ADVANCES IN BIOSCIENCES

VOLUME 1

Enzymes are macromolecular biological catalysts that is important to speed up reaction. Enzymes can be found naturally from the microorganisms such as bacteria, fungi and yeast which may not be genetically modified. In this perspective, resorting to bioremediation to clean-up such environment may prove feasible and beneficial as microorganisms effective in degrading such substances. The liberation of excess halogenated compounds into the environment is becoming a major global issue, since the toxic contaminants tend to accumulate and persist in the biosphere. Some of the microbes that able to utilise these toxic compounds have been successfully isolated from the soil contaminated environment and were further characterised. Apart from pollutant degradation, commercial enzyme like protease will be discussed that has potential for commercialization in many manufacturing processes.

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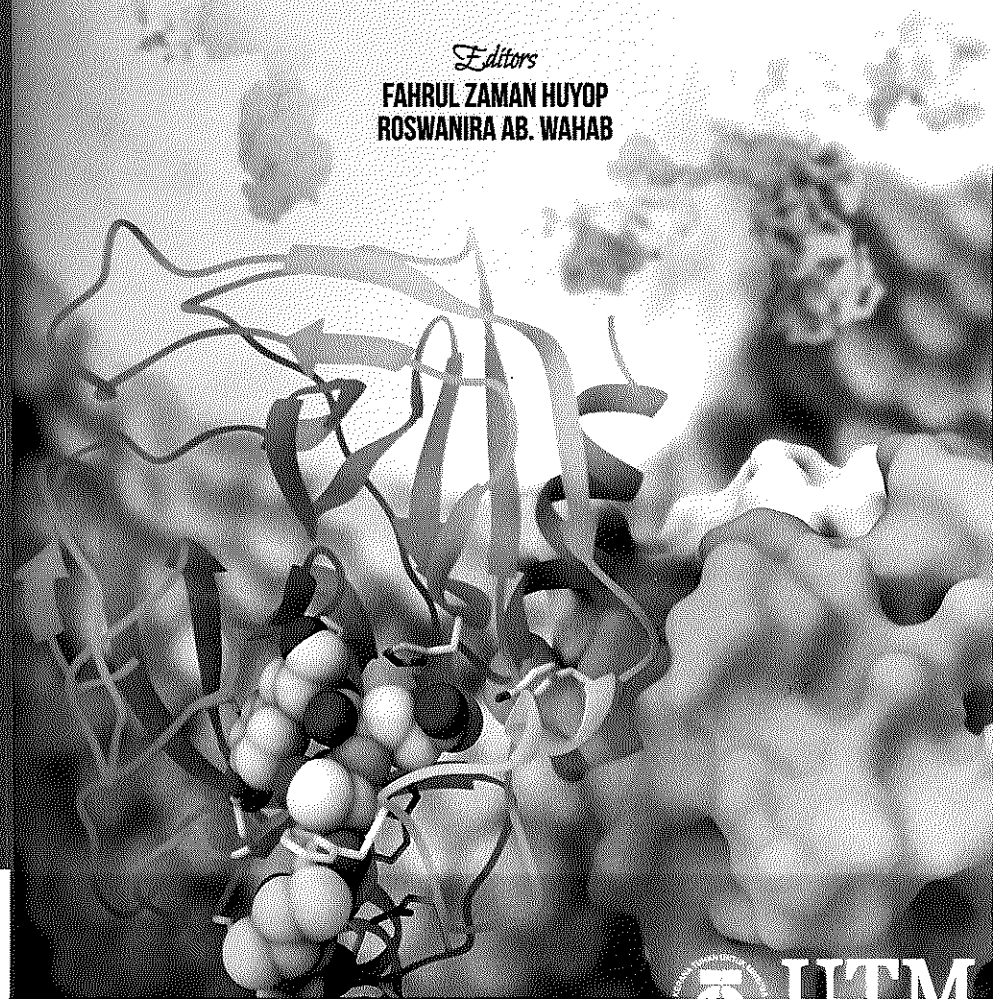

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Basic Analysis of L-specific Microbial Dehalogenases

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Azzmer Azzar Abdul Hamid

2.1 INTRODUCTION

The term xenobiotic was derived from the Greek words xenos (foreigner, stranger) and bios or vios (life) (Mansuy, 2013). However, it was very often used in the context of pollutants and their effect on the entire biological system. Xenobiotics may be classified as antioxidants, carcinogens, drugs, environmental pollutants, food additives, hydrocarbons, and pesticides.

2.2 HALOGENATED COMPOUNDS

Carbon-halogen compounds were largely used as herbicides, pharmaceuticals, fungicides, insecticides and intermediate in organic synthesis, produced in industrial-scale chemical processing and naturally were released in the biosphere. Halogenated compounds are one of the persistent productions that have been caused the environmental contaminations (Gribble, 1992; Swanson, 1999). Stockholm Convention had recognized Persistent Organic Pollutants (POPs), include polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and tributylin (TBT) which survived in the environment for a long time,

bioaccumulate and biomagnified, and in many cases were toxic to both wildlife and humans (Voulvoulis and Georges, 2015).

2.3 MICROBIAL DEGRADATION OF HALOGENATED COMPOUNDS

Generally, biodegradation often been affected due to the lack of efficient metabolic pathways resulting to recalcitrance. Nevertheless, a huge number of microorganisms have the capability to degrade organic pollutants. Biodegradation was an effective approach for the removal of contaminants in the environment (Uhlik *et al.*, 2013). Yang *et al.* (2013) had described BDE-209 degradation by microbial electricity generation. There were two important features to study dehalogenases, first to identify with the variety of α or β haloalkanoic dehalogenase (Hill *et al.*, 1999). Second, a number of isolated microorganisms able to produce multiple dehalogenases and this is important to control dehalogenase gene regulation (Huyop and Cooper, 2014).

2.4 TYPE OF HALOACID DEHALOGENASE

In general, classification of dehalogenases comprised of three major grouping based on reaction mechanisms or by substrate specificities (Figure 2.1). The structurally characterized as haloalkane dehalogenases (Pries *et al.*, 1994), haloalkanoic acid dehalogenases (Slater *et al.*, 1997) and 4-chlorobenzoyl-coenzyme A dehalogenases (Yang *et al.*, 1994) used substitution mechanisms that proceed via a covalent aspartyl intermediate. Hydrolytic dehalogenases were very common among all dehalogenases. Figure 2.1 shows an overview of dehalogenase division.

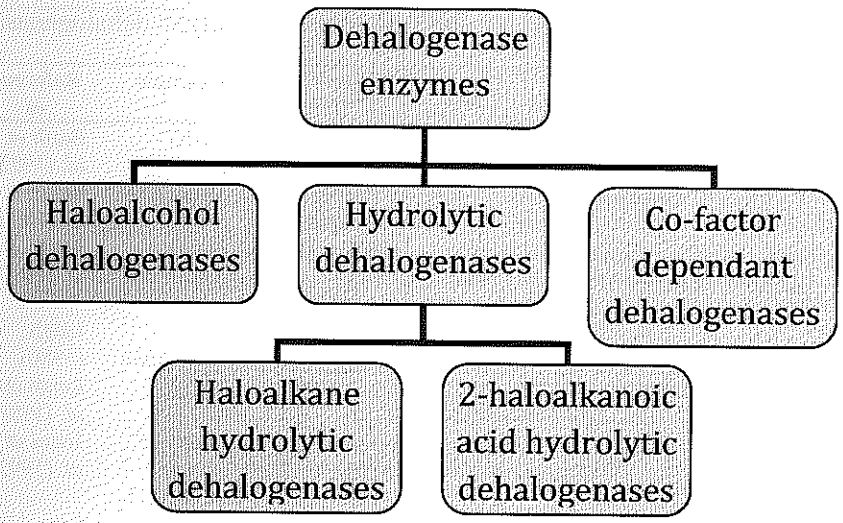


Figure 2.1 Type of dehalogenase enzymes

2.5 CLASSIFICATION OF DEHALOGENASES BY SUBSTRATE SPECIFICITY

The most common dehalogenation mechanism was hydrolysis reaction for removal of halogens from aliphatic compounds. Hydrolytic dehalogenase comprised of two subgroups which were 2-haloalkanoic acid hydrolytic and haloalkane hydrolytic dehalogenases. 2-haloalkanoic acid dehalogenases were divided into class 1 (stereospecific) or class 2 (non-stereospecific) and further subdivided into Class 1D, Class 1L, Class 2I and Class 2R, respectively, as in Table 2.1 (adapted from Slater *et al.*, 1995; Slater *et al.*, 1997).

This biological method is economical, safer and environmentally friendly, hence give a suitable choice to dispose these environmental contaminants. Considering the negative effect to man and the environment associated with the abundant deposition of these halogenated compounds, there is need to isolate and identify more species of microorganisms that can degrade these compound to aid in their removal from the environment. However, various species of microorganisms that can degrade halogenated compounds from contaminated soil have been documented but very few from contaminated water.

5.2 GROWTH CONDITION OF BACTERIA SA1

Bacteria SA1 previously isolated from contaminated waste water was successfully sub-cultured onto nutrient agar (NA) at 30°C for 24 hours. The growth of the bacterial strain was observed on nutrient agar plate (Figure 5.1). The bacterial culture was then inoculated into three flasks containing, 10 mM, 20 mM and 30 mM 2,2-dichloropropionic acid (2,2-DCP) as the sole source of carbon and energy. After 24 hours of incubation, 0.1 mL of aliquot from each flask were spread onto their respective solid minimal media containing 10 mM, 20 mM and 30 mM 2,2-DCP as a carbon source. After 5 to 8 days of incubation, the colonies formed was further characterised as reported by Abel *et al.* (2012).

5.3 GRAM- AND SPORE STAINING

The Gram-staining and spore staining were observed under light microscope at 100X magnification using oil immersion (Figure 5.2). Table 5.1 summarised all observations. Spore staining indicated that strain SA1 has no spore. Spore forming bacteria like *Clostridium*and *Bacillus* possess a green endospore enclosed in a pink sporangium (Cowan *et al.*, 2004). However, bacteria SA1 when observed under microscope showed only pink colour, no

green colour was seen as reported in previous literature (Figure 5.2 b.).

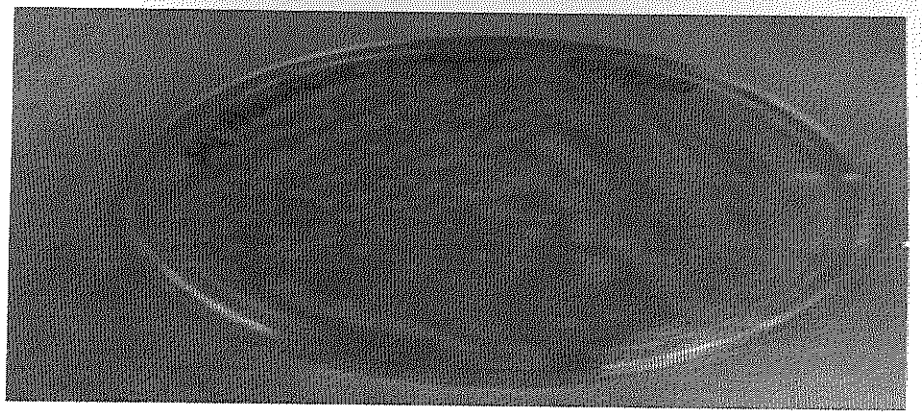
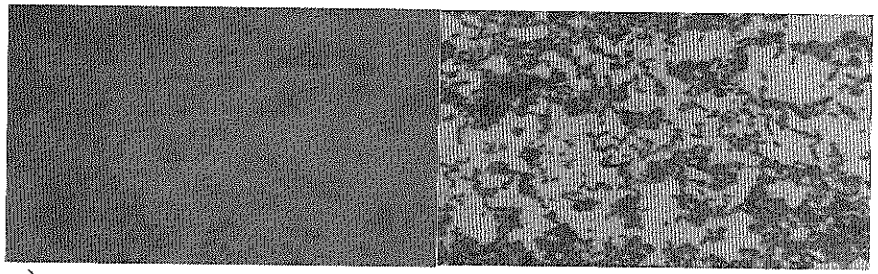


Figure 5.1 Bacteria SA1 on nutrient agar media growth at 30°C after 16 hours incubation



a) b)

Figure 5.2 Bacteria SA1 (a) Gram staining (b) spore staining

Table 5.1 Strain SA1 characterisation

Characteristic	Observation
Gram	Negative
colour	Pink
shape	Rod
spore	Negative