ADVANCES IN BIOSCIENCES

VOLUME 2

Salts of 2,2-dichloropropionic acid (2,2DCP) or Dalapon are well known active components of herbicides, regularly found as contaminants in the water system and soil. Nonetheless, continuous consumption of water contaminated with such substances may pose significant threat to living organisms especially mammals. Fortunately, these toxic substances can only be eliminated from the environment by the reaction of certain microbes. These microbes that possess the ability to degrade such substances have been successfully isolated from contaminated environment and characterized by molecular analysis for identification of their genus and species. Since salts of 2,2DCP have been found toxic, the substance has been used as model substrate for studying the catalytic and non-hydrolytic properties of microbial dehalogenases.

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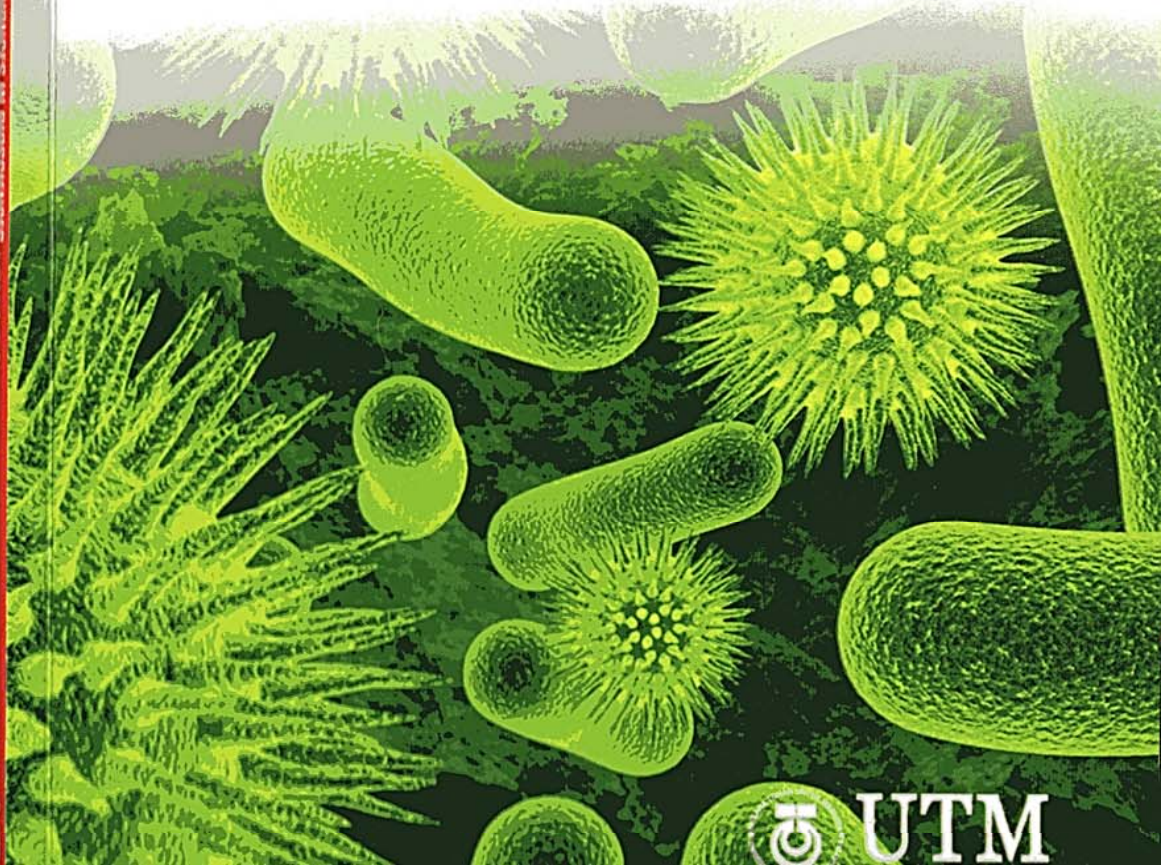

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Contents

List of Contributors

Fahrul Zaman Huyop
Mabrok Ali DauSheha
Mohamed Faraj Edbeib
Nastaran Rizan
Nur Amina Anis Abd Manan
Wong Wen Yong
Universiti Teknologi Malaysia,
Skudai, Johor

Rohaya Megat Abdul Wahab
Shahrul Hisham Zainal Ariffin
Wong WoanYeen
Universiti Kebangsaan Malaysia,
Bangi, Selangor

Azzmer Azzar Abdul Hamid
Mohamad Noorshafik Shudin
Tengku HaziyaminTengku Abdul Hamid
International Islamic University Malaysia,
Kuantan, Pahang

Intan Zarina Zainol Abidin
Centre for Graduate Studies
Research & Commercialization, Cyberjaya University College
of Medical Sciences, Cyberjaya (CUCMS),
Selangor

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Taxonomical Analysis of β-Haloalkanoic Acid-Degrading Bacteria

Mohamad Noorshafik Shudin, Tengku Haziyamin Tengku Abdul Hamid and Azzmer Azzar Abdul Hamid

2.1 INTRODUCTION

The advancement in industrial technology and rapid progression of agricultural practices has caused the production of various xenobiotic compounds that function as solvents, hydraulic or transfer fluids, herbicides, pesticides, fungicides, and insecticides (Fetzner and Lingens, 1994). Interestingly, many environmentally important xenobiotic compounds that are present in the pesticides and herbicides are halogenated, especially chlorinated (Chaudhry and Chapalamadugu, 1991). The widespread use of pesticides and herbicides containing derivatives of halogenated organic compounds such as α- and β-haloalkanoic acid are the main contributors to the environmental pollution especially to the soil, groundwater and water reservoir due to their recalcitrance and resistance to microbial degradation (Chaudhry and Chapalamadugu, 1991; Fetzner, 1998; Hill *et al.*, 1999; Olaniran *et al.*, 2001; Slater *et al.*, 1995).

In addition, the toxicity level of these halogenated organic compounds promotes possible adverse health effects on human and also ecosystem (Sinha *et al.*, 2011; Van Pee and Unversucht, 2003). Therefore, the concerns to maintain the xenobiotic concentration in soil at low level have become

popular among scientists and environmentalist (Jing *et al.*, 2008). The halogenated organic compounds can be transformed into harmless products using non-biological or biological degradation. Biological degradation by microorganisms are favoured because they are economical, safer and environmentally friendly (Mesri *et al.*, 2009). In this preceding chapter, three different isolates were identified from the agricultural area in Kuantan, Pahang which could grow on 3-Chloropropionic acid (3CP) or known as β-haloalkanoic acid. It was then further characterised by basic growth analysis and identification of bacteria was conducted by using molecular identification technique supported by morphological and biochemical tests.

2.2 THE GROWTH OF 3-CHLOROPROPIONIC ACID DEGRADING BACTERIA

Several morphologically different colonies were observed after conducting the spread and streak plate techniques. Colonies were formed after 4 to 5 days of incubation at 30°C. After repeatedly subcultured, only three of them grew well in 10 mM of 3-Chloropropionic acid (3CP) solid minimal medium. These bacteria were designated as bacterium SS1, bacterium SS2 and bacterium SS3. All three isolated bacteria were able to grow well on the plate, proving the presence of dehalogenase enzyme and their ability to degrade 3CP as their sole carbon and energy source. Colonies morphologies were summarized in Table 2.1.

Table 2.1 Colony morphologies of bacteria on 10 mM of 3-Chloropropionic acid minimal media

Morphology	Bacteria		
	SS1	SS2	SS3
Age	48 hours	24 hours	48 hours
Size	Punctiform	Large	Punctiform
Shape	Circular	Irregular	Circular
Pigmentation	Pale yellow	Cream	White
Elevation	Raised	Raised	Raised
Margin	Entire	Irregular	Entire
Optical property	Opaque	Transclucent	Transclucent

In subsequent analysis, bacterium SS1, bacterium SS2 and bacterium SS3 were grew in duplicate in 100 mL of 10 mM of 3CP liquid minimal media at 30°C at 200 rpm in shaker incubator for five days. Figure 2.1 illustrated the growth profile curve of bacterium SS1, bacterium SS2 and bacterium SS3. From the growth curve, the exponential phases of each growth profile curve were different based on the curve. Both bacteria, SS1 and SS3 were analysed to be in the exponential phase for about 60 hours from 36 hours to 96 hours, and from 48 hours to 108 hours respectively. Additionally, both bacteria reached their death phase at 120 hours. However, a different trend was observed for bacterium SS2. Bacterium SS2 showed the shortest exponential phase, from 72 hours up to 108 hours which about 36 hours as compared to bacterium SS1 and bacterium SS3.

The maximum turbidity of all the isolated bacteria was measured at similar hour, which was 108 hours. Based on Table 2.2, bacterium SS3 revealed the highest maximum absorbance of 0.1432 ± 0.0007 at A_{600nm}, absorbance of 0.1406 ± 0.0001 for bacterium SS2 as second highest and the lowest was bacterium SS1 that was 0.1100 ± 0.0033. The exponential phases of each isolated 3CP degrading bacteria were used to construct the doubling time curve. Gradient values were taken and the doubling time were calculated and recorded in Table 2.2.

Comparison was made between the doubling times of the three isolated bacteria. Data (Table 2.2) stated that, bacterium SS2 had the shortest doubling time, 26.41 hours when compared to bacterium SS1 and bacterium SS3. Bacterium SS1 was found to have longest doubling time, 35.42 hours whereas bacterium SS3 showed doubling time of 28.13 hours.

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