 3: Dehalogenase producing organisms from alkaliphiles and acidophiles conditions.

Environmental Factors	Organism	pH	Reference
High pH (Alkaliphile)	*Pseudomonas putida* strain AJ1/23	9.5	Smith *et al.* (1989)
	Pseudomonas putida strain AJ1/23	6 to 9	Smith *et al.* (1990)
	Pseudomonas	9.5 to 10	Hasan *et al.* (1994)
	Rhodococcus sp.	9	Jing and Huyop (2007)
	Pseudomonas sp. strain S3	9.5	Thasif *et al.* (2009)
	Agrobacterium tumefaciens C58	9.8	Hasan *et al.* (2011)
	Paracoccus sp. DEH99	10	Zhang *et al.* (2014)
Low pH (Acidophile)	*Sulfolobus tokodaii*	4 to 10	Bachas-Daunert *et al.* (2009)
	Pseudomonas fluorescens DSM 8341	4 to 10	Donnelly and Murphy (2009)
	Alcanivorax dieseloleistrain B-5	5.0 to 10.0	Li and Shao (2014)

Table 4: Dehalogenase producing organisms from halophilic conditions.

Environmental Factor	Organism	Salinity	References
Extreme salinity (Halophiles)	*Roseobacter* sp., *Paracoccus homiensis* sp., *Pseudomonas putida* sp. *Pseudomonas stuzeri* sp.	15%	Huang *et al.* (2011)
	Serratia sp.	1%	Abel *et al*. (2012)
	Raoutella ornithilolytica	1 %	Niknam *et al.* (2014)
	Bacillus sp., *Rhodococcus* sp., *Lysinibacillus* sp., *Microbacterium* sp. and *Aminobacter* sp.	8%	Khosrowabadi and Huyop (2014)
	Gelliodes sp.	-	Sufian *et al.* (2015)
	Pseudomonas aeruginosa MX1	-	Edbeib *et al.* (2016b)

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et al., 2009). Hasan *et al.* (2011) discovered a novel haloalkane dehalogenase, DatA that was isolated from the plant pathogen *Agrobacterium tumefaciens* C58. The effect of pH on the activity of DatA was examined by using 1, 3-Dibromopropane. The activity was accessed in the range of pH from 5 to 11, with the maximal activity at pH 9.8. Zhang *et al.* (2014) investigated 2-haloacid dehalogenase, Deh99 purified from *Paracoccus* sp. DEH99, derived from marine sponge *Hymeniacidon perlevis*. The optimal pH for Deh99 activity was pH 10.0.

A putative dehalogenase, $_L$-HAD$_{ST}$, identified by Bachas-Daunert *et al.* (2009) extracted from the thermophile microorganism *Sulfolobus tokodaii* was able to tolerate extreme pH conditions ranging from 4 to 10. Donnelly and Murphy (2009) studied the degradation of fluoroacetate. A *Pseudomonas fluorescens* DSM 8341 was isolated from soil and the dehalogenase enzyme was purified from cell-free extracts and was characterized. The enzyme was stable between pH 4 and 10. DadB enzyme was obtained from *Alcanivorax dieseloleistrain B-5.* The enzyme was active at low pH from pH 5.0 to 10.0. Furthermore, DadB was stable in buffers with pH above pH 7.5 (Li and Shao, 2014).

Halophiles

Halophiles can survive in hypersaline habitats by their ability to maintain osmotic balance. They can be found in lakes, oceans, salt pans or salt marshes. They accumulate salts such as sodium or potassium chloride (NaCl or KCl), up to concentrations that are isotonic with the environment. Proteins from halophiles have to cope with very high salt concentrations such as KCl concentrations close to 4 M and NaCl concentrations of > 5 M (Kumar *et al.*, 2011). According to the optimal salt concentration for growth, they are classified in three categories such as extreme halophile (grows in an environment with 3.4-5.1 M (20% to 30%) NaCl), moderate halophile (grows in an environment with 0.85-3.4 M (3% to 25%) NaCl) and slightly halophile (grows in an environment with 0.2-0.85 M (1% to 5%) NaCl) (Pikuta *et al.*, 2007). Halotolerant microorganisms do not show an absolute requirement for salt to grow but grow well in high salt concentrations (Siglioccolo *et al.*, 2011). Members of the family *Halobacteriaceae* have been isolated from different habitats including alkaline and salt lakes, marine salterns, the Dead Sea and saline soils. Some halophiles also are thermostable and tolerant to a wide range of pH (Dalmaso *et al.*, 2015). In addition, halophilic bacteria can be isolated from many sources such as fish, animal hides, domestic dishwasher, polar ice, spider webs in desert caves and anchovies (Edbeib *et al.*, 2016a).

There are several properties of halophiles to withstand in extreme condition. Halophiles have developed different adaptive strategies to support the osmotic pressure induced by the high NaCl concentrations in the environments they inhabit. Some extremely halophilic bacteria accumulate inorganic ions (K^+, Na^+, Cl^-) in the cytoplasm, which is a type of “salt-in” strategy to balance the osmotic pressure of the environment, and they have also developed specific proteins that are stable and active in the presence of salts (Dalmaso *et al.*, 2015). The high-salt-in strategy is an adaptation that protects halophiles from a saline environment in which they accumulate inorganic ions intracellularly to balance the salt concentration in their environment. This process involves Cl^- pumps that are found only in halophiles that transport Cl^- from the environment into the cytoplasm. Arginines and/or lysines are positioned at both ends of the channel to facilitate Cl^- uptake and release (Edbeib *et al.*, 2016a). Halophilic organisms contain enzymes that maintain their activity at high salt concentrations, alkaline pH and high temperatures (Siglioccolo *et al.*, 2011). Most proteins and enzymes denature when suspended in high salt concentrations. It is because, charge balance is important for protein stability, because a protein uses charged residues (as well as other factors) to fold and stay folded. Halophilic proteins bind significant amounts of salt and water. This characteristic is dependent on the number of acidic amino acids on the surface of the protein. The amino acid composition of halophilic enzymes generally contains abundant of acidic amino acid- like aspartic and glutamic acids, a low frequency of lysine, and a high occurrence of amino acids with a low hydrophobic character. Structural analyses between halophilic and mesophilic proteins reveal that the major differences are concentrated on the surface of the protein. These characteristics allow cooperation with electrostatic interactions and the presence of a higher number of salt bridges (Tadeo *et al.*, 2009).

Moreover, one of the halophile adaptation mechanism developed is the lipid composition. Structural adaptations have been observed in the surface layers (S-layers) of halophiles. The extreme halophile contains sulfated glucuronic acid residues and a higher degree of glycosylation, leading to an increased density in surface charges. This characteristic demonstrates an adaptation in response to the higher salt concentrations experienced by *Halobacterium salinarum.* Moreover, in *Haloarchaea*, some S-layer glycoproteins are enriched in acidic residues (Eichler, 2003).

The production of halophilic enzymes, such as xylanases, amylases, proteases and lipases has been reported for some halophiles belonging to the genera *Acinetobacter*, *Haloferax*, *Halobacterium*, *Halorhabdus*, *Marinococcus*, *Micrococcus*, *Natronococcus*, *Bacillus*, *Halobacillus* and *Halothermothrix* (Kumar et al., 2011). Table 4 summarized the dehalogenase bacteria that can withstand halophilic condition. Huang et al. (2011), isolated DEH 66, DEH 99, DEH125 and DEH138 from the marine sponge *Hymeniacidon perlevis* for degradation of 2-CP. Seven of the 11 isolates were able to degrade 2-CP at 8% salt, and four isolates (DEH 66, DEH 99, DEH 125 and DEH 138) degraded 2CP at 15% salt. The DEH 66 belongs to *Roseobacter* sp., DEH 99 was Paracoccus homiensis sp., DEH 125 was Pseudomonas putida sp. and DEH 138 belongs to *Pseudomonas stuzeri* sp.

Serratia sp. was isolated from soil surrounding lake water located in Universiti Teknologi Malaysia (UTM). *Serratia* sp. that was cultured in Luria-Bertani (LB) agar

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plates containing 1.0% sodium chloride (NaCl) has the ability to degrade 2,2-dichloropropionic acid (Abel *et al.*, 2012).

Niknam *et al.* (2014), isolated new bacteria species which are capable to utilizing 2,2-dichlropropionic acid (2,2DCP) as a sole carbon source from the wastewater sample that was taken from Tioman Island off the coast of Malaysia. The bacteria that was cultured in 1.0% sodium chloride (NaCl) was designated as an aerobic bacillus *Raoutella ornithilolytica.*

Khosrowabadi and Huyop (2014) isolated bacterial from marine sediments collected at Danga Bay and east coast of Singapore. The growth medium was spread on plates containing 20 mM 2,2DCP plus NaCl (8%). The 16S rRNA analysis suggested that the isolated bacteria had more than 96% sequence identity to the sequence in the NCBI database, therefore, they were designated as *Bacillus* sp., *Rhodococcus* sp., *Lysinibacillus* sp., *Microbacterium* sp., and *Aminobacter* sp.

Sufian *et al.* (2015) isolated bacteria from a marine organism. In this study, a 3CP-degrading bacterium designated as *Bacillus* sp. strain H4 was successfully isolated from sponge, *Gelliodes* sp. that capable of degrading 3CP as the sole carbon and energy source. The bacteria growth on on solid minimal media containing 10 mM 3CP was the evident for 3CP utilisation.

Edbeib *et al.* (2016b) presented a study of characterization of *Pseudomonas aeruginosa* MX1 that can degrade 2,2DCP. *Pseudomonas aeruginosa* MX1 was isolated from seawater at Desaru, located in the southern coast of Malaysia. There was a high tendency of contamination with halogenated compounds within this stipulated area due to the surrounding agricultural wastewater flow into the sea. The MX1 strain grew best in a 20 mM 2,2DCP minimal medium as the sole carbon source and illustrated a 44 ± 0.2 h cell-doubling time as well as a 38 mmol Cl^-/mL maximum rate of chloride ion release. However, no attempt was made to test on the ability to grow in extreme salinity.

RESEARCH PERSPECTIVE

Several extremophiles microbes with the ability to degrade halogenated compound have been elucidated because of their potential to survive at extreme conditions. It was evident that the use of enzymes isolated from extremophiles offers the prospect to access enzymes that are stable in various conditions such as high temperatures, low temperatures, extreme salt concentration and pH, which can make them more suitable to the industrial environments. Thermophiles, psycrophiles, acidophiles, alkaliphiles and halophiles generally have molecule and membrane stability, produce enzymes or protein and have suitable mechanisms that can survive at extreme conditions. The discovery of new extremophilic species provides a route to new enzymes, with the possibility that this will lead to novel applications. Furthermore, extremophilic enzymes are becoming an essential source of new industrially robust biocatalysts. Nevertheless, this knowledge is still lacking. Only a minor fraction of the microorganisms on earth have been exploited. Thus, the most significant is a current lack of information in basic research, development of appropriate molecular tools as well as looking into a better insight of structure and functions of these extremophile enzymes for biotechnological interest to combat pollution.

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