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Research Article 研究报告

Biochemical and structural characterization of a monoethylhexyl phthalate hydrolase from *Gordonia alkanivorans* strain YC-RL2

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Abstract: [Objective] This study aimed to investigate the effect of environmental factors on a monoethylhexyl phthalate hydrolase (MehpH) activity, model the 3-D structure of the enzyme and the interaction of the catalytic amino acid residues with the substrate. **[Methods]** The effect of environmental factors was determined by the standard enzyme assay. Primary structure analysis and 3-D model prediction were completed by DNAMAN (Version 2.1) and SWISS-MODEL server respectively and the results visualized by PyMOL software. Autodock tools, Swiss-PDB viewer and PyMOL were used to investigate the interactions between the enzyme and monoethylhexyl phthalate (MEHP). **[Results]** The primary structure of this enzyme was similar to MehpH from *Gordonia* sp. P8219 with different optimum temperature and pH (40 °C and 8.0, respectively). The enzyme was stable in presence of organic solvents, detergents and ions. However, it was inhibited by 2 mol/L of Ni^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} ions, 1 mol/L phenylmethylsulfonyl fluoride (PMSF), 0.5 mol/L paraoxon, 1 mol/L phenyl glyoxal (PGO), 2 mol/L diethyl pyrocarbonate (DEPC) and 5 mol/L eserine. The pentapeptide motif GXSXG and catalytic triad HSD conserved in serine hydrolases were present in the sequence. The docking result showed that the amino residues Thr152 and Ser230 were much conserved among the hydrolases and closely associated with MEHP (5.8 Å and 3.6 Å respectively) and thus may play important roles in the catalytic process. However, MEHP was not very close to the catalytic triad acid residues Ser125, His291, Asp259. **[Conclusion]** This study showed that MehpH in YC-RL2 was fairly stable in presence of organic solvents, detergents and metal ions indicating its application potential. The structural and catalytic analysis provides important information for further investigation of catalytic mechanism and enzymatic modification.

Keywords: monoethylhexyl phthalate, *Gordonia alkanivorans* YC-RL2, biochemical characteristics, hydrolase, structural characteristics

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Phthalic acid esters (PAEs) are high volume compounds not only applied as softeners of plastics, but also used as additives in the manufacture of adhesives, paints, lacquers, cardboard and cosmetics[1]. To provide the required flexibility, PAE plasticizers do not bond covalently to the plastic matrix and are thus capable of leaching into the environment[2]. PAEs are also one group of widely reported endocrine-disrupting compounds which could disrupt the endocrine system by interacting with estrogen receptors[3]. Di (2-ethylhexyl) phthalate (DEHP) is one of the predominant PAEs in the environment and various degradation methods for DEHP have been reported[4–5]. During degradation, DEHP is hydrolyzed to phthalic acid (PA) via MEHP. MEHP, as the metabolite of DEHP, was usually detected in urine of mammals[6]. It is an environmental contaminant of its own, and has been detected in many environmental media[7]. For instance, MEHP was detected in intravenous solutions stored in medical grade polyvinyl chloride (PVC) bags[8], sediment and soil[9]. Just like DEHP, MEHP is an active metabolite that has a potential to induce ovarian toxicity, inhibit hepatocyte protein synthesis in rats and to disrupt thyroid endocrine system in fish[7].

Microbial degradation of PAEs is the preferred choice of degradation but only a few certain species of bacteria and fungi have been reported as potent pollutant degraders and mostly under laboratory conditions[5]. Their application, however, is hampered by the complexity of natural environments. Although microorganisms can exist in extreme conditions, it is difficult to achieve optimal growth conditions of these degraders in the natural environment. These challenges can be overcome by using enzymes extracted from these microbes[10]. Enzymes are easier to handle and with the development of biotechnology methods such as directed evolution, they can be engineered to increase stability and broaden specificity[11–12].

Esterases are key enzymes involved in PAEs degradation. DEHP is de-esterified to MEHP which is further hydrolyzed to form phthalic acid (PA)[13]. PAE hydrolases were reported such as an esterase from *Micrococcus* sp. YGJ1[14], mono-2-ethylhexyl phthalate hydrolase from *Gordonia* sp. P8219[15], ester hydrolase PatE from *Rhodococcus jostii* RHA1[16], dibutyl phthalate (DBP) hydrolase from *Camelimonas*[17], DBP hydrolase from *Acinetobacter* sp. M673[18], and mono-2-ethylhexyl phthalate hydrolase from *Rhodococcus* sp. EG-5[19].

In a previous study, we isolated a DEHP degrading bacterium *Gordonia alkanivorans* strain YC-RL2, and cloned and expressed a monoethyl hexyl phthalate hydrolase gene (*mehpH*) in *Escherichia coli*. The enzyme (MehpH) could catalyze the transformation of a wide range of monoalkyl phthalates to PA. However, systematic characterization of this enzyme was not completed. The aim of this study was to systematically investigate the effect of environmental factors on enzyme activity, model the 3D structure of the enzyme and the interaction of the catalytic amino acid residues and the substrate.

1 Materials and methods

1.1 Chemicals, solutions and media

1.1.1 Chemicals: The chemicals used in this study: MEHP, PA, paraoxon, eserine, phenylmethylsulfonyl fluoride (PMSF), phenyl glyoxal (PGO), diethyl pyrocarbonate (DEPC), Tween-20, Tween-80, Triton-X 100 were all >96% purity and of analytical grade.

1.1.2 Solutions: 100 mol/L of citric acid-sodium acetate (pH 4.0–6.0), Na_2HPO_4-NaH_2PO_4 (pH 6.0–8.0), Tris-HCl (pH 8.0–9.0), and Glycine –NaOH (pH 9.0–10.0) were used.

1.1.3 Media: Luria-Bertani (LB) medium (g/L of distilled water): NaCl (10.0), peptone (10.0), yeast extract (5.0). For solidification of the LB, 15 g/L agar was added. The pH value was adjusted with NaOH or HCl (2 mol/L) to 7.0 ± 0.2 before autoclaving at 121 °C for 30 minutes.

1.2 Protein purification

The cloning and expression of *mehpH* gene was conducted as previously[20]. To obtain the high concentration and high quality of recombinant MehpH, the crude enzyme was concentrated and then purified by immobilized metal (Ni^{2+}) affinity chromatography (ÄKTA Avant, GE health sciences, USA). The concentration of purified enzyme was quantified using the protein absorbance at 480 nm using BSA as a standard.

The concentration of PA formed was measured by high performance liquid chromatography (HPLC) (Agilent 1200 series; Agilent technologies, USA) using methanol and 0.1% acetic acid as mobile phases in a ratio of 60%:40%.

1.3 Enzyme activity assays

1.3.1 Experimental setup and operation: The effect of environmental factors on purified enzyme activity was determined by using MEHP as the substrate. The produced PA was detected by HPLC. The total reaction mixture (1.0 mL) consisted of Tris-HCl buffer (pH 8.0), 50 μL (70 μg/μL) of enzyme solution and 50 mg/mL MEHP-methanol stock solution. The reaction was stopped by addition of 1mL of 6 mol/L HCl. Parameters may be changed for the determination of different factors. Three replications were taken for each measurement and the mean value calculated.

1.3.2 The effects of pH and temperature: The effect of pH on the enzyme activity was examined at 40 °C in the pH range (5.0–10.0). The following buffers (100 mol/L) were used: citric acid-sodium citrate (pH 4.0–6.0), Na_2HPO_4-NaH_2PO_4 (pH 6.0–8.0), Tris-HCl (pH 8.0–9.0), and Glycine-NaOH (pH 9.0–10.0).

The effect of temperature on the enzyme activity was investigated at optimum pH (pH 8.0) by incubating the reaction mixture at temperatures ranging from 5 °C to 60 °C with an interval of 5 °C.

1.3.3 The effects of organic reagents and metal ions: The stability of the enzyme in several organic reagents such as SDS, Triton X-100, Tween-20, Tween-80, methanol and ethanol was investigated by the measurement of the enzyme activity using the standard enzyme assay as described above. The reaction was then incubated with each compound at 40 °C for 60 minutes. Each measurement was carried out with two different concentrations of the compounds: 1% and 5% (*W*/*V*) detergents and organic solvents. Control treatments were prepared with the enzyme in the buffer solution lacking the above solvents and incubated in the same way as described above.

To investigate the effect of different cations on the enzyme activity, 2 mol/L of cations: Ca^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Mg^{2+}, Co^{2+}, Cd^{2+}, Al^{3+}, Ni^{2+} and Zn^{2+} were separately added to the enzymatic reaction and incubated for 60 min at 40 °C.

1.3.4 The effect of chemical amino acid modifiers: The inhibitory effect of the chemical modifiers, which are specific to particular amino acids, such as PGO to Arg, DEPC to His, and PMSF to Ser was examined. Enzyme activity was measured by adding different concentrations to the standard enzyme assay and then incubated for 60 min at 40 °C. Inhibitors such as paraoxon and eserine (0.5 mol/L and 5 mol/L respectively) which are known as indicators for the classification of esterases were also examined under the same conditions as described above.

1.4 Enzyme structure modeling

1.4.1 Analysis of MehpH primary structure: Blastn and Blastp were used to search for related nucleotide sequences and amino acid sequences (www.ncbi.nlm.nih.gov/Blast), respectively. For primary structure analysis, the amino acid sequences of MehpH and other related amino acid sequences were aligned using DNAMAN (version 2.1).

1.4.2 Prediction of MehpH three-dimensional structure: Homology modeling prediction was performed by using SWISS-MODEL server (https://swissmodel.expasy.org/). The models were analyzed using the PyMOL software (version 1.5.0.3). SWISS-MODEL provided us several

templates, and only model with the highest global model quality estimation values and the highest values from the QMEAN server were used for further investigation.

1.4.3 Molecular docking: Molecular docking was performed by docking the chemical substance MEHP obtained from the PubChem Compound Database (https://www.ncbi.nlm.nih.gov/pccompound) to the active site of the MehpH protein using AutoDock Tools software (version 1.5.6). Polar hydrogen atoms were added to MehpH using the Autodock Tools software. The protein receptor (MehpH) and ligand MEHP were converted from PDB format to PDBQT format. Six bonds within the ligand were set to allow rotation as the fewest atoms and the flexible and rigid files were saved respectively. A grid box was used around the active site to cover the entire enzyme binding site. The best conformation with the lowest docked energy was chosen from the docking search. The interaction of complex MehpH-MEHP conformation was analyzed using Swiss-PdbViewer v4.0 and PyMOL software.

2 Results

2.1 Protein purification

The crude enzyme was purified and concentrated by ÄKTA avant protein purification systems. The protein was analyzed by SDS-PAGE and the result is shown in Figure 1.

2.2 Enzyme activity assays

2.2.1 The effects of pH and temperature: The optimum pH and temperature of the MehpH activity was determined using MEHP as a substrate. The activity was determined by measuring the amount of PA produced by HPLC. Just like the P8219-MehpH and EG-5-MehpH, it showed preference for high temperature (40 °C) and slightly alkaline pH (8.0) (Figure 2-A) and (Figure 2-B) respectively. The relative activity of the enzyme sharply declined after 40 °C.

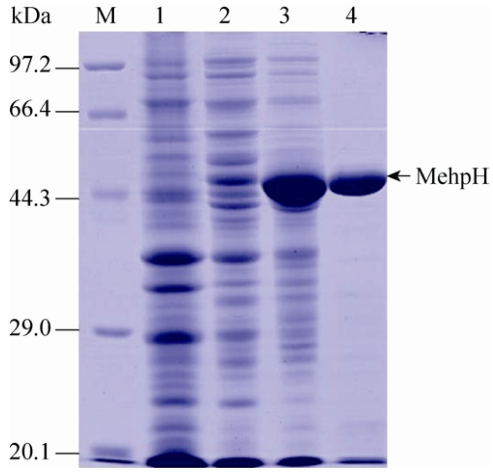

Figure 1. SDS-PAGE result of recombinant MehpH[20]. M: protein molecular marker; lane 1: before induction, lane 2: after induction, lane 3: extracted crude protein, lane 4: purified recombinant MehpH.

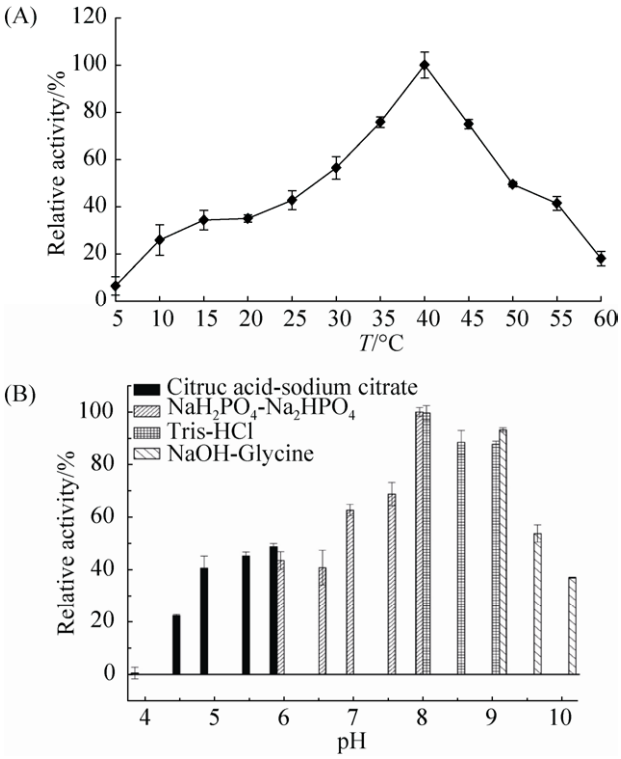

Figure 2. The effect of temperature (A) and pH (B) on the activity of MehpH. The activity was determined by measuring the concentration (mg/mL) of PA produced in 60 min. Relative activity was calculated as a percentage of the highest measurement. Error bars represent standard deviation from the mean of three measurements.

2.2.2 The effects of organic reagents and metal ions: The effect of detergents and organic solvents on MehpH activity was examined using the standard enzyme assay and the results shown in Table 1. After incubation at 40 °C for 60 min with 1% and 5% non-ionic detergents (Triton X-100, Tween-20 and Tween-80), the enzyme retained > 50% of its activity. Incubation with 1% SDS slightly inhibited the enzyme.

The effect of organic solvents was investigated by incubating the enzyme separately with 1% and 5% ethanol and methanol.

The effect of various ions is shown in Table 2. A concentration of 2 mol/L Zn^{2+}, Cu^{2+}, Fe^{3+} and Ni^{2+} inhibited enzyme activity (Residual activities; 44.13%, 42.59%, 56.85% and 50.67 % respectively).

2.2.3 The effect of chemical amino acid modifiers: In order to investigate the catalytic mechanism of MehpH, the inhibitory effect of various amino acid modifiers was measured using the standard enzyme assay after incubation at 40 °C for 60 min as described by Nishioka *et al* (2006)[15]. As shown in Table 3, the enzyme activity was inhibited by 1 mol/L PMSF, and 0.5 mol/L paraoxon, 1 mol/L PGO, 2 mol/L DEPC and 5 mol/L eserine.

Table 1. Effect of organic solvents and detergents on enzyme activity

Treatment	Concentration/%	Relative activity/%
CK	0	100
TritonX-100	5	55.150±1.880
	1	74.170±1.247
Tween-20	5	76.800±3.360
	1	86.650±4.437
Tween-80	5	73.070±5.216
	1	88.670±2.666
Ethanol	5	76.790±5.320
	1	93.130±3.080
Methanol	5	83.540±3.628
	1	99.010±4.450
SDS	1	89.230±2.050

Table 2. Effect of different ions (2 mol/L) on enzyme activity

Ions	Relative activity/%	Ions	Relative activity/%
CK	100	Mn^{2+}	72.38±0.56
Fe^{2+}	89.56±1.86	Zn^{2+}	44.13±0.98
Co^{2+}	79.86±0.64	Cu^{2+}	42.59±1.07
Al^{3+}	77.16±0.11	Fe^{3+}	56.85±0.67
Cd^{2+}	83.37±2.98	Ni^{2+}	50.67±2.07
Mg^{2+}	71.63±2.55	Ca^{2+}	83.09±0.66

2.3 Enzyme structure modeling

2.3.1 Primary structure of MehpH: The YC-RL2 MehpH showed high identity with several hydrolases of Abhydrolase superfamily and the amino acid sequence of YC-RL2-MehpH was totally the same with P8219 MehpH (Figure 3). The MehpH has the consensus pentapeptide motif GXSXG (X indicates an unconserved amino acid residue).

2.3.2 Prediction of MehpH 3D structure: The absence of a 3D structure for MehpH in PDB prompted us to construct a 3D model. Homology modeling prediction was performed by using SWISS-MODEL server (https://swissmodel.expasy.org/). Among the several models, one model based on a MhpC enzyme (PDB ID: 1u2e) which was a C-C bond hydrolase was selected as the prediction model of YC-RL2-MehpH (Figure 4).

The structure of template protein and constructed model were analyzed using PyMOL software. The template protein MhpC contained the active site in a buried channel including Ser110, His263 and Asp235, and they were postulated contributors to a serine protease-like catalytic triad in homologous enzymes. The constructed model based on MhpC contained the similar amino acid residues Ser125, His291 and Asp259 (Figure 5). The

Table 3. Effect of amino acid-specific inhibitors (Eserine, PMSF and Paraoxan, DEPC and PGO)

Treatment	Concentration/(mol/L)	Relative activity/%
Control	0	100
Eserine	5.0	25.10±3.00
DEPC	2.0	35.90±0.40
Paraoxon	0.5	51.80±0.05
PGO	1.0	42.30±4.00
PMSF	1.0	50.00±0.05

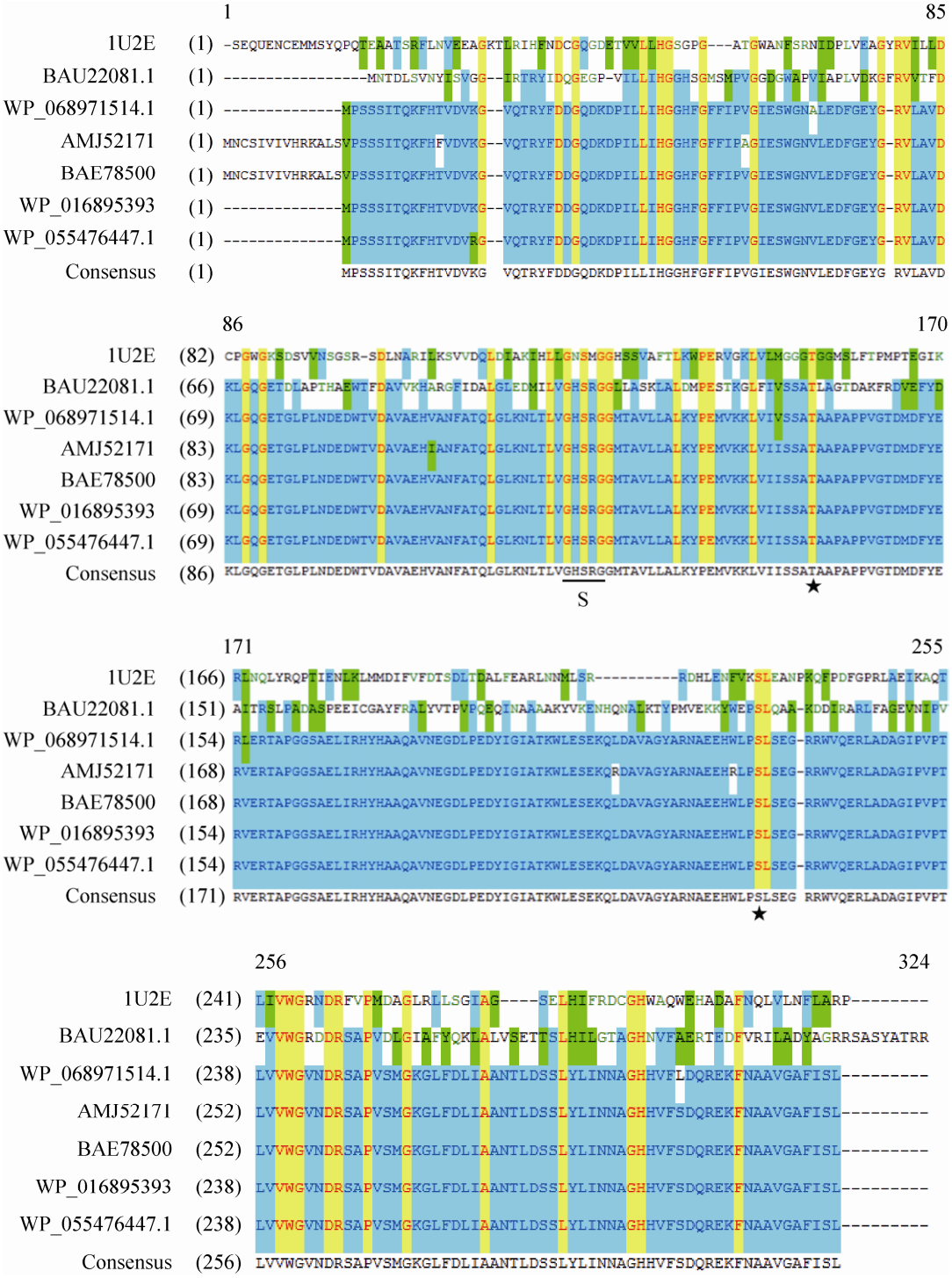

Figure 3. Protein sequences alignment between MehpH and Abhydrolases. KU212213.1: MehpH (*Gordonia* sp. YC-RL2); AB214635.1: MehpH (*Gordonia* sp. P8219); BAU22081.1: MehpH (*Rhodococcus* sp. EG-5); 1U2E: MhpC (*E. coli*); WP_016895393.1: α/β hydrolase (Corynebacteriales); WP_055476447.1: α/β hydrolase (*Gordonia* sp. HS-NH1); WP_068971514.1: hydrolase (*Nocardia farcinica*). A conserved pentapeptide (GXSXG) motif is underlined. Pentacle marked the possible active site of the MehpH.

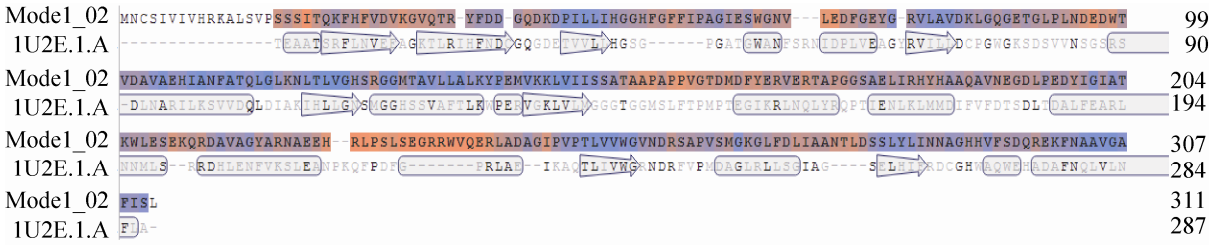

Figure 4. The result of model-template alignment. The colours mean the quality of QMEAN (orange gradual changing to blue means low quality to high quality).The box indicates secondary structure of the sequence is α helix and arrow means it is β sheet.

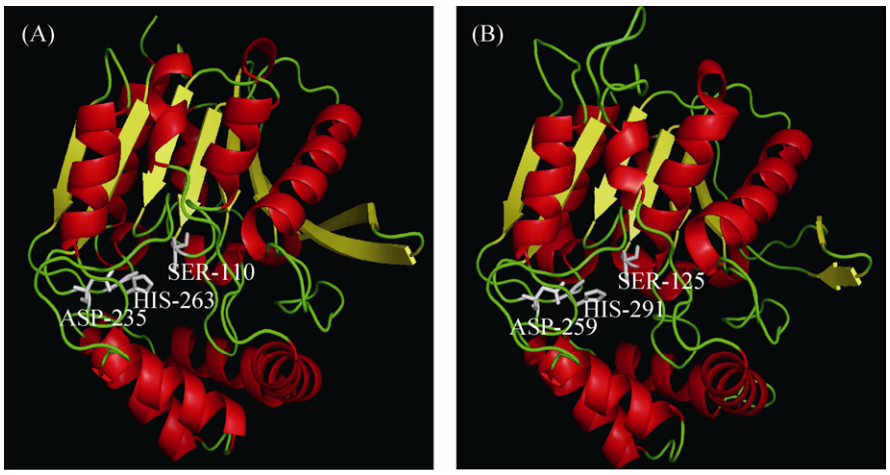

Figure 5. Secondary structure of MhpC (PDB ID:1u2e) (A) and MehpH (B). The serine protease-like catalytic triad (Ser110, His263 and Asp235) marked in white in figure A. The space similar amino residues (Ser125, His291 and Asp259) marked in white in figure B.

model contained the lid domain similar as the four helices of MhpC. The constructed model consisted of 11 helices and 8 sheets.

2.3.3 Molecular docking: Molecular docking could predict the interactions between the MehpH protein and the suitable ligand MEHP. Docking analysis was finished using AutoDock Tools software (Figure 6).

Analyzing the interactions of complex MehpH-MEHP conformation was completed using Swiss-PdbViewer v4.0. Selecting the atoms C and O of ester bond in MEHP as the center, a search the possible reactive amino residues of MehpH in the range of 6 Å was conducted. The result was analyzed with PyMOL software. There were nine amino residues namely Met129, Ser150, Thr152, Ala153, Tyr166, Leu228, Pro229, Ser230 and Met266 around MEHP in the range of 6 Å (Figure 7).

According to the result of docking, the MEHP was not very close to the serine protease-like catalytic triad amino acid residues Ser125, His291 and Asp259. Thr152 and Ser230 were much conserved among the hydrolases and the template enzyme. And by analyzing the docking result and primary alignment result, we predicted the amino residues Thr152 and Ser230 may play important roles in the catalytic process. Thr152 and Ser230 were much conserved among the hydrolases and the template enzyme. Thr152 was 5.8 Å from MEHP and Ser230 was 3.6 Å from MEHP (Figure 8).

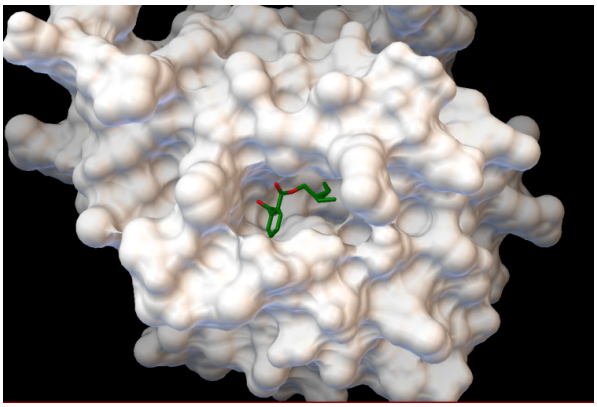

Figure 6. The docking result.

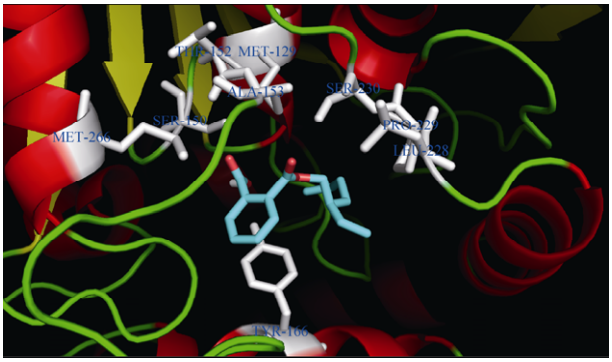

Figure 7. The possible reactive amino residues of MehpH in the range of 6 Å.

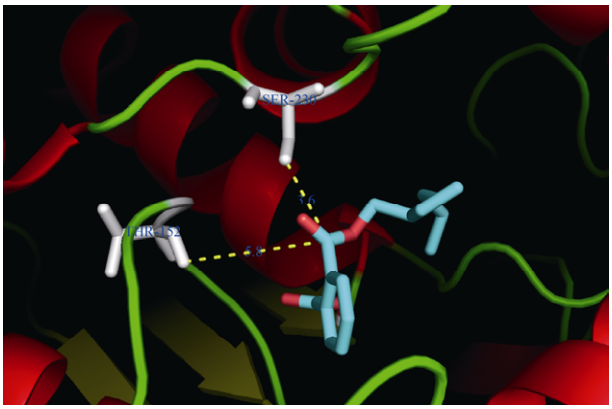

Figure 8. The distance between MEHP and the amino residues (Thr152 and Ser230).

3 Discussion

Enzyme activity depends on defined conditions with respect to temperature, pH, nature and strength of ions[21]. The enzyme catalyzing the hydrolysis of monoalkyl phthalates to PA was previously identified from strain YC-RL2. The recombinant enzyme was resolved by SDS-PAGE and had a molecular weight above 44 kDa[20]. In comparison, P8219-MehpH was a 32 kDa homodimeric protein.

The optimum temperature and pH were 40 °C and 8.0 respectively. The optimum pH was similar to that from EG-5 MehpH. However, unlike EG-5-MehpH, CarEW and P8219-MehpH (optimum temperature and pH: 50 °C, 8.0; 45 °C, 7.5 and 45 °C, 7.5 respectively) the enzyme's activity reduced beyond 40 °C and preferred a slightly alkaline pH.

The enzyme showed a great stability in organic solvents and detergents. It was inhibited by Triton X-100 (residual activities of 74.17% and 55.15% at 1 and 5% respectively). High concentrations of the organic solvents alter the native structure of proteins by disrupting hydrophobic interactions between the non-polar side chains of amino acids[22]. 2 mol/L of Fe^{2+}, Ca^{2+}, Co^{2+}, Al^{3+}, Mg^{2+} and Mn^{2+} had no significant inhibition on the enzyme while the same concentration of Ni^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+} severely inhibited the enzyme (residual activities 50.67%, 56.85%, 42.59% and 44.13% respectively). The stability of the enzyme in presence of solvents, ions and detergents is critical if the enzyme is to be used in industrial applications. Ions influence the enzyme activity both by means of their ionic strength and by their nature which may influence the catalytic process directly especially if required as essential cofactors or are inhibitors[21].

The results of enzyme inhibition by paraoxon, eserine and PMSF confirmed that MehpH is a serine hydrolase. Inhibition by DEPC and PGO showed that Arg and His residues maybe at or around active site and are related to catalytic activity of the enzyme or involved in the stabilization of the enzyme structure[15]. Multiple sequence alignment results showed that YC-RL2-MehpH is completely similar to P8219-MehpH and closely related to

hydrolases like EG-5-MehpH and CarEW. Nishioka *et al*. (2006) proposed that P8219-MehpH was a novel serine hydrolase phylogenetically related to the meta-cleavage compound hydrolases[15]. Our results showed that these hydrolases contain the conserved pentapeptide motif (GX_1SX_2G) and catalytic triad (DHS) typical of serine hydrolases. However, X_2 is a basic Arg residue unlike the typical hydrophobic residues such as Phe, Ala and Met usually found in this position. Iwata *et al*. (2016) investigated the role of this residue and found out that mutation of Arg to Phe, Ala or Met reduced the activity of EG-5-MehpH towards MEHP but had no effect on the activity towards 1-naphthyl esters. These results suggested that the Arg was involved in stabilizing the structure of the active site[19].

Serine hydrolases belong to the big family of α/β hydrolases which use Asp-His–Ser catalytic triad where Ser acts a nucleophile while Asp/Glu and His act as acid and basic residues respectively. Variants where Ser is replaced by Thr have been reported[23–24]. The Thr residue also acts as a nucleophile and has been identified in other hydrolytic enzymes of α/β fold for instance aspartylglucosaminidase[23]. YC-RL2-MehpH had the catalytic triad Ser125, His291, Asp259 in its sequence but the docking result showed that these residues are not in close proximity to the ligand. Instead Thr152 and Ser230 were closely associated with MEHP with distances of 5.8 Å and 3.6 Å respectively (Figure 8).Thomas *et al*. (1999) revealed the presence a second conserved Ser in carboxylesterases whose function was stabilize the orientation of the catalytic triad via hydrogen-bonding rather than being an essential catalytic amino acid[25–26]. In YC-RL2-MehpH, this could be Ser230 that is in close proximity to the ligand (Figure 3). Furthermore, nine predominantly hydrophobic amino acid residues (Figure 7) were found in the range of 6 Å of MEHP. This is not surprising considering the hydrophobic nature of the substrate[25].

This study showed that YC-RL2-MehpH was fairly stable in presence of organic solvents, detergents and metal ions indicating its application potential. This study provides important information for further investigation of catalytic mechanism and enzymatic modification. However, the actual catalytic mechanism of MehpH remains unknown and therefore determining the atomic structure would elucidate further on the relationship between the ligand and active site residues.

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MehpH 的生化与结构特性

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摘要：【目的】本研究旨在系统探究环境因素对邻苯二甲酸单乙基己基酯水解酶(monoethylhexyl phthalate hydrolase，MehpH)活性的影响，模拟该酶的 3D 结构并分析活性中心氨基酸残基与底物之间的相互作用。【方法】根据标准的酶学实验验证了环境因素对酶活性的影响。该酶的基本结构分析由 DNAMAN (2.1)分析所得，同时利用 SWISS-MODEL 对其 3D 模型进行预测，并通过 PyMOL 软件对相关结果作了可视化处理。Autodock 工具，Swiss-Pdb 以及 PyMOL 均用以酶与邻苯二甲酸单乙基己基酯(monoethylhexyl phthalate，MEHP)的相互作用分析。【结果】该酶的初级结构与已报道 *Gordonia* sp. P8219 菌株中的酯类水解酶相似，该酶的最适温度与 pH (分别为 40 °C 和 8.0)和菌株 P8219 有所不同；该酶在有机溶剂、洗涤剂和金属离子环境中表现出良好的稳定性；然而，其酶活受 2 mol/L 的 Ni^{2+}、Fe^{3+}、Cu^{2+}和 Zn^{2+}离子，1 mol/L 苯甲基磺酰氟(PMSF)，0.5 mol/L 的对氧磷，1 mol/L 苯基醚(PGO)，2 mol/L 焦碳酸二乙酯(DEPC)和 5 mol/L 的毒扁豆碱所抑制。丝氨酸水解酶中高度保守五肽基序 GXSXG 与催化三联体 HSD 结构均存在于该酶的编码序列中。根据分子对接结果，Thr152 与 Ser230 在水解酶与其模板之间也显得更为保守，且与 MEHP 联系较为紧密(分别为 5.8 Å 和 3.6 Å)，可能起着重要的催化作用。而 MEHP 与催化氨基酸残基 Ser125、His291、Asp259 并不十分靠近。【结论】YC-RL2 中 MehpH 在有机溶剂、洗涤剂以及金属离子环境中具有稳定的生物活性，表明其具有良好的应用潜力。酶的结构及活性中心得到了初步的分析，对于催化机理及酶改造研究具有重要意义。

关键词：邻苯二甲酸单乙基己基酯，食碱戈登氏菌 YC-RL2，生化特性，水解酶，结构特性

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