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Isolation of *Klebsiella pneumoniae* from Sungai Skudai and *in silico* analysis of putative dehalogenase protein

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ABSTRACT

Aims: The surplus use of herbicide Dalapon® contains 2,2-dichloropropionic acid (2,2-DCP) poses great danger to human and ecosystem due to its toxicity. Hence, this study focused on the isolation and characterization of a dehalogenase producing bacteria from Sungai Skudai, Johor, capable of utilizing 2,2-DCP as a carbon source and *in silico* analysis of its putative dehalogenase.
Methodology and results: Isolation of the target bacteria was done by using 2,2-DCP-enriched culture as the sole carbon source that allows a bacterium to grow in 20 mM of 2,2-DCP at 30 °C with the corresponding doubling time of 8.89 ± 0.03 h. The isolated bacterium was then designated as *Klebsiella pneumoniae* strain YZ based on biochemical tests and basic morphological examination. The full genome of *K. pneumoniae* strain KLPN_25 (accession number: RRE04903) which obtained from NCBI database was screened for the presence of dehalogenase gene, assuming both strains YZ and KLPN_25 were the same organisms. A putative dehalogenase gene was then identified as type II dehalogenase from the genome sequence of strain KLPN_25. The protein structure of the type II dehalogenase of KLPN_25 strain was then pairwise aligned with the crystal structure of L-2-haloacid dehalogenase (L-DEX) *Pseudomonas* sp. strain YL as the template, revealing the existence of conserved amino acids residues, uniquely known to participate in the dehalogenation mechanism. The finding thus implies that the amino acid residues of type II dehalogenase possibly shares similar catalytic functions with the L-DEX.
Conclusion, significance and impact of the study: In conclusion, this study confirmed the presence of new dehalogenase from the genus *Klebsiella* with potential to degrade 2,2-DCP from the river water. The structural information of type II dehalogenase provides insights for future work in designing haloacid dehalogenases.

Keywords: 2,2-DCP, degradation, dehalogenase, *Klebsiella pneumoniae*, halogenated compound, type II dehalogenase

INTRODUCTION

The advancement of technology has resulted in the formation of synthetic compounds known as xenobiotics which can cause environmental pollution when present in high concentration. Their ubiquitous distribution in this biosphere is due to extensive usage in industry and agricultural activity (Jing and Huyop, 2008; Oyewusi *et al.*, 2021). Apart from its widespread use as pesticides, insecticides and antibiotics (Abel *et al.*, 2012), these man-made compounds are frequently found in herbicides as agrochemicals used to prevent the growth of undesired weeds. 2,2-dichloropropionic acid (2,2-DCP) often market as Dalapon, is a synthetic halogenated chemical extensively used as an active compound in the production of herbicides in controlling a wide variety crops (Ismail *et al.*, 2016). 2,2-DCP is a plant growth regulator that have been used in controlling specific annual and perennial grasses, such as quick grass, Johnson grass, Bermuda grass as well as rushes and cattails (Jing and Huyop, 2008).

However, the implementation of chemically synthesized herbicide in agricultural fields can cause negative environmental problems. Rivers and lakes become polluted by herbicide due to its persistence and resistance in nature. The bioaccumulative of these compounds and their toxicity are threat to human health and environment (Fetzner and Lingens, 1994). The accumulation of 2,2-DCP due to its resistance to degradation has led to river water pollution and has adverse effect on aquatic habitat.

Recently, microbial degradation of toxic compounds has become a prominent path to transform toxic compounds into harmless products. A large number of

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dehalogenases producing bacteria have been previously isolated, purified and characterized by widely distributed microorganisms that utilize halogenated compounds as their primary sources of carbon and energy (Ismail *et al*., 2016; Heidarrezaei *et al.*, 2020; Oyewusi *et al.*, 2020; Wahhab *et al.*, 2021). The enzyme possessed by the microbes to detoxify harmful halogenated compounds has also been well studied (Huyop and Nemati, 2010; Hamid *et al.,* 2011; Adamu *et al.*, 2020). The halogenated compounds are degraded by dehalogenases synthesize by bacteria through dehalogenation process which catalyze the cleavage of carbon-halogen bond (Fetzner, 1998; Alomar *et al.*, 2014). Their capability in removing pollutants helps reduce the environmental halogen-associated pollution.

To combat environmental problems, biological method is a suitable choice as various halogenated degrading bacteria have been studied in the last few years. However, there are limited research on bacteria isolated from river water samples that able to degrade 2,2-DCP. Therefore, the main objective of this study is to isolate 2,2-DCP degrading bacteria from Sungai Skudai river water. The isolated bacterium that capable in degrading 2,2-DCP was further characterized. Besides, the genetic properties of the isolated bacterium were analyzed based on the genome sequence of the similar bacterium genus and species available from the National Centre for Biotechnology Information (NCBI) database.

MATERIALS AND METHODS

Media preparation

A total of 0.5 L of stock solution of "Paul John Chapman" or 10× PJC basal salts (containing 16.22 g K_2HPO_4, 5.0 g $NaH_2PO_4{\cdot}2H_2O$ and 12.5 g $(NH_4)_2SO_4$) and 10× PJC trace metals (containing 0.5 g nitrilotriacetic acid, 1.0 g $MgSO_4{\cdot}7H_2O$, 0.06 g $FeSO_4{\cdot}7H_2O$, 0.015 g $MnSO_4{\cdot}4H_2O$ and 0.015 g $ZnSO_4{\cdot}H_2O$) were prepared (Hareland *et al.*, 1975; Heidarrezaei *et al.*, 2020). The growth media contained 10 mL of 10× basal salts and 10 mL of 10× trace metals per 100 mL of distilled water and were autoclaved at 121 °C for 5 min, 15 psi. Sodium 2,2-dichloropropionate (2,2-DCP) (1 M) stock solution was prepared by dissolving 1.65 g of sodium 2,2-DCP in 10 mL of distilled water and filter-sterilised (with 0.2 µm pore size of nylon filter. Then, the 2,2-DCP stock solution was added aseptically into the growth media to get the desired final concentration. As for solid minimal media preparation, Oxoid bacteriological agar (1.5% w/v) was added prior sterilization.

The bacteria were also allowed to grow in 20 mM pyruvate growth solution containing 10 mL of each 10× basal salts and trace metals in 100 mL of distilled water. Pyruvate (1 M) stock solution was prepared by dissolving 5.5 g of sodium pyruvate in 50 mL distilled water and filter-sterilised using 0.2 µm pore size of nylon filter. An approximately 2 mL of pyruvate stock solution was added aseptically into the growth media.

Bacteria cultivation

River water sample was collected from Sungai Skudai, Johor Bahru, Malaysia. About 3 mL of water sample was added into 100 mL liquid PJC minimal medium containing 20 mM of 2,2-DCP and incubated at 30 °C overnight in a rotary shaker set at 150 rpm. A bacteria culture (0.1 mL) was spread onto solid PJC minimal media supplemented with 20 mM of 2,2-DCP and incubated at 30 °C for next 7 days. The potential bacterial isolates that grow were selected and transferred onto a fresh PJC minimal media supplemented with the same media component to obtain pure culture.

A single colony of the bacterium was then inoculated into a 100 mL liquid minimal medium containing 10 mM of 2,2-DCP and incubated at 30 °C overnight to prepare for the inoculum. After an overnight incubation period, the inoculum (25 mL from the overnight culture at A_{680nm} 1.2) was then inoculated into a fresh PJC liquid minimal media containing 10 mM, 20 mM, 30 mM and 40 mM concentrations of 2,2-DCP, respectively and 20 mM of pyruvate served as control. The cultures were prepared in triplicates and incubated at 30 °C for 24 h on a rotary shaker at 150 rpm. The cells doubling time was determined by checking the turbidity at A_{680nm} at specific time intervals quantitated based on changes in the turbidity from the initial time.

Bacteria characterization

A standard Gram-staining procedure was performed. The basic biochemical tests were based on standard API-20E test kit for bacteria identification according to Bergey's Manual of Systematic Bacteriology (Holt *et al.*, 1994).

Database screening for the genome sequence belongs to *K. pneumoniae*

The basic biochemical tests proposed strain YZ belongs to the genus *K. pneumoniae*. Therefore, all *K. pneumoniae* in the NCBI database were screened for dehalogenase gene in their full genome sequence assuming that strain YZ and other/selected *Klebsiella* sp. in the data base will carry similar gene in the genomes.

Computational analysis

Dehalogenase amino acid sequence

Klebsiella pneumoniae strain KLPN_25 selected from the NCBI database (https://www.ncbi.nlm.nih.gov/) with accession number RRE04903 was found to carry putative dehalogenase gene that belongs to haloacid dehalogenase type II. The amino acid sequence was then retrieved in FASTA format for further analysis. The sequence was subjected to ProtParam analysis via Expasy (Expert Protein Analysis System) Proteomic Server (https://web.expasy.org/protparam/) for primary structure analysis (Gasteiger *et al.*, 2005; Harisna *et al.*, 2017). A NCBI-BLASTp (Altschul *et al.*, 1990) of the

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amino acids of the haloacid was performed and five similar dehalogenase proteins from various species of dehalogenase producing bacteria were matched to putative dehalogenase belongs to *K. pneumoniae* strain KLPN_25.

Multiple sequence alignment

The multiple amino acid sequence alignment was carried out using MultAlin web-based sequence alignment at http://multalin.toulouse.inra.fr/multalin/ (Corpet, 1988).

Secondary structure prediction

The secondary structure prediction for haloacid dehalogenase type II of *K. pneumoniae* strain KLPN_25 was conducted using Chou and Fasman Secondary Structure Prediction (CFSSP), http://www.biogem.org/tool/chou-fasman/ (Kumar, 2013)

Homology modelling

The amino acid sequence of haloacid dehalogenase type II from *K. pneumoniae* strain KLPN_25 was submitted to SWISS-MODEL program at https://swissmodel.expasy.org/ to obtain a recommended template for three-dimensional structure building (Biasini *et al.*, 2014; Harisna *et al.*, 2017; Waterhouse *et al.,* 2018) and was visualized using PyMol version 2.4.0. The pairwise amino acid sequence alignment between haloacid dehalogenase type II from *K. pneumoniae* strain KLPN_25 and other established templates was conducted using Bioedit version 7.2.5 (Harisna *et al.*, 2017).

RESULTS AND DISCUSSION

Bacteria growth on 2,2-DCP solid and liquid minimal media

After 7 days incubation period kept at 30 °C, two colonies were seen on the plate culture (Figure 1). The isolate which has bigger size was streaked again onto fresh 20 mM 2,2-DCP solid minimal media as sole carbon source to obtain a pure colony. The pure colony of the bacterium was designated as strain YZ.

Since the bacterium strain YZ isolated from the river water has the ability to grow on a selective media supplemented with 2,2-DCP, it is suggested that they are likely to metabolize or utilize 2,2-DCP as a carbon source. The bacterium that can effectively dechlorinates 2,2-DCP as seen in the previous study reported by Jing *et al.* (2008). Both chloride ions were released from 2,2-DCP and converted into pyruvate. For each molecule of pyruvate formed may be taken as a measure of dehalogenase activity. Apart from turbidity measured at A_{680nm}, the cells growth can be measured by two chloride ions being released from 2,2-DCP.

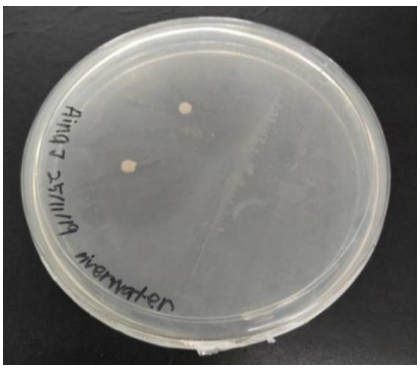

Figure 1: The growth of bacteria on solid minimal media containing 20 mM of 2,2-DCP as a carbon source after 7 days of incubation.

Bacteria growth measurement

The growth profiles of strain YZ at different concentrations of 2,2-DCP (10 mM, 20 mM, 30 mM and 40 mM) were constructed (Figure 2). The maximum growth was exhibited in 20 mM 2,2-DCP (A_{680nm} 1.074 ± 0.04) at 16 h incubation, while no growth was observed at 16 h in 40 mM 2,2-DCP (0.190 ± 0.04). This was due to the toxicity effect of the substrate above 40 mM 2,2-DCP to the bacterium, thereby inhibiting growth. Although the bacteria exhibited significant growth at 10 mM (0.826 ± 0.01) and 30 mM (0.528 ± 0.01) 2,2-DCP, it is still lower compared to 20 mM concentration. The poor growth and/or slow growth of cells could be attributed to several reasons, (i) the poor uptake system of the substrate and (ii) low expression of the dehalogenase gene by the bacteria or (iii) dehalogenase gene system was silent/cryptic due to lack of inducer (Hill *et al.*, 1999). However, it was hypothesized that the lack of growth may possibly be due to the toxicity of the substrate to the bacteria and/or due to the lack of expression of permease uptake system gene, which co-transport the substrate into the cell. It was previously reported that for dehalogenation to occur, the halogen atom of the substrate should fit in a position within the active site that favors its interaction with a basic amino acid residue within the catalytic site for water molecules activation to react with the substrate (Adamu *et al.*, 2020). Therefore, from the results, it shows the optimum concentration of 2,2-DCP to grow and replicate is 20 mM, which implying the optimum concentration to degrade the 2,2-DCP is 20 mM.

The cells doubling time of strain YZ in different concentrations of 2,2-DCP were calculated and summarized in the Table 1. The doubling time for strain YZ in 20 mM 2,2-DCP was fastest. It has been reported that *Ralstonia solanacearum* MK121002 and *Chromobacterium violaceum* MK121009 (Abel *et al.*, 2012; Ismail *et al.*, 2017) showed an impressive cell doubling time of 7.2 h and 6.2 h in 20 mM 2,2-DCP, respectively. However, slow growth was observed for *Raoutella ornithinolytica* (Niknam *et al.*, 2014) and *Acinetobacter baumanni* MK121007 (Abel *et al.*, 2012)

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with cells doubling time of 23.11 h in 20 mM 2,2-DCP for both microorganisms, but *Citrobacter* sp. AZZ2 exhibited a cell doubling time of 15 h (Hamid *et al.*, 2010). *Labrys* sp. Wy1 (Wong and Huyop, 2012) and *Pseudomonas aeruginosa* MX1 (Edbeib *et al.*, 2016) showed a rather longer cells doubling time than any other reported α-halogenated compounds degrading microorganisms with reported cells doubling time of 34.6 h and 44 h, respectively. Nevertheless, both *A. tumefaciens* RS4, and *A. tumefaciens* RS5 were found to grow on 2,2-DCP with cells doubling time of 8.5 and 4.7 h, respectively (Schwarze *et al.*, 1997).

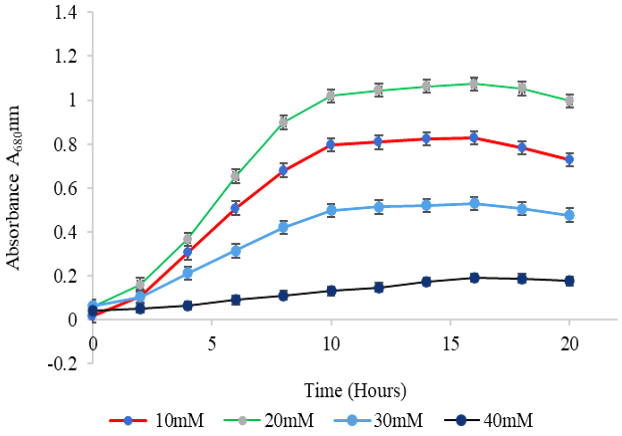

Figure 2: Growth rate of strain YZ in different concentrations of 2,2-DCP. Readings were taken in triplicates + standard deviation.

Table 1: The doubling time of strain YZ in different concentrations of 2,2-DCP.

Concentration (mM)	Doubling time (h)
10	9.36 ± 0.038
20	8.89 ± 0.030
30	9.75 ± 0.057
40	No growth

Morphological observation of isolates

The morphological and physiological features of strain YZ were observed following 24 h of incubation at 30 °C on 20 mM of 2,2-DCP solid agar minimal medium. The colony features are summarized in Table 2. The strain YZ was a Gram-negative bacterium with rod shape (Figure 3).

Biochemical characterization and bacterium identification

The API-20E for strain YZ was summarized in Table 3. The results were compared with Bergey's Manual of Systematic Bacteriology (Holt *et al.*, 1994) in order to identify the genus and species of the strain YZ. The

Table 2: Colony observations of strain YZ on 20 mM of 2,2-DCP solid minimal media over 24 h.

Parameters	Results
Pigmentation (pure colony)	creamy
Margin	entire
Colour	milky
Elevation	convex
Texture	mucoid
Shape	punctiform

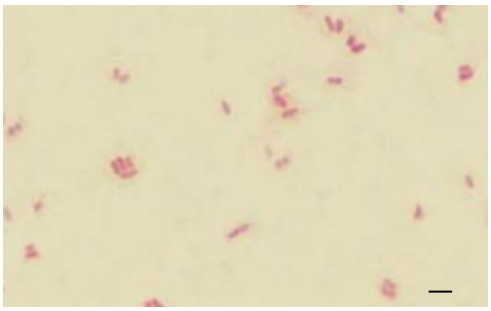

Figure 3: Strain YZ was observed under 1000× magnification using light microscope. The bacteria were Gram-negative, rod in shape. Scale bar (–) in the figure indicates 2 µm length.

biochemical tests were selected based on the Gram-negative bacteria and non-motile. According to the previous study, *Klebsiella* sp. have typical Gram-negative bacilli characteristics with citrate, oxidase, urease and indole positive as well as non-motile (Alves *et al.*, 2006). In addition, *Klebsiella* sp. have shown catalase positive and formed pink colonies indicating the fermentation of lactose on MacConkey agar (Patel *et al.*, 2017). Meanwhile, negative result was shown in starch hydrolysis test for *Klebsiella* sp. (Kumar *et al.*, 2019). Based on current API-20E biochemical characteristics results, it was proposed strain YZ belongs to *K. pneumoniae*. An API-20E was applied in this study for rapid identification of the bacteria from total bacterial population. The test sensitive enough for the identification of microorganisms up to the genus and species level (Juang and Morgan, 2001; O'hara, 2005). However, in future an additional method like 16S rRNA may be necessary.

Klebsiella sp. belongs to the family *Enterobacteriaceae* which is widely found in nature (soil, plants, waters) and in the gastrointestinal tracts of a wide range of animals. *Klebsiella* sp. is usually involved in nosocomial infections, such as urinary and respiratory tract infections as well as infections of wounds and soft tissue (Herridge *et al.*, 2020). *Klebsiella pneumoniae* and *Klebsiella oxytoca* are the most common pathogens in genus *Klebsiella* that have rapidly become global threat. Apart from their pathogenicity properties, *Klebsiella* sp. have been reported in utilizing or degrading hydrocarbons (Ismail *et al.*, 2017). The influence of organic fertilizers on the bioremediation of hydrocarbon-contaminated soil was

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previously studied and *Klebsiella* sp. was found as the isolated microorganism (Ibiene *et al.*, 2011). This shows that genus *Klebsiella* sp. has the ability to perform bioremediation.

Database search of genome sequence belongs to *K. pneumoniae* KLPN_25

Since the current isolated bacteria which proposed as *K. pneumoniae* strain YZ was able to grow in minimal media supplemented with 2,2-DCP as sole source of carbon and energy, therefore, it was hypothesized that strain YZ contain dehalogenase gene. Genomic content of the same genus and species might contain similar DNAs and some other properties. Hence, the NCBI database search was carried out to observe whether any other *K. pneumoniae* that have its full genome sequence to screen for the presence of dehalogenase gene(s).

The genomic sequence of *K. pneumoniae* strain KLPN_25 with database number of RCZV01000004 (Tamma *et al.*, 2018) was selected from NCBI and was further analysed. In this study, only one haloacid dehalogenase type II (deh type II) gene from *K. pneumoniae* strain KLPN_25 was detected from its full genome sequence with accession number RRE04903. The corresponding dehalogenase gene FASTA format was then converted into its amino acid sequence for further protein analysis.

Table 3: API-E20 biochemical tests result for strain YZ.

Tests	Results
Catalase	positive
Ornithine decarboxylase test	negative
Lactose utilization	positive
Citrate	positive
Oxidase	positive
Urease	positive
Indole	negative
Methyl-red test	negative
Voges-Proskauer test	positive
Hydrogen sulfide production (TSI)	negative
Urea hydrolysis test	positive
Lysine decarboxylase test	positive
D-Glucose (acid/gas)	positive/ positive
D-mannitol fermentation	positive
Sucrose fermentation	positive
Arginine dihydrolase test	negative
D-sorbitol fermentation	positive
Cellobiose	positive
Esculin hydrolysis	positive
ONPG Test	positive

Computational studies

Computational analysis of haloacid dehalogenase type II from *K. pneumoniae* strain KLPN_25 have been carried out and its amino acid sequence was compared to other sequences in NCBI database using BLASTp. The amino acid sequence showed homology with various dehalogenase amino acid sequences from other bacterial species as summarized in Table 4.

Primary structure analysis

The primary structure of dehalogenase type II from *K. pneumoniae* strain KLPN_25 was generated using Protparam tool. The protein sequence consists of 235 amino acid residues with molecular weight of 25,595.05 Da with a positive GRAVY value (grand average of hydrophobicity) of 0.003. This indicates that dehalogenase type II was hydrophobic. The theoretical isoelectric point (pI) of dehalogenase type II was 4.85 suggesting its acidic amino acids were more than basic amino acids. There was a total of 30 negatively charged and 19 positively charged residues in the amino acid sequence. Furthermore, the total number of atoms was 3591 with a molecular formula of $C_{1143}H_{1790}N_{298}O_{352}S_8$ and aliphatic index of 93.91.

Multiple sequence alignment

The multiple sequence alignment of dehalogenase type II amino acid from five different species were performed as shown in Figure 4. This alignment has a minimum sequence length of 151 amino acid from *Klebsiella michiganensis* and a maximum sequence length of 235 from *Enterobacteriaceae* and *Salmonella enterica* subsp. *enterica*. Both *K. pneumoniae* strain KLPN_25 and *Pantoea deleyi* have a sequence length of 231, while *P. ananatis* has a sequence length of 226 amino acid. The multiple sequence alignment provides information on the conservation of core residues, includes hydrophobic core residues and regions that are important for protein function. The amino acids residues highlighted in red colour are the amino acid residues with high consensus and those low consensus value and neutral are in black. The conserved regions are G (Gly12), (Gly67), (Gly82), (Gly108), (Gly116), L (Leu14), (Leu22), (Leu23), (Leu28), (Leu36), (Leu51), (Leu55), (Leu74), (Leu85), (Leu92), (Leu109), (Leu112), (Leu119), (Leu122), (Leu134), (Leu147), F (Phe15), (Phe16), (Phe47), (Phe66), (Phe143), D (Asp17), (Asp24), (Asp104), V (Val18), (Val56), (Val59), (Val73), (Val105), (Val120), (Val149), N (Asn19), (Asn124), E (Glu20), (Glu71), (Glu75), (Glu87), (Glu150), T (Thr21), (Thr48), (Thr60), (Thr77), (Thr123), R (Arg35), (Arg39), (Arg80), W (Trp46), S (Ser49), (Ser54), (Ser62), (Ser125), (Ser148), H (His52), (His64), (His102), I (Ile69), (Ile83), A(Ala70), (Ala72), (Ala78), (Ala101), (Ala106), (Ala121), (Ala127), (Ala130), (Ala137), M (Met76), Y (Tyr81), P (Pro100), (Pro103) and Q (Gln114), (Gln115), (Gln133).

Secondary structure prediction

The prediction of the secondary structure of the protein done using CFSSP program showed that the number of predicted quantities of amino acid residues to form helix structure (H) are 195 equals to 83%, while 150 amino

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Table 4: List of dehalogenases enzyme and its sources.

Organism	Dehalogenase	Accession number	Percentage identity	References
Multispecies: *Enterobacteriaceae*	Deh type II	WP_040063703	100.00%	(van der Ploeg *et al*., 1991; Hill *et al*., 1999; Tsang and Sam, 1999; Janssen, 2001)
S. enterica subsp. *enterica*	Deh type II	EBH2621797	99.57%	Unpublished https://www.ncbi.nlm.nih.gov/protein/EBH2621797.1
P. ananatis	Deh type II	WP_024472286	72.32%	(van der Ploeg *et al*., 1991; Hill *et al*., 1999; Tsang and Sam, 1999; Janssen, 2001)
K. michiganensis	Deh type II	PLL41259	100.00%	(Yang *et al.*, 2019)
P. deleyi	Deh type II	WP_128086185	63.48%	(van der Ploeg *et al*., 1991; Hill *et al*., 1999; Tsang and Sam, 1999; Janssen, 2001)

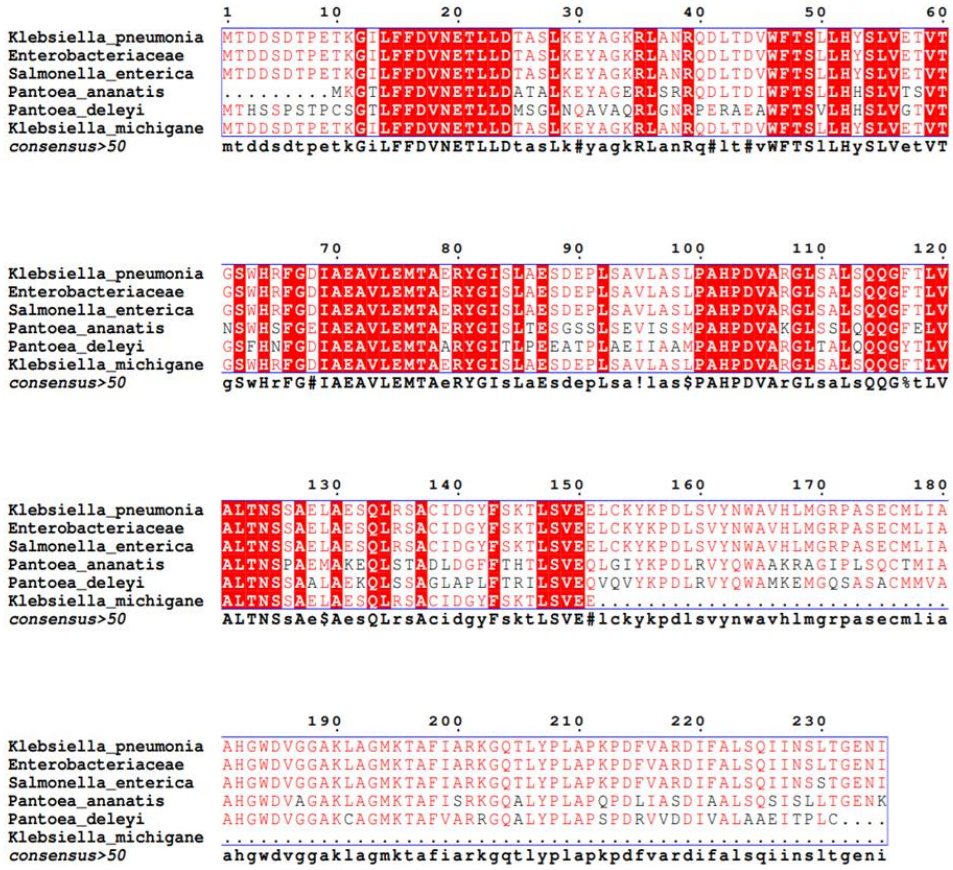

Figure 4: Multiple sequence alignment of haloacid dehalogenase type II from *K. pneumoniae* strain KLPN_25 with other five sequence from different species. The alignment was generated by MultiAlin program. Conserved amino acids residues are D (Asp), R (Arg), S (Ser), H (His), I (Ile), A (Ala), M (Met), Y (Tyr), P (Pro) and Q (Gln). The sequence were downloaded with accession number: *K. pneumoniae* strain KLPN_25 (RRE04903) (Tamma *et al.*, 2018), *Enterobacteriaceae* (WP_040063703) (van der Ploeg *et al.*, 1991; Hill *et al.*, 1999; Tsang and Sam, 1999; Janssen, 2001), *S. enterica* subsp. *enterica* (EBH2621797), *P. ananatis* (WP_024472286) (van der Ploeg *et al.*, 1991; Hill *et al.*, 1999; Tsang and Sam, 1999; Janssen, 2001), *P. deleyi* (WP_128086185) (van der Ploeg *et al.*, 1991; Hill *et al.*, 1999; Tsang and Sam, 1999; Janssen, 2001) and *K. michiganensis* (PLL41259) (Yang *et al.*, 2019).

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Figure 5: Prediction of the secondary structure of haloacid dehalogenase type II from *K. pneumoniae* strain KLPN_25.

acid residues form beta sheet (E), (63.8%) and 30 amino acids residue form turns (T), (12.8%) as shown in Figure 5. This predicted secondary structure of Deh type II by CFSSP server is in agreement for construction of homology model using SWISS-MODEL, whereby the enzyme is mainly made up from alpha helices and beta sheets but the loops are also significant.

Homology modelling

In the present study, three-dimensional model of dehalogenase type II from *K. pneumoniae* strain KLPN_25 was constructed based on the suggested template L-2-haloacid dehalogenase (L-DEX) from *Pseudomonas* sp. strain YL (PDB ID: 1JUD) by using SWISS-MODEL, a modelling server as shown in Figure 6. The template possesses 23.64% of sequence identity, 0.33 of sequence similarity and a coverage score of 0.94. The predicted structural model showed the “Global Model Quality Estimation” (GMQE) value of 0.61 and “Qualitative Model Energy Analysis” (QMEAN) is -3.27. GMQE is a parameter in estimating the model quality based on the target–template alignment and the template search method, ranging from 0 to 1 (Benkert *et al.*, 2008). The model built for dehalogenase type II from *K. pneumoniae* strain KLPN_25 based on the template L-DEX from *Pseudomonas* sp. strain YL reflects a considerably high in reliability with a 0.61 score in the expected accuracy of a model built with that alignment and template. On the other hand, QMEAN score indicates an estimation of the "degree of nativeness" of the structural features observed in the model on a global scale, in which the score around zero suggests good agreement between the model structure and experimental structures of similar size while a score of -4.0 or lower is an indication of model with low quality (Benkert *et al.*, 2008). Therefore, the model built with -3.27 QMEAN score is still considered quite good in the quality.

Pairwise analysis

The pairwise of both dehalogenase type II from *K. pneumoniae* strain KLPN_25 and L-DEX from *Pseudomonas* sp. strain YL showed only 24% sequence identity (41% similarity), however, some important amino acids are compared as well, for example D (Asp10), T (Thr14), R (Arg41), S (Ser118), K (Lys151), Y (Tyr157), S (Ser175), N (Asn177), and D (Asp180) (Figure 7). These are key amino acid residues that important for catalysis in the crystallised dehalogenases of L-DEX strain YL. In this study, these amino acids were predicted to have similar function with dehalogenase type II KLPN_25. The L-DEX YL amino acids are best aligned at location Asp10, Thr14, Arg41, Ser118, Lys151, Tyr157 and D180 are relatively equivalent to Asp17, Thr21, Arg39, Ser125, Lys156, Tyr162 and Asp185, respectively. Conserved amino acid residues often provide functional conservation. Therefore, it can be hypothesized that the catalytically important residues of L-DEX YL that are conserved dehalogenase type II KLPN_25 may also be catalytically important and probably have similar functions.

The key amino acids Asp10 in L-DEX YL (Asp17 in dehalogenase type II KLPN_25) have shown to play a role as a nucleophile α-carbon of the substrate to form an ester intermediate in the L-haloacid dehalogenase mechanism (Kondo *et al.*, 2015). The role of Thr14 in L-DEX YL (Thr21 in dehalogenase type II), is not yet

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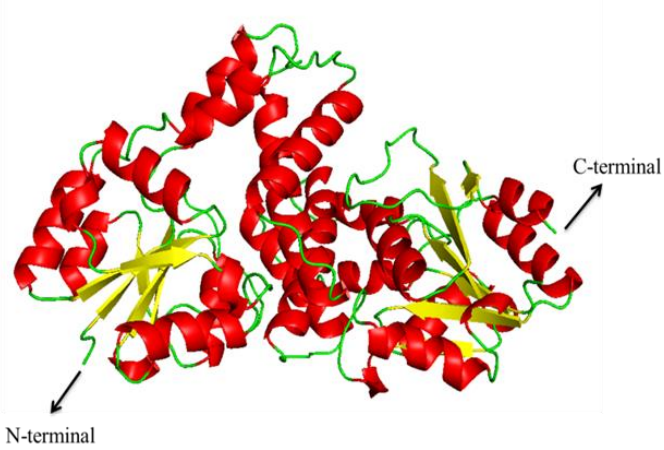

Figure 6: The structure of dehalogenase type II from *K. pneumoniae* strain KLPN_25 built according to L-DEX from *Pseudomonas* sp. strain YL as a template showing its secondary structures consisting of α-helix (red), β-sheet (yellow) and loop (green).

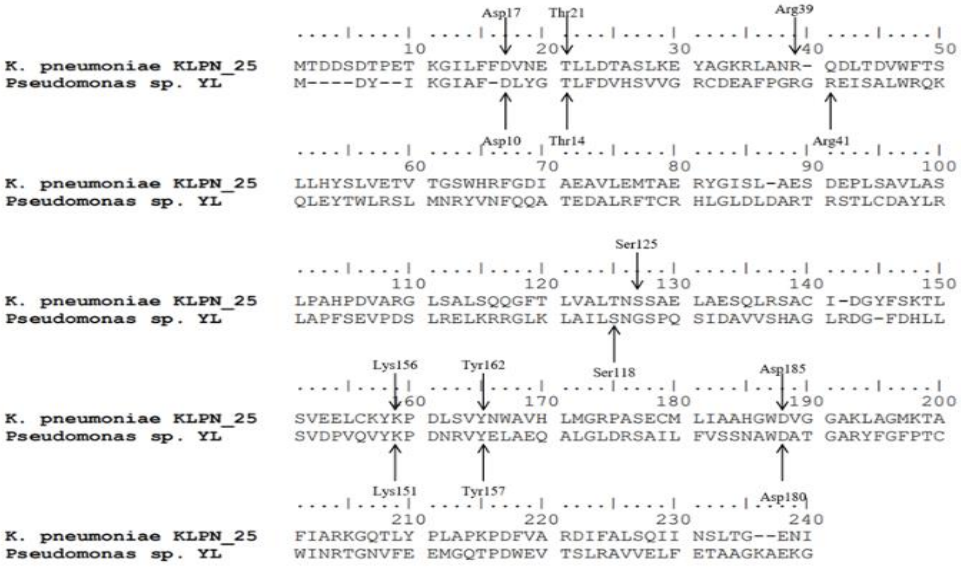

Figure 7: The pairwise sequence alignment results within haloacid dehalogenase type II from *K. pneumoniae* strain KLPN_25 and L-DEX from *Pseudomonas* sp. strain YL. Conserved regions are D (Asp17), T (Thr21), R (Arg39), S (Ser125), K (Lys156), Y (Tyr162) and D (Asp185).

determined, however it was reported to firmly hold the Asp10 in a position that favours the nucleophilic attack (Ridder *et al.*, 1997). Arg41 in L-DEX YL which corresponds to Arg39 in dehalogenase type II KLPN_25 act as a substrate uptake, by accepting the chloride ion released (Adamu *et al.*, 2016). Ser118 in L-DEX YL (Ser125 in dehalogenase type II KLPN_25) acts to stabilize the substrate hydrolysis of ester carboxyl moiety (Harisna *et al.*, 2017). In addition, Lys151 and Tyr157 in L-DEX YL corresponds with Lys156 and Tyr162 in dehalogenase type II KLPN_25, are proposed to be hydrogen-bonded to Asp180, in order for Asp180 keeps the orientation to activate the water molecule (Hisano *et al.*, 1996). Furthermore, Asp180 in L-DEX (Asp185 in dehalogenase type II), activate the hydrolysis of ester intermediate.

On the other hand, another two catalytically important residues of L-DEX YL which are S175 and N177, are not conserved in dehalogenase type II KLPN_25 and may probably the same as those in L-DEX but in different positions in the active site or substituted by similar residues. To fully elucidate the dehalogenation

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mechanism of dehalogenase type II from *K. pneumoniae* strain KLPN_25 and the contributions of the specific residues, additional work is needed. Although, dehalogenase type II KLPN_25 might dehalogenates the same substrates as L-2-haloacid dehalogenases from other organisms, its overall amino acid sequence is quite different from those of the other enzymes. Results of our pairwise and structural superposition of dehalogenase type II KLPN_25 amino acid sequence comparison with L-DEX from *Pseudomonas* sp. strain YL suggest that Asp17, Thr21, Arg39, Ser125, Lys156, Tyr162 and Asp185 are conserved in dehalogenase type II KLPN_25 and may be catalytically important in dehalogenation reaction.

CONCLUSION

Current study focused on isolation and characterization of bacteria able to utilize 2,2-DCP as a sole carbon source, a model substrate to detect the presence of dehalogenases. A bacterium strain YZ was successfully isolated from the river water sample of Sungai Skudai, Johor. The bacterium grown well in both liquid and solid PJC minimal media containing 10 mM, 20 mM and 30 mM 2,2-DCP, but growth was inhibited at 40 mM of 2,2-DCP. The biochemical tests and morphological examination revealed that bacteria strain YZ shared similarity with *K. pneumoniae*. The isolated bacterium was designated as *K. pneumoniae* strain YZ. Genomic DNA (obtained from NCBI data bases) of *K. pneumoniae* strain KLPN_25 was further analysed assuming strain YZ and KLPN_25 strains carried similar gene functions. Dehalogenase gene type II was found in strain KLPN_25. A three-dimensional model of haloacid dehalogenase type II from *K. pneumoniae* strain KLPN_25 was constructed based on template L-DEX from *Pseudomonas* sp. strain YL with conserved amino acid residues that play important roles in dehalogenation process. It is hypothesized that dehalogenase type II of *K. pneumoniae* strain KLPN_25 may have similar functions with crystallized L-DEX from *Pseudomonas* sp. strain YL, which is also present in strain YZ.

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