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Isolation and identification of bacteria isolated from ruminant animal waste that able to degrade 2,2-dichloropropionic acid (2,2-DCP)

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ABSTRACT

Aims: A 2,2-dichloropropionic acid (2,2-DCP) naturally degrading bacterial species, strain SN1 was successfully isolated from cow dung capable of utilizing the substance as the sole carbon source and energy.
Methodology and results: Strain SN1 was preferred over other strains (SN2, SN3 and SN4) following observations on its rapid growth in 20 mM 2,2-DCP liquid minimal media. Since strain SN1 clearly exhibited tolerance towards 2,2-DCP, its growth in various concentrations (10 mM, 20 mM, 30 mM and 40 mM) of the substance was evaluated. The study found the bacteria grew particularly well in 20 mM 2,2-DCP with the highest chloride release of 39.5 µmole Cl^-/mL while exhibiting a remarkably short doubling time of 3.85 h. In view of such notable characteristics, species identification *via* Biolog GEN III system and 16S rRNA analysis was performed and established strain SN1 as *Bacillus cereus*.
Conclusion, significance and impact of study: Considering the rapid growth of *B. cereus* strain SN1 in such medium, its employment as a bioremediation agent to treat 2,2-DCP contaminated soils may prove beneficial. Moreover, this is the first reported case of a *Bacillus* sp. isolated from cow dung capable of utilizing 2,2-DCP. Therefore, further assessment into its ability to degrade other types of haloalkanoic acids merit special consideration.

Keywords: Cow dung, halogenated compound 2,2-dichloropropionic acid, bioremediation *Bacillus cereus*

INTRODUCTION

The ever growing human population has resulted in the mass production of a myriad of halogenated compounds pertinently used in pharmaceutical and agricultural management (herbicides, fungicides, insecticides) as well as intermediates in organic solvents etc. However, the large presence of such substances in the environment may lead to undesirable consequences pertaining to health problems in humans and pollution due to their toxicity and recalcitrance, respectively (Pee and Unversucht, 2003). This problem has been further exacerbated by the fact that such halogenated chemicals i.e. 2,2-dichloropropionic acid (2,2-DCP) has been extensively use as the prevailing active components in the production of herbicides (Dalapon) for weed management in agricultural practices. Pertinently, it has been indicated in literature that the heavy reliance on the chemical means to control weed in agricultural sectors may cause long term adverse effects to our existing fragile ecosystem (Reyes-Franco *et al.*, 2006; Srivanasan *et al.*, 2009). Therefore, alternative methods that would overcome such disadvantages need to be suggested. In this context, the enzymatic degradation of 2,2-DCP using dehalogenases may prove to be a promising alternative means to the prevailing chemical route to control weed in agriculture.

The continuing search for novel biocatalysts is a matter of great interest as such only a fraction of the microbial species present in nature has been reported, encompassing only 0.2 to 0.6% bacteria, 5% fungi and a maximum of 24% of known algae (Wubbolts *et al.*, 2000). Therefore, such available wealth of undiscovered microorganisms out there has led in concerted efforts in search of novel microorganism with “unnatural” abilities to synthesize compounds that are not normally produced by “normal metabolic” routes (Adamczak and Krishna, 2004; Gomes and Steiner, 2004). Hence, the hunt for such biocatalysts specific for a particular application remains a challenge. To date, two common approaches are used to select microorganisms suited for a particular task. The first approach involves screening for novel microorganisms by taking advantage of the largely unexplored biodiversity; and the second option is screening for new activities amongst the existing microorganisms (Adamczak and Krishna, 2004; Gomes and Steiner, 2004). These approaches may also include works in the improvement of catalytic properties of known

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enzymes through protein engineering, either through molecular biology or direct protein modification (Hamid *et al.*, 2011). Moreover, chances of finding enzymes with extraordinary properties is fairly reasonable considering numerous reports pertaining to microbial isolation studies (Jing *et al.*, 2008; Roslan *et al.*, 2011; Abel *et al.*, 2012b; Bagherbaigi *et al.*, 2013; Niknam *et al.*, 2014). Such findings have since open doors on explorations of different properties of dehalogenases from countless microorganisms capable of hydrolyzing 2,2-DCP (Jing *et al.*, 2008; Roslan *et al.*, 2011; Abel *et al.*, 2012b; Bagherbaigi *et al.*, 2013; Niknam *et al.*, 2014).

Over the years, microorganisms displaying interesting properties have been isolated from extreme environments *viz.* high salt concentration or temperature as well as in polluted water bodies. Interestingly, several studies have indicated that the dung of a cow may prove to be a rich source of pollutant degrading microflora bacteria (Joshi and Pandey, 2011; Geetha and Fulekar, 2008; Randhawa and Kullar, 2011). In effect, previous studies have shown that the major portion in the daily diet of ruminant animals mostly consisted of forage (Hendriks, 2012; Camboim *et al.*, 2012a, 2012b) which may contain naturally occurring halogenated compounds (Siuda and De Bernardis, 1973; Fuge 1988). Hence, it could not be ruled out that such animals feeding on a daily diet of halogen contaminated forage may inadvertently influence the microflora in their digestive tract. Pertinently, recent studies have supported the hypothesis that the microflora in the digestive tract of cattle may develop special adaptations to counteract the effects of halogenated chemicals in their diet (Singh and Fulekar, 2007; Hendriks, 2012; Camboim *et al.*, 2012a, 2012b; Arunkumar and Chandrasekaran, 2013). Reports by Hendriks (2012) and Camboim (2012a) indicated that bacteria found in the digestive tract of herbivores which fed on naturally occurring halogenated compound-monofluoroacete in gifblaar (*Dichapetalum cymosum*) were effective in treating cows affected by the toxicity of monofluoroactetae (Hendriks, 2012; Camboim *et al.*, 2012a, 2012b). A previous work by Singh and Fulekar (2007) described several microorganisms namely *Pseudomonas* sp., *Streptococcus* sp., *Sarcina* sp., *Escherichia coli*, *Penicillium* sp., *Rhizopus* sp., *Mucor* sp. *and Nocardia* sp. isolated from the dung of cow were effective in degrading phenol. Correspondingly, endosulfan (Arunkumar and Chandrasekaran, 2013) and palm oil degrading microorganisms (Ojonoma and Udeme, 2014) isolated from similar sources have been reported.

Considering the abundance of forage fed cattle in the rural areas of Peninsular Malaysia, specific studies on isolating dehalogenase producing microbes from cow dung merits certain consideration. This present investigation attempted to isolate, characterize and identify such microorganism from the dung of free range cow capable of utilizing 2,2-DCP as sole source of carbon. It is pertinent to highlight here that isolation studies on bacterial species from cow dung able to utilize 2,2-DCP is yet to be reported in Malaysia.

MATERIALS AND METHODS

Growth media preparation

Stock solution for growing bacteria was prepared as 10x concentrated of basal salts containing $K_2HPO_4{\cdot}3H_2O$ (42.5 g/L), $NaH_2PO_4{\cdot}2H_2O$ (10.0 g/L) and $(NH_4)_2SO_4$ (20.0 g/L). The trace metals salts solution was a 10x concentrated that contained nitriloacetic acid $C_6H_9O_6$ (1.0 g/L), $MgSO_4{\cdot}7H_2O$ (2.0 g/L), $FeSO_4{\cdot}7H_2O$ (120.0 mg/L), $MnSO_4{\cdot}4H_2O$ (30.0 mg/L), $ZnSO_4{\cdot}7H_2O$ (30.0 mg/L) and $CoCl_2{\cdot}6H_2O$ (10.0 mg/L) in distilled water (Hareland *et al.*, 1975). The liquid minimal media contained 10 mL of 10× basal salts and 10 mL of 10× trace metal salts per 100 mL of distilled water and were autoclaved (121 °C for 15 min, 15 psi). 2,2-DCP (1 M) was prepared by filter sterilized and added to the 100 mL of growth medium appropriately.

Bacteria isolation and growth in halogenated compound

Cow dung sample (5 g) from rural area in state of Kelantan, Malaysia was mixed into liquid minimal media supplied with 20 mM 2,2-DCP. The mixture was incubated at 30 °C on rotary shaker (150 rpm) overnight. After incubation period, 100 µL from the mixture was spread onto solid 20 mM 2,2-DCP minimal medium followed by incubation at 30 °C. After 4 days, the bacterial colonies were formed, whereby only four large colonies were chosen to be repeatedly streaked onto fresh 20 mM 2,2-DCP minimal medium to obtain the pure colonies.

All four isolates were separately grown in 20 mM 2,2-DCP liquid minimal media and shaken at 150 rpm at 30 °C. Aliquots of the samples were removed periodically and the bacterial growth was determined by turbidity measurement at A_{680nm}. The bacterial strain that exhibited the fastest growth in the 20 mM 2,2-DCP liquid minimal media was selected for further characterization.

Chloride ion released in growth medium

Enzyme activity was measured colorimetrically by determining the release of chloride ion during dehalogenation (Bergman and Sanik, 1957). Sample (1 mL) was added into a test tube containing 0.25 M (100 µL) ammonium ferric sulphate (FAS) prepared in 9 M nitric acid. The solution was mixed prior to addition of mercuric thiocyanate-saturated ethanol (100 µL) and inverted several times for proper mixing. The color was allowed to develop for 10 min at room temperature and liberation of chloride ion was observed at A_{460nm}.

BIOLOG^{TM} GEN III MicroPlate identification

An important breakthrough for determining the species or strains of bacteria was achieved following the development of the Biolog GEN III, a commercial tool for identifying unknown bacterium using 94 phenotypic tests, consisting of 71 carbon source utilization assays and 23 chemical sensitivity assays. The test panel provides a

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"Phenotypic Fingerprint" of the microorganism which can then be used to identify up to species level. The isolate to be identified was grown on agar medium and suspended in a special "gelling" inoculating fluid (IF) at the recommended cell density. Then, the cell suspension was inoculated into the GEN III MicroPlate, 100 µL per well and the MicroPlate was incubated to allow the phenotypic fingerprint to form. All of the wells start out colorless when inoculated. During incubation, the increased respiration in the wells following utilization of the carbon source and/or cell growth causes reduction of the tetrazolium redox dye, hence forming a purple color. The negative wells remain colorless, as does the negative control well without carbon source. A positive control well was used as a reference for the chemical sensitivity assays. After incubation, the developed phenotypic fingerprint of purple wells was compared to the BIOLOG's extensive species library in order to find a match and identify the species level of the isolates. Most identification will be obtained within 13 to 24 h period.

16S rDNA gene sequencing

Bacterial DNA was extracted from bacterial cultures grown on 20 mM 2,2-DCP minimal liquid media using Wizard® Genomic DNA Purification Kit. The polymerase chain reaction (PCR) was carried out to amplify the target DNA fragments using universal primers, Fd1 (5' - AGA GTT TGA TCC TGG CTC AG - 3') and rP1 (5' - ACG GTC ATA CCT TGT TAC GAC TT - 3') (Fulton and Cooper, 2005). DNA amplification was performed for 30 cycles: Initial denaturation (94 °C for 5 min), cooling, denaturation (94 °C for 1 min), annealing (55 °C for 1 min) and final extension (72 ºC for 10 min). Amplicons were purified using QIAGEN® QIAquick PCR purification kit and sequenced by 1st Base® company (Malaysia).

RESULTS

Growth analysis in 20 mM 2,2-DCP minimal medium

The maximum growth profile of the four bacterial isolates, namely strains SN1, SN2, SN3 and SN4were evaluated in 20 mM 2,2-DCP liquid culture medium (Figure 1). It was revealed that strain SN1 achieved the highest maximum growth at A_{680nm} as compared to that of other strains, with a remarkably short doubling time of 3.85 h. Table 1 shows the growth curves for the estimation of cell doubling time for bacterial strains SN1, SN2, SN3 and SN4. Considering the rapid doubling time of strain SN1 (3.85 h) as compared to that of SN2 (7.79 h), SN3 (7.84 h) and SN4 (7.73 h), respectively, the bacterial strain was selected for subsequent basic morphological examinations. The morphological properties of strain SN1 are depicted in Table 2. The growth of strain SN1 in various concentrations (10 mM, 20 mM, 30 mM and 40 mM) of 2,2-DCP liquid minimal media was evaluated. The study found concentrations of 30 and 40 mM of 2,2-DCP were toxic to the SN1 bacteria which consequently inhibited their growth. Apart from the growth experiment, analysis of chloride ion released was carried out to ascertain the utilization of 2,2-DCP by bacterial strain SN1, monitored by halide ion assay. Figure 2 shows the comparison of chloride ion released and growth of strain SN1 in 20 mM 2,2-DCP liquid minimal media determined at A_{460nm} and A_{680nm}, respectively. The highest release of chloride ion attained by strain SN1 was 39.5 µmolCl^-/mL at 28 h, suggesting complete utilization of the 20 mM 2,2-DCP in the growth medium.

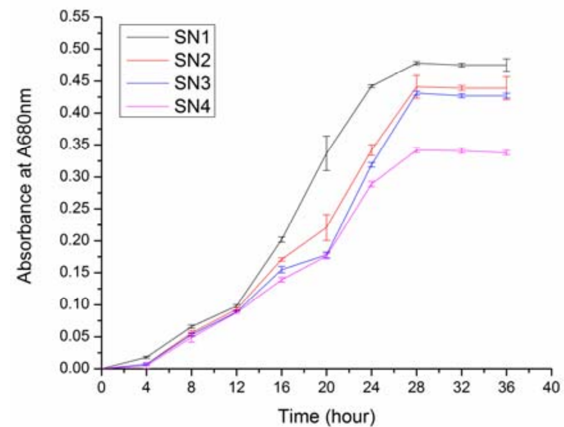

Figure 1: Growth profiles of different bacterial strains (SN1, SN2, SN3, SN4) on 20 mM 2,2-DCP liquid minimal media incubated at 30 °C and shaken at 150 rpm.

Table 1: The estimated doubling time of the different bacterial strains.

Bacterial strains	Doubling time (h)
SN1	3.85 ± 0.18
SN2	7.79 ± 0.25
SN3	7.84 ± 0.11
SN4	7.73 ± 0.03

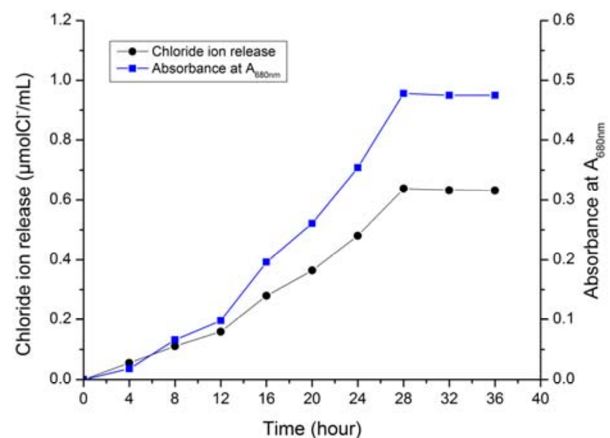

Figure 2: The correlation between the chloride ion released measured at A_{460nm} and growth profiles of strains SN1, SN2, SN3 and SN4, respectively, measured at A_{680nm}.

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Table 2: Basic morphological properties of strain SN1.

Properties	Details
Length	5μm-10μm
Color	White creamy
Physical morphology	Rod shape
Gram staining	Positive
Motility	Positive

Identification of SN1 strain by biochemical tests and DNA analysis

BIOLOG GEN III MicroPlate and analysis of 16S rRNA gene were the two key experiments used to identify the SN1bacterial strain. Table 3 shows the positive results of carbon source utilization and chemical sensitivity assays by BIOLOG's Microbial Identification Systems software. The phenotypic fingerprints of the positive wells were compared the BIOLOG's extensive species library and strain SN1 was indicated as *Bacillus cereus*. The 16S rRNA analysis afforded the gene sequence of strain SN1, in which a phylogenetic tree may be constructed using the Neighbour-joining method. The selected sequences of bacteria in the NCBI database identical to that of strain SN1 was obtained from BLASTn analysis. It was observable from the constructed phylogenetic tree that strain SN1 was clustered together with *Bacillus cereus* (Figure 3), hence, in concurrence with the analyses results of the BIOLOG's GEN III.

Table 3: Extensive biochemical analysis of strain SN1 using BIOLOG™ GEN III Microplate.

Test	Result	Test	Result
Dextrin	+	Gelatin	+
D-Maltose	+	Glycyl-L-Proline	-
D-Trehalose	+	L-Alanine	-
D-Cellobiose	+	L-Arginine	-
Gentiobiose	-	L-Aspartic Acid	-
Sucrose	-	L-Glutamic Acid	-
D-Turanose	-	L-Histidine	-
Stachyose	-	L-Pyroglutamic Acid	-
D-Raffinose	-	L-Serine	-
α-D-Lactose	-	Pectin	-
D-Melibiose	-	D-Galacturonic Acid	-
β-Methyl-DGlucoside	-	L-Galactonic Acid Lactone	-
D-Salicin	-	D-Gluconic Acid	+
N-Acetyl-DGlucosamine	+	D-Glucuronic Acid	-
N-Acetyl-β-DMannosamine	-	Glucuronamide	-
N-Acetyl-DGalactosamine	-	Mucic Acid	-
N-Acetyl Neuraminic Acid	-	Quinic Acid	-
α-D-Glucose	+	D-Saccharic Acid	-
D-Mannose	-	p-Hydroxy-Phenylacetic Acid	-
D-Fructose	-	Methyl Pyruvate	+
D-Galactose	-	D-Lactic Acid Methyl Ester	-
3-Methyl Glucose	-	L-Lactic Acid	-
D-Fucose	-	Citric Acid	-
L-Fucose	-	α-Keto-Glutaric Acid	-
L-Rhamnose	-	D-Malic Acid	-
Inosine	+	L-Malic Acid	+
D-Sorbitol	-	Bromo-Succinic Acid	+
D-Mannitol	-	Tween 40	-
D-Arabitol	-	γ-Amino-Butyric Acid	-
myo-Inositol	-	α-Hydroxy-Butyric Acid	-
Glycerol	-	β-Hydroxy-D,LButyric Acid	-
D-Glucose-6-PO_4	-	α-Keto-Butyric Acid	-
D-Fructose-6-PO_4	+	Acetoacetic Acid	-
D-Aspartic Acid	-	Propionic Acid	-
D-Serine	-	Acetic Acid	-
		Formic Acid	-
pH 6	+	Lincomycin	-
pH 5	-	Guanidine HCl	+
1% NaCl	+	Niaproof 4	-
4% NaCl	+	Vancomycin	-
8% NaCl	+	Tetrazolium Violet	-
1% Sodium Lactate	+	Tetrazolium Blue	-
Fusidic Acid	-	Nalidixic Acid	-
D-Serine	+	Lithium Chloride	+
Troleandomycin	-	Potassium Tellurite	+
Rifamycin SV	-	Aztreonam	+
Minocycline	-	Sodium Butyrate	+
		Sodium Bromate	+

+: for positive result; -: for negative results.

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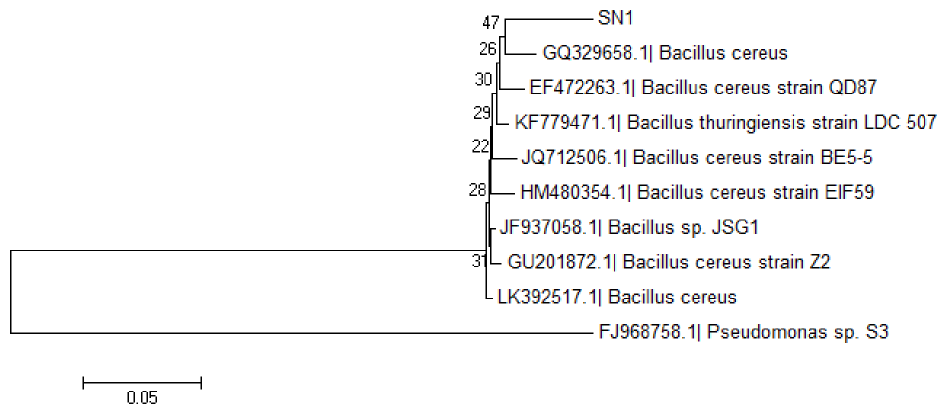

Figure 3: Phylogenetic tree based on 16S rRNA gene sequence analysis constructed using the neighbour-joining method showing the position of strain SN1 among related taxa. The figure shows the relationship of related taxa (GQ329658, EF472263, KF779471, JQ712506, HM480354, JF937058,GU201872, LK392517) with that of the outgroups *Pseudomonas* sp. S3. Numbers at nodes indicate bootstrap values based on a neighbour-joining analysis. The bootstrap consensus tree inferred from 500 replicates to represent the evolutionary history of the taxa analyzed. The bar at the bottom, provides a scale and the line segment with the number '0.05' shows the length of branch that represents an amount genetic change of 0.05.

DISCUSSIONS

To date, a variety of bacteria able to grow on 2,2-DCP as sole source of carbon and energy has been successfully isolated (Table 4), with observably varied doubling time between each species. The current study was focused on isolating and characterizing the bacteria from dung of ruminant animals that fed on forage in rural area in Malaysia. Scientifically, it is believed that the local forage may contain naturally occurring halogenated substance, hence the intestinal microbes of these animals were also similarly exposed (Siuda and De Bernardis, 1973). According to previous works by Camboim *et al.* (2012a) and Camboim *et al.* (2012b), the microflora in cattle that fed on halogen-contaminated forage may develop special adaptations, in which they could be used as microbial inoculum in animal feed to treat sick cows poisoned by monofluoroactetae-containing forage (Lee *et al.*, 2014).

All five bacterial strains SN1, SN2, SN3 and SN4 isolated from cow dung were found capable of utiliziing 2,2-DCP as their source of carbon and energy. However, only strain SN1 was found most effective, inferred from its short cell doubling time (3.85 h) in liquid minimal media containing 20 mM 2,2-DCP. Although the doubling time of strain SN1 seemed impressive, a 2,2-DCP degrading *Burkholderia* sp. isolated from *Axonopus compressus* leaf exhibited a much shorter doubling time of 2.7 h (Yong *et al.*, 2014) (Table 4). Likewise, Khosrowabadi and Huyop (2014) discovered a marine *Microbacterium resistens* able to degrade 2,2-DCP, but its growth was particularly slow (cell doubling time of 41.23 h), suggesting sluggish uptake of the substance into the cells. *Bacillus megaterium* isolated from volcanic environment with an 8 h cell doubling time (Roslan *et al.,* 2011) was also reported. Hence, it can be construed that the SN1 strain was relatively fast growing as compared to other reported *Bacillus* sp.. Correspondingly, the observable slow growing strains SN2, SN3 and SN4 were possibly due to the same reason mentioned above.

According to a previous report by Huyop and Cooper (2012), the slow process of nutrient transport into cells may result in slow development of bacterial colonies. However, certain bacteria are able to grow on halogen contaminated environment, attributable to their ability to produce haloacid permease which could improve the uptake of halogenated substances into their cells (Yu *et al.*, 2007). Production of haloacid permease in bacteria may aid in facilitating the transport processes as most substances do not passively enter their cell. For example, dissolved nutrients entering the aquatic environment can be integrated into microorganisms due to their permeability and active transport through the cytoplasmic membrane. The transfer of matter in the environment into the bacterial cells may be possible by the presence of cellular appendages (prostheca) in some bacteria. Such adaptation improves transport of substances by increasing the surface area of the cell and cytoplasmic membrane (Huyop and Cooper, 2012).

The growth of strain SN1in concentrations of 2,2-DCP up to 20 mM observable in this study is in concurrence with a previous report by Roslan *et al.,* (2011). No growth of bacterial colonies was seen in concentrations 30 mM and 40 mM 2,2-DCP, respectively, suggesting such concentrations were particularly toxic to the SN1 strain. According to Sikkema *et al.* (1995) culturing bacteria in high concentrations of 2,2-DCP will result in accumulation of the substance within the lipid bilayer of the bacterial cells, hence affecting the structural and functional

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Table 4: The reported bacterial that can grow on 2,2-DCP minimal media as sole source of carbon.

Source	Microorganism	Concentration of 2,2-DCP	Doubling Time (TD) (hours)	References
Agricultural soil UTM	*Methylobacterium* sp.	20 mM	23	Jing and Huyop (2007)
Soil (mixed culture) UTM plantation	*Methylobacterium* sp.	5 mM	25.41	Jing (2007)
		10 mM	19.50	
		20 mM	20.32	
Agricultural soil	*Methylobacterium* sp.	20 mM	14	Jing *et al*. (2008)
Soil from volcanic area	*Citrobacter* sp.	20 mM	15	Hamid *et al*. (2010)
Soil from garden	Aminobacter	20 mM	7	Amini *et al*. (2011)
Soil from rubber plantation estate	*Labrys* sp.	30 mM	33.44	Wong and Huyop (2011)
Soil from volcanic area	*Bacillus megaterium*	20 mM	8	Roslan *et al*. (2011)
Soil from rubber estate	*Burkholderia* sp.	10 mM	7.48	Wong and Huyop (2012)
		20 mM	6.16	
		40 mM	6.96	
	Enterobacter cloacae	10 mM	11.59	
		20 mM	12.24	
		40 mM	11.44	
Soil from lake water	*Serratia marcescens* sp. SE1	20 mM	5	Abel *et al*. (2012a)
Gut of pond-reared rohu (*Labeo rohita*)	*Ralstonia solanacearum*	20 mM	7.2	Abel *et al*. (2012b)
	Acinetobacter baumannii		23.3	
	Chromobacterium violaceum		6.2	
Soil from the seaside area of Tigbauan, Iloilo, Philippines.	*Enterobacter cloacae*	20 mM	10	Nemati *et al*. (2013)
Axonopus compressus leaf	*Burkholderia* sp.	10 mM	2.7	Yong (2013)
		20 mM	2.7	
		40 mM	4.8	
Marine sediment	*Bacillus* sp.	20 mM	39.60	Khosrowabadi and Huyop (2014)
	Rhodococcus equi		36.60	
	Lysinibacillus sphaericus		30.71	
	Microbacterium resistens		41.23	
	Aminobacter sp.		36.70	
Wastewater from Tioman Island	*Raoutella ornithilolytica*	20 mM	23.11	Niknam *et al*. (2014)

properties of the cell membrane. As such, the membrane loses its integrity and may result in an uncontrolled permeability of protons and ions into the bacterial system, thereby rapid dissipation of the proton motive force and impairment of intracellular pH occur. However, at normal concentrations, 2,2-DCP is not toxic to the cells and may even trigger the production dehalogenase in bacteria (Sikkema *et al*., 1995). Apart from living organisms, the toxicity of 2,2-DCP may also be transferred into plants. The leaves and roots of plants may absorb the 2,2-DCP and translocate the substance through both the symplastic and apoplastic systems. The symplastic (living) route to the vascular stele involves cell to cell transport by plasmodesmata, allowing 2,2-DCP to move from cell to cell without passing through the cell wall. In contrary, the apoplastic (non-living) pathway provides a route into the vascular stele through free spaces and cell walls of the epidermis and cortex. It acts by precipitation of protein that inhibits production of pantothenic acid, and leads to physiological malfunctions of plants (Tseng *et al*., 2004).

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Yu *et al.* (2007) suggested that certain bacteria may be able to utilize 2,2-DCP as a growth substrate, hence such ability was positively observed in the SN1 strain. Similarly, *B. cepacia* MBA4 (Yu *et al.*, 2007) and *Rhizobium* sp. RC1 (Jing *et al.*, 2010) demonstrated tolerance towards haloacids and was able to utilize these toxic compounds as growth substrate. This was possibly due to evolution of a haloacid operon that contains both genes of a dehalogenase and permease (Jing *et al.*, 2010). According to the body of literature, the ability of strain SN1 to grow on 2,2-DCP as sole source of carbon and energy observable in this study may be described by four basic factors. Firstly, such organism should possess or produce dehalogenase in response to the presence of halogenated substances in the surrounding environment. Secondly, the product of the dehalogenation reaction should simply be converted to an intermediate of the organism's central metabolic pathway and must be non-toxic. Thirdly, the halogenated substance should be able to enter the cell passively or by active transport to reach the site of dehalogenase activity. Lastly, the halogenated substance must be non-toxic to the organism at normal intracellular concentrations (Slater *et al.*, 1996). In this context, the results of the chloride ion assays (A_{680nm}) exemplified in Figure 2 indicated that strain SN1 grew rapidly in 20 mM 2,2-DCP, inferred from the predominantly exponential growth curve. It can be seen that the rate of chloride ion release was directional proportional to the growth of strain SN1 in the minimal media, thereby strongly suggesting that 2,2-DCP was an acceptable substrate for its growth.

A species level identification of the isolate was successfully made when a 100% matched was found in the BIOLOG's GEN III library (Table 3). The positive utilization of various sugars (dextrin, D-maltose, D-trehalose, D-cellobiose, N-acetyl-D-glucosamine,α-D-glusoce inosine and D-fructose-6-PO_4) were expected as sugars are generally transported into the bacterial cells against a concentration gradient (Zhang *et al.*, 2006). Nevertheless, the observable inability of strain SN1 to utilize D-galactose as a carbon source was in agreement with a previous work by Lindner *et al.* (1994). Additionally, positive oxidization of methyl pyruvate, L-malic acid and bromo-succinic acid are similar to that reported by Zhang and co-worker (2006) for *Bacillus macauensis*. Hence, the identification of strain SN1 as *Bacillus cereus* was quite anticipated as such the genus has been frequently reported by many studies which include Boricha and Fulekar (2009), Pradhan and Babu (2012), Ojonoma and Udeme (2014), and Joshi and Pandey (2011). In fact, the *Bacillus* species is widely distributed in the environment and has been known to demonstrate broad phenotypic differences and pathological effects. In the case of *B. cereus*, it is an ubiquitous soil bacterium as well as an opportunistic pathogen that has been linked to many cases of food poisoning. The pathogenicity of *B. cereus* may be attributed to production of sturdy spores that allow the bacteria to survive in harsh conditions during commercial treatment. In effect, contamination of dairy products from spores of *B. cereus* has been reported (Helgason *et al.*, 2000).

CONCLUSIONS

The present study indicates that *B. cereus* isolated from cow dung may have potential application as bioremediation agent to treat environment contaminated with halogenated substances. The bacteria may act as purifier as well as an excellent source of microflora for therapeutic treatment of sick cattle that fed on toxic plants containing monofluoroacetate and other similarly halogenated compounds. Considering the beneficial uses that *B. cereus* may have on our livelihood, it is recommended that empirical studies on the properties of dehalogenase of *B. cereus* are carried out, as well as evaluating the ability of the bacteria in utilizing different types of halogenated substances as growth substrate.

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