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Characterization of Crude DPEase (D-Psicose 3-Epimerase) from Recombinant *Escherichia coli*

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Abstract: *D-psicose is a unique monosaccharide sugar with prospective health benefits and physiologically safer than fructose. D-psicose is an enzymatic product of DPEase, converting fructose as substrate. The DPEase holds an important mechanism to produce D-psicose in laboratory and industrial scale. The DPEase is known to bind with certain cofactors in performing optimum enzymatic reaction. This study aimed to characterize the crude DPEase obtained from recombinant Escherichia coli in order to obtain the optimum condition for converting fructose to D-psicose in laboratory scale. Environmental parameters such as pH, temperature, and presence of metal ions were adjusted to observe any changes in DPEase activity. The results showed that optimum condition for DPEase activity were at 35^{o}C, pH 8.0, and addition of Co^{2+} dan Mn^{2+} enhanced the enzyme activity. Meanwhile, the presence of metal ions, e.g Ni^{2+} , Cu^{2+}, Zn^{2+} , Ca^{2+}, Mg^{2+} and Mo^{2+} were known as inhibitors to enzyme activity*.

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I. Introduction

D-psicose is a ketose sugar by having a carbon 3-epimer in D-fructosestructure [1]. This distinguishing character makes D-psicosesafe for consumption in health perspectives [2]. There are several health benefits of low-calorie D-psicose sugar, e.g it is readily absorbed in human body metabolism or in the small intestine, therebyenhances sugar consumption by diabetic patients [3], prevent the postprandial hyperglycemia in patients [4], when eating foods containing sucrose and maltose [5], [6]. Other benefits, D-psicose may be consumed for fat and weight losses [7], nerve protectors [8], antioxidant activity [9], pancreatic protector [10], with no side effects [11]. D-psicose from isalso reported to lower the hypertension [12].

D-psicoseis obtained from enzymatic reaction between the DPease enzyme and its substrate, the fructose. [13]. DPEase enzyme is the most important element in D-psicose production. In the three-dimensional structure of DPEase, the residues involved in binding of substrates or fructise are E156, W112, R215, I66 and H186, while metal binding sites are H209, D183, E150 and E244.Theresidue charactersdistinguisheDPEase from other epimerases [14]. The DPEase was originally classified as DTEase (D-tagatose 3-epimerase) [15], but recent finding on its main product, the D-psicose, has clarified its position and was grouped into the DPEase enzymes [14]. The DPEase is a metalloproteinwhich requires metal ions to increase its enzyme activity [16], especially Co^{2+} andMn^{2+} [17]. Due to possible nature of different metal binding properties by different source of DPEase, we need to investigate the environmental conditions which may directly improve or inhibit the enzyme activity in laboratory scale by using the recombinant *E. coli* as a model in this study.

II. Material And Methods

Recombinant *E. coli* containing the *dpe* gene from *Agrobacterium tumefaciens* was used as D-psicose producing strain in this study [18]. Crude enzymes were obtained from fermentation medium containing containing fructose as substrate. Enzyme assay or DPEase activity was determined based on the yield of D-psicose from D-fructose. The enzyme reaction contained a mixture of crude enzyme, fructose, Tris-HCL buffer (pH 8.0), and distilled water, reacted at 35°C for 10 minutes. The enzyme reaction was stopped in boiling water.The remaining fructose is removed or converted by adding *Saccharomyces.cereviceae* into the reaction mixture and analyzed by the Cysteine-carbazole method [19]. One unit of DPEase activity is determined as the amount of μmol D-psicose formed in a reaction. The protein content (mg/mL) was determined using the Lowry method [20], and bovine serum albumin was used as a standard. Characterization of DPEase based on varying environmental conditions, e.g temperature, pHand presence of metal ions.

Temperature condition Determination of optimum temperature was conducted from 30 to 50°C.
Acidity (pH) Condition Determination of optimum pH was tested in the range of 6.0 to 10.0. at 35°C for 10 minutes.
Presence of Metal Ions Determination of metal-binding capacity by DPEase was tested under the presence of Ni^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Mo^{2+}, and Mn^{2+} at 35 ° C for 1 hour incubation period. Results of metal-binding capacity by different metal ions were designated as cofactors or inhibitors based on their effects to enzyme activity. All enzyme activity was calculated as percentage of relative activity (%) in respect to standard condition or before characterization assay.

III. Result

Temperature Condition
The crude DPEase showed the highest relative activity at 35°C. Upon reaching the optimum temperature, the relative activity declined slowly at 40, 45, and 50°C (Figure 1).

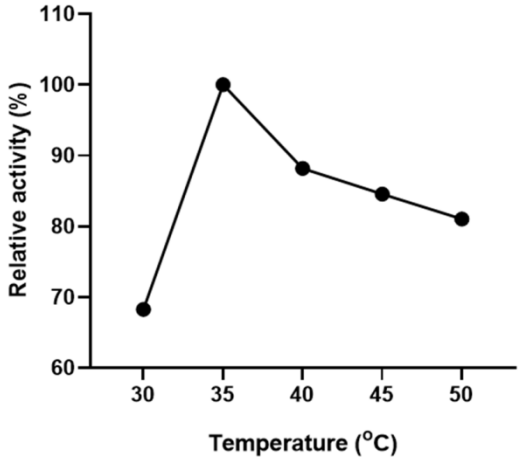

Fig. 1. Effect of temperature on relative activity of DPEase

Acidity (pH) Condition
The crude DPEase showed the highest activity at pH 8.0. Upon reaching the optimum pH, the relative activity declined steadily between pH 8.5 and 9, and drastically declined at pH 10.0 (Figure 2).

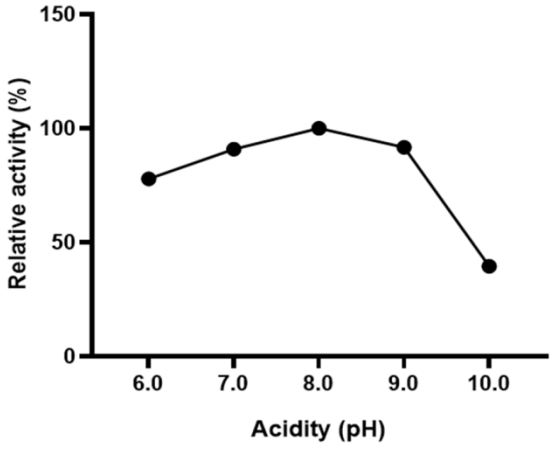

Fig. 2. Effect of acidity (pH) on relative activity of DPEase

Presence of Metal Ions
The presence of the metal ions, Co^{2+} and Mn^{2+} are known to act as cofactors. In contrary, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, and Mo^{2+} are inhibitors in this study (Figure 3).

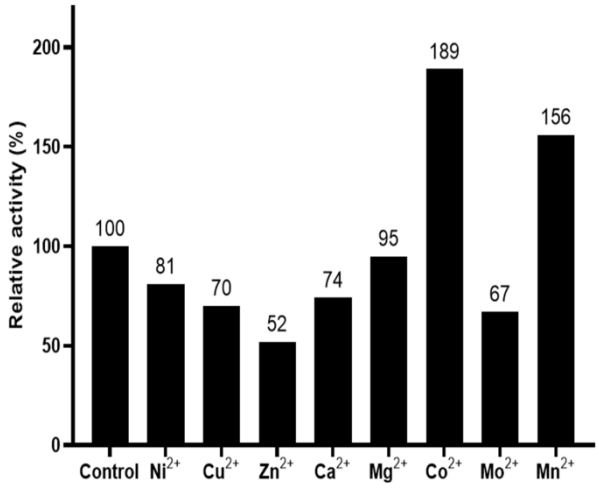

Fig. 3. Effect of metal ions to relative activity of DPEase

IV. Discussion

Several studies have been conducted to determine the temperature characteristics of the DPEase enzyme by using different microorganisms, such as *Clostridium bolteae* (55ºC) [16], *C. cellulolyticum* H10 (55ºC) [21] and *Treponema primitia* ZAS-1 (70ºC) [22]. Of all reported temperature characteristics, the DPEase produced by recombinant *E. coli* may be considered as relatively different by being active in mesophilic temperature. This may be due to differences in amino acids composition of each DPEase. Determination of optimum temperature of an enzyme is needed since the temperature affects the speed of the enzymatic reaction by increasing substrate binding through collision in aqueous solution. In contrary, enzyme reacts in outer temperature range may result into decline of activity or even loss of activity or denaturation [23], rendering enzyme on binding to substrate [24].

Different pH condition may affect the enzyme activity of DPEase as reported from previous studies, such as *C. bolteae* (pH 7.0) [16], *C. cellulolyticum* H10 (pH 8.0) [21], and *T. primitia* ZAS-1 (pH 8.0) [22]. Similar results were also obtained for the DPEase activity which resulted in the highest relative activity at 8.0. The DPEase in this study performed a stable enzymatic reaction in a wide spectrum of pH, ranging from 6.0 to 9.0. Environmental pH may be adjusted to achieve optimum activity of an enzyme, otherwise a higher or lower pH may result in change of chemical structure of enzyme yet decreasing or even losing its enzyme activity [25].

Most enzymes require cofactors to increase their activity while some enzymes do not need cofactors. Several studies have approved that DPEase is strongly dependent on Co^{2+} as cofactor [16], [21], [22]. Cobalt is an essential cofactor to DPEase enzyme in binding to the D-fructose to produce D-psicose. The function of the cofactor is to strengthen the bonds between the substrate and the enzyme by transferring electrons arise during the catalysis process. Inhibitors are inhibitory molecules that work by competing with the substrate to penetrate the active side of the enzyme, maintaining the shape of the enzyme which result in loss of activity [26].

V. ConclusionAcknowledgement

The DPEase enzyme studied from recombinant *E. coli* has optimum characteristics of temperature at 35ºC, pH 8.0, and increased its activity by the presence of Co^{2+} and Mn^{2+}. Other metal ions tested such as Ni^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+} and Mo^{2+} are known as inhibitors.

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