Journal of Engineering and Applied Sciences 12 (17): 4306-4309, 2017
ISSN: 1816-949X

Isolation and Characterization of a Novel 2, 2-DCP-Degrading *Achromobacter* sp. M×2 Isolated from Contaminated Seawater of Desaru Beach, Southern Malaysia

[1]Mohammed Faraj Edbeib, [2]Roswanira Abdul Wahab and [1]Fahrul Huyop
[1]Department of Biotechnology and Medical Engineering,
Faculty of Biosciences and Medical Engineering,
Universiti Teknologi Malaysia (UTM), 81310 Johor Bahru, Malaysia
[2]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM),
81310 Johor Bahru, Malaysia

Abstract: Halogenated Organic Compounds (HOC) are major pollutants in the environment due to their wide usage as biocides in the agricultural industry. Hence, it is important that safe methods to eliminating such compounds are developed. Consequently, the goal of this study is to isolate and identify bacterial species capable of utilizing 2,2 Dichloropropionic acid (2,2-DCP). Using enrichment culture, a bacterium strain designated as M×2 was isolated from contaminated seawater located at desaru beach, Malaysia. The isolate grew best at 20 mM of 2,2-DCP minimal media with doubling time of 13.9 h. More than 0.59 mmol/L chloride ions were detected in the growth medium. Morphological and biochemical characteristics of M×2 were closely matched to those of *Achromobacter* sp. and the 16S rDNA sequence (GenBank accession number KP336491) 98% identical to that of several species of the genus *Achromobacter*. This is one of the first studies detailing the ability of *Achromobacter* sp. to utilize 2,2-DCP as the sole source of carbon and energy.

Key words: Bioremediation, 2,2-dichloropropionic acid, *Achromobacter* sp., 16S rDNA, phylogenetic analysis, sole source

INTRODUCTION

The accumulation of xenobiotic compounds in the environment is the result of both natural and human activities. Halogenated organic compounds constitute one of largest group of these pollutants (Haggblom *et al*., 2000) and are used extensively as biocides. According to World Health Organization (1990) approximately 2-3% of the applied pesticides are used in mitigating pest in agriculture industry. However, the rest of pesticides remain in soil and can potentially pollute the underground water and harm the ecosystem.

Among the halogenated organic compounds, 2,2-Dichloropropionc acid (2,2-DCP) or commercially known as dalapon is widely used to control the growth of seasonal weeds especially grasses and monocots (Ware, 1989). The compound 2,2-DCP is highly toxic and resistant to degradation (Van Pee *et al*., 2003) and which can adversely affect marine habitats including commercial and marine fisheries located along the coastal areas (Islam and Tanak, 2004). Hence, the recalcitrance of such substance is as much a local problem as well as a global concern as its persistence may prove detrimental to human health.

Fortunately, certain microorganisms are reportedly able to utilize 2,2-DCP as sole source of carbon and energy, owing to their ability to produce dehalogenases that remove halogens (Hardman, 1991). These microorganisms are widely distributed and are usually found in soils that contain naturally occurring halogenated compounds (Mesri *et al*., 2009). Previous studies have shown that 2,2-dichloropropionc acids can be degraded by a variety of dehalogenase producing microorganisms via a biotransformation process that cleaves the carbon-halogen bond (Jing and Huyo, 2008). Once the halogen is liberated, the carbon atoms can then be utilized for the microbial growth and energy.

MATERIALS AND METHODS

Growth conditions: The 100 mL of minimal media containing 2,2-DCP as the only source of carbon and energy was used to incubate the bacterial culture. Liquid minimal media was made up of 10X concentration basal salts containing $NaH_2PO_4.2H_2O$ (10.0 g/L), $K_2HPO_4.3H_2O$ (42.5 g/L) $(NH_4)_2SO_4$ (25.0 g/L). Minimal media also consisted of 10X concentration trace metals containing $C_6H_9NO_6$ (1.0 g/L), $MgSO_4$ (2.0 g/L)

Corresponding Author: Roswanira Abdul Wahab, Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM), 81310 Johor Bahru, Malaysia

$FeSO_4.7H_2O$ (120.0 mg/L), $MnSO_4.4H_2O$ (30.0 mg/L), $ZnSO_4.H_2O$ (30 mg/L) and $CoCl_2.6H_2O$ (10 mg/L). All components were dissolved in distilled water (Hareland *et al*.,1975). Basal salts (10 mL) and trace metals (10 mL) were added to 80 mL of distilled water and autoclaved (12°C for 15 min at 15/psi). The carbon source (2,2-DCP) was filter sterilized separately using a 0.2 im nylon-membrane and added aseptically to the media. Using DELTA 320 pH meter, the pH of the media was adjusted to 7.5±0.2 by addition of sodium hydroxide (NaOH) and/or Hydrochloric acid (HCl). The flask was incubated at 30°C in a rotary incubator at 150 rpm.

Growth measurements and halide ion assay: Growth rate of the isolate was determined colorimetrically. After inoculation an aliquot of sample (1 mL) was taken periodically (every 12 h) and turbidity was measured at $A_{600\ nm}$ using pye-unicam SP 1750 series spectrophoto meter. Measurement of released halide ions was carried out in accordance with the method of (Bergmann and Sani, 1957). The sample (1 mL) was added to a mixture containing 100 μL of 0.25 M ammonium ferric sulphate and 9 M nitric acid. After thorough mixing, 100 μL of mercuric thiocyanate-saturated ethanol solution was added and vortexed. The color was allowed to develop for 10 min and measured at $A_{460\ nm}$.

Morphological screening and biochemical tests: In order to ascertain the identity of the isolate, staining and biochemical tests were carried out. Gram staining and motility observation were among the morphological investigation. Whereas, biochemical analysis covered the following tests: citrate, catalase, urease, oxidase, indole, gelatin liquefaction, methyl red, vogues proskaeur, nitrate reduction and carbohydrate fermentations.

Amplification of the 16S rDNA gene and building of phylogenetic tree: Polymerase Chain Reaction (PCR) was carried out using BIO-RAD™MyCycler thermal cycler. 16S rDNA gene sequence was targeted by universal primers as proposed by Fulton and Cooper (2005) Fd1 (5'-AGA GTT TGA TCC TGG CTC AG-3') and rP1 (5'-ACG GTC ATA CCT TGT TAC GAC TT-3'). Total volume of the amplification reactions was 25 μL which consisted of 12 μL PCR master mix (Fermentas Inc. USA) 1 μL template DNA, 1 μL forward primer (Fd1),1 μL reverse primer (rP1) and 10 IL nuclease free water. The 16S rDNA Gene amplification was performed for 30 cycles whereby each cycle was set as initial denaturation 94°C for 5 min, followed by denaturation 94°C for 1 min, annealing 55°C for 1 min and final extension 72°C for 10 min. Obtained 16S rDNA sequences were aligned with other homologus sequences using ClustalW server. The output data was used to reconstruct the phylogenetic tree using MEGA 6 Software (Tamura *et al*., 2013).

RESULTS AND DISCUSSION

Bacterial isolation, screening, morphological and biochemical analysis: A bacterium designated as M×2 was isolated and grew aerobically at 30°C in liquid minimal media containing 20 mM of 2,2-DCP as the only source of carbon. No growth was observed in the control medium (without 2,2-DCP). Morphological and biochemical characteristics of M×2 are listed in Table 1.

Degradation of 2,2-DCP and halo ion assay: To determine the optimum degradation c oncentrations of the M×2, 4 different concentrations of 2,2-DCP were prepared to quantify the release of chloride ions in the growth culture. Summary of the M×2 growth profile in various concentrations of 2,2-DCP is listed in Table 2.

The 16S rDNA gene analysis and phylogenetic tree construction: The length of 1392 bp 16S rDNA gene of M×2 (GenBank accession number KP336491) was amplified (Fig. 1). The PCR product was purified and sequenced. Similarity search revealed that M×2 shares

Table 1: Morphological and biochemical properties of strain M×2

Tests	Results
Culture characterization on agar plate	
Colonies	Watery
Color	Gray-translucent
Form	Convex
Margins	Entire
Elevation	
Growth	Abundant
Density	Opaque
Appearance	Mottled-mucoid
Morphological characterization	
Shape	Rod, curved
Arrangement	Single, diploid
Gram staining	-
Spore staining	-
Consistency	Butyrous
Motility	+
Growth on broth	
Clouding	Heavy
Surface growth	Distributed
Sediment	Small amount
Biochemical tests	
Catalyze	+
Urease	+
Oxidase	+
Nitrate reduction	+
Methyl red	-
Gelatin liquefaction	-
Indole production	-
Vogues proskaeur	+
Citrate utilization	+
Carbohydrate/fermentation	
Maltose	
Mannitol	
Glucose	+
Sucrose	-

Table 2: Growth profile of M×2 at various 2,2-DCP concentrations and correspondent chloride ion released

	10 mM			20 mM			30 mM			50 mM
Isolate	[1]M.A.	[2]D.T.	[3]Cl.$^-$	[1]M.A.	[2]D.T.	[3]Cl$^-$	[1]M.A.	[2]D.T.	[3]Cl.$^-$	[4]NG
M×2	0.388±0.002	14.3	0.23	0.577±0.002	13.9	0.59	0.150±0.002	15.3	0.14	

[1] Maximum absorbance at A_{600nm}; [2] Doubling time (h); [3] Chloride ion released (mmol/L) at A_{460nm}; [4]NG: No growth

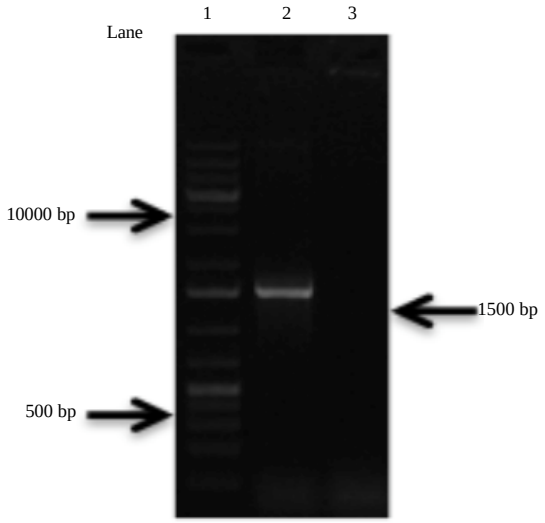

Fig. 1: PCR amplification of 16S rDNA gene on agarose gel (0.5%). Lane 1: 1 kb DNA ladder; Lane 2: an approximately 1.5 kb amplified 16S rDNA fragment; Lane 3: control (without Fd1 primer)

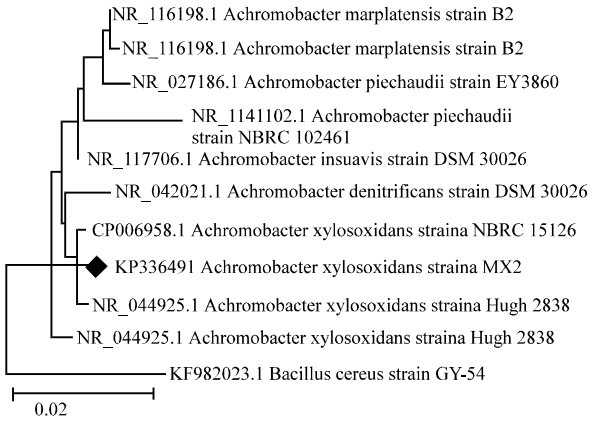

Fig. 2: phylogenetic trees showing the evolutionary relationship between M×2 and other bacteria in the same genera. The sequence of *Bacillus cereus* GY-54 was used as the out-group. Scale bar represents 0.02 substitutions per site

98% identity to the genus of Archomobacter. The first ten species were selected and used to construct the phylogenetic tree (Fig. 2).

In the current investigation, 2,2-DCP degrading bacterium (M×2) was successfully isolated from contaminated seawater at the Desaru Beach, Malaysia. Morphological and biochemical characteristics (Table 1) of M×2 were very similar to those of the Achromobacter reported strains (Guo *et al*., 2008; Farajzadeh and Heidari, 2012). Further, investigations using 16S rDNA sequence and phylogenetic tree analysis revealed the gene sequence of M×2 was highly similar to *Achromobacte* sp.

And was closely related to *Achromobacter* xylosoxidanas (CP_006958.1) (Fig 2). Thus, strain M×2 was identified as *Achromobacter* xylosoxidanas M×2. The determined 16S rDNA sequence of strain M×2 was deposited in the GenBank nucleotide sequence databases under accession number KP336491.

The *Achromobacter* sp. degraded different concentrations of 2,2-DCP at varying efficiencies. Based on the bacterial growth profile, the highest degradation measured by the presence of chloride ion in the media, occurred at 20 mM 2,2-DCP corresponding to a doubling time of 13.9 per hour.

Increasing the concentration of 2,2-DCP resulted in the decline of the degradation rate to 0% at 80 mM (Table 2). These findings are in accordance with previous studies concerning inhibition of bacterial growth at 2,2-DCP concentrations of >50 mM (Wong and Huyop, 2012) presumably due to the increased toxicity of the substrate. The maximum chloride ion released (0.59 mmol/L) occurred at the concentration of 20 mM (Table 2) which suggests the highest concentration of 2,2-DCP tolerated by the *Achromobacter* sp.

Earlier studies have reported several strains of *Achromobacter* sp. isolated from sediments and soil exhibited abilities to degrade xenobiotics, i.e., *Achromobacter xylosoxidans* Ns capable of assimilating p-nitrophenol (Wan *et al*., 2007) the *A. xylosoxidans* B-16 utilized bisphenol A as its only source of carbon and energy (Zhang *et al*., 2007) and *A. xylosoxidans* CS5 successfully degraded xenobiotics in both water and soil environments (Li *et al*., 2009). The *Achromobacter* species are well known for their ability to degrade xenobiotics at various terrestrial environments (Ronen *et al*., 2000; Janbandhu and Fuleka, 2011) but are unsuccessful in degrading halogenated compounds in marine environments. The inability of the bacterial species to degrade these substances could be attributed to the relative oligotrophy and high salinity of the marine

environment. Thus, strain M×2 is the first reported *Achromobacter* species able to degrade 2,2-DCP in marine environment.

CONCLUSION

A marine 2,2-DCP-degrading bacterium M×2 identified as *Achromobacter* sp. was isolated from contaminated seawater located at Desaru Beach, Malaysia. The growth profile of the *Achromobacter* sp. showed the bacteria possess an excellent 2,2-DCP degrading ability and exhibited good adaptability to saline environment. Therefore, this novel *Achromobacter* sp. may be used as a cost-effective and eco-friendly biocatalyst to eliminate hazardous halogenated compounds that persist and contaminate the marine environment.

ACKNOWLEDGEMENT

This research was supported by the Exploratory Research Grant Scheme from the Ministry of Higher Education Malaysia (FRGS 4F649). We would also like to acknowledge valuable help and suggestions provided by our colleagues.

REFERENCES

Bergmann, J.G. and J.J. Sanik, 1957. Determination of trace amounts of chlorine in naphtha. Anal. Chem., 29: 241-243.

Farajzadeh, Z. and K.H.R. Heidari, 2012. Isolation and characterization of a new *Achromobacter* sp. strain CAR1389 as a carbazole-degrading bacterium. World J. Microbiol. Biotechnol., 28: 3075-3080.

Fulton, C.K. and R.A. Cooper, 2005. Catabolism of sulfamate by Mycobacterium sp. CF1. Environ. Microbiol., 7: 378-381.

Guo, W., D. Li, Y. Tao, P. Gao and J. Hu, 2008. Isolation and description of a stable carbazole-degrading microbial consortium consisting of Chryseobacterium sp. NCY and *Achromobacter* sp. NCW. Curr. Microbiol., 57: 251-257.

Haggblom, M.M., V.K. Knight and L.J. Kerkhof, 2000. Anaerobic decomposition of halogenated aromatic compounds. Environ. Pollut., 107: 199-2070.

Hardman, D.J., 1991. Biotransformation of halogenated compounds. Crit. Rev. Biotechnol., 11: 1-40.

Hareland, W.A., R.L. Crawford, P.J. Chapman and S.T.A.N.L.E.Y. Dagley, 1975. Metabolic function and properties of 4-hydroxyphenylacetic acid 1-hydroxylase from Pseudomonas acidovorans. J. Bacteriol., 121: 272-285.

Islam, M.S. and M. Tanaka, 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. Mar. Pollut. Bull., 48: 624-649.

Janbandhu, A. and M.H. Fulekar, 2011. Biodegradation of Phenanthrene using adapted microbial consortium isolated from petrochemical contaminated environment. J. Hazard. Mater. 187: 333-340.

Jing, N.H. and F. Huyop, 2008. Enzymatic dehalogenation of 2,2-dichloropropionic acid by locally isolated Methylobacterium sp. HJ1. J. Biol. Sci., 8: 233-235.

Li, W., Y. Dai, B. Xue, Y. Li and X. Peng et al., 2009. Biodegradation and detoxification of endosulfan in aqueous medium and soil by *Achromobacter* xylosoxidans strain CS5. J. Hazard. Mater., 167: 209-216.

Mesri, S., R.A. Wahab and F. Huyop, 2009. Degradation of 3-chloropropionic acid (3CP) byPseudomonas sp: B6P isolated from a rice paddy field. Ann. Microbiol., 59: 447-451.

Ronen, Z., L. Vasiluk, A. Abeliovich and A. Nejidat, 2000. Activity and survival of tribromophenol-degrading bacteria in a contaminated desert soil. Soil Biol. Biochem., 32: 1643-1650.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar, 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol., 30: 2725-2729.

Van Pee, Karl-Heinz and S. Unversucht, 2003. Biological dehalogenation and halogenation reactions. Chemosphere, 52: 299-312.

Wan, N., J.D. Gu and Y. Yan, 2007. Degradation of p-nitrophenol by *achromobacter* xylosoxidans Ns isolated from wetland sediment. Int. Biodeterior. Biodegrad., 59: 90-96.

Ware, G.W., 1989. Drinking Water Health Advisories for Pesticides. Springer, Berlin, Germany,.

Wong, W.Y. and F. Huyop, 2012. Molecular identification and characterization of dalapon-2, 2-dichloropropionate (2, 2DCP)-degrading bacteria from a Rubber Estate Agricultural area. Afr. J. Microbiol. Res., 6: 1520-1526.

World Health Organization, 1990. Public Health Impact of Pesticides in Agriculture. WHO., Geneva.

Zhang, C., G. Zeng, Y. Li, J. Yu and J. Li *et al*., 2007. Aerobic degradation of bisphenol A by *Achromobacter* xylosoxidans strain B-16 isolated from compost leachate of municipal solid waste. Chemosphere, 68: 181-190.