UNIVERSITI PUTRA MALAYSIA

ISOLATION OF TRANSKETOLASE GENE, SUBCELLULAR LOCALIZATION, AND TRANSKETOLASE PROTEIN STRUCTURE STUDIES OF SUGARCANE (Saccharum officinarum. L)

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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May 2013

Chairman: Rosimah Nulit, PhD

Faculty: Science

This study focused on isolation, identification, subcellular localisation, protein structure prediction of transketolase enzyme from sugarcane, *Saccharum officinarum*. Pentose phosphate pathway is composed of two functionally-connected phases, the oxidative and non-oxidative phase. Both phases are catalysed by a series of enzymes. One of these enzymes is transketolase which play an important role in non-oxidative phase of pentose phosphate pathway (PPP). Synthesis of sugar phosphate intermediate is the main role of this enzyme in non-oxidative phase of pentose phosphate pathway. Transketolase transfer two carbon units from fructose-6-phosphate to erythrose-4-phosphate and convert two carbon fragments from glyceraldehyde-3-phophate (G3P) to ketose of xylulose-5-phosphate. Erythrose-4-phosphate then enters the shikimate pathway that produced many secondary metabolites such as aromatic amino acids, lignin and flavonoid. Transketolase also play important role in photosynthesis and glycolysis. Although the contribution of transketolase in plant system is important, study of this enzyme is still limited. Until now, *TKT* genes had been isolated only from seven plants so far, thus this leads to

the first objective to isolate *TKT* gene from sugarcane to compare its identity with other organisms. Unlike bacteria, fungi and all animals, PPP is complete in the cytosol of these living system and all enzymes of this pathway localised in the cytosol. However, in plant system, the first phase of pentose phosphate pathway is complete in the cytosol of plant and the sub localisation for non-oxidative pentose phosphate pathway in still remains unclear. Thus, the second objective is to determine its subcellular localization of sugarcane transketolase. The final objective is to predict modelling protein structure of sugarcane transketolase. The isolation of *TKT* by was done by RT- PCR, followed by cloning and sequencing. The present study has isolated 2327 bp length of sugarcane *TKT*. Similarity studies using CLUSTALW2 revealed that sugarcane *TKT* gene showed highest identity with *Zea mays* (99%). Classification analysis revealed that both plants are in the same family, Poaceae. However, the identity of sugarcane *TKT* compared with other plants species are low (<65 %) due to these plants are only in the same phylum, Strephytophyte. Moreover, sugarcane *TKT* showed lower similarity (<60%) compared with bacteria, yeast and animals because these organisms from different kingdom, animalia. Analysis by Target P 1.1 followed by Chloro P revealed that sugarcane transketolase is localised in the chloroplast which is 85 amino acids are plant plastid target sequence. The nucleotide sequence of sugarcane *TKT* was converted to amino acid sequence using Expasy.org, Phyre2 and YASARA to predict secondary, tertiary structure and the quality of protein structure of sugarcane transketolase. This present study revealed that sugarcane transketolase protein is 100% similarity with Thiamin diphosphate-binding fold, from *Zea mays* (d1itz). In conclusion, sugarcane transketolase was successfully isolated and was found plastidic in plant system. This led to conclusion that the OPPP is incomplete in the

cytosol of sugarcane. This present study also similarity sequence of sugarcane TKT closely related with classification or taxonomy level of plants and other organisms. This present study also found that sugarcane transketolase protein is 100% similarity with Thiamin diphosphate-binding fold, from *Zea mays* (d1itz).

Abstrak tesis yang dikemukakan kepada Senate Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains

PEMENCILAN, PENENTUAN PENEMPATAN SUBSELLULAR DAN KAJIAN STRUKTUR PROTEIN TRANSKETOLASE DARIPADA TEBU, (*Saccharum officinarum*. L)

Oleh

NAHID KALHORI

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Pengerusi: **Rosimah Nuli, PhD**

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Kajian ini memfokuskan kepada pemencilan, pengenalpastian, penentuan penempatan subsellular dan kajian struktur protein enzim transketolase daripada tebu, *Saccharum officinarum*. Tapak Jalan Pentosa Fosfat terdiri daripada dua fasa, iaitu, oksidatif dan non-oksidatif. Kedua-dua fasa ini dikawal oleh satu siri enzim. Salah satunya adalah transketolase yang memainkan peranan penting dalam fasa non-oksidatif di mana menghasilkan beberapa pengantara gula fosfat. Transketolase memindahkan dua unit karbon daripada fruktosa-6-fosfat kepada eritrosa-4-fosfat dan menukarkan dua fragmen karbon daripada gliseraldehid-3-fosfat kepada kumpulan ketosa xylulosa-5-fosfat, Eritrosa-4-fosfat akan memasuki tapak jalan shikimate di mana tapak jalan ini merupakan tapak jalan yang penting dalam penghasilan metabolit sekunder dalam sistem tumbuhan seperti, aromatik asid

amino, lignin dan flavonoid. Transketolase juga memainkan peranan penting dalam proses fotosintesis dan glikolisis. Walaupun peranan transketolase dalam tumbuhan adalah penting, kajian mengenai enzim ini dalam tumbuhan adalah terlalu terhad. Sehingga kini, pemencilan gen transketolse telah dilakukan hanya dari tujuh spesis tumbuhan. Maka, objektif pertama kajian ini ialah untuk memencilkan gen TKT daripada tebu, dan untuk membandingkan persamaannya jujukan nukletotida transketolase dengan organisma lain. Berbeza dengan bakteria, kulat dan haiwan, tapak jalan pentosa fosfat adalah lengkap di dalam sitosol. Walaubagaimanapun, dalam sistem tumbuhan, fasa pertama adalah di dalam sitosol, tetapi fasa kedua masih dalam kajian. Jadi, objectif kedua adalah untuk menentukan penempatan subsellular transketolase dalam pokok tebu. Manakala, objektif ketiga adalah untuk meramalkan struktur protin transketolase.P emencilan *TKT*daripada pokok tebu dilakukan dengan RT-PCR, diikuti dengan pengklonan dan penjujukan nukleotida. Kajian telah berjaya memencilkan jujukan nukleotida *TKT* sebanyak 2327 bp. Analisa persamaan menggunakan CLUSTALW2 mendapati jujukan nukleotida TKT daripada pokok tebu menunjukkan persamaan yang tertinggi (99%) dengan transketolase dari pokok jagung, *Zea mays*. Ini adalah kerana analisis klasifikasi tumbuhan mendapati kedua-dua tumbuhan adalah daripada famili yang sama, Pocaceae. Walaubagaimanapun, persamaan jujukan nukleotida TKT tebu adalah rendah (<65%) berbanding dengan spesie lain memandangkan tumbuhan ini berada dalam filum yang sama, Strephytophyte. Ia menunjukkan persamaan jujukan nukleotida terendah (<60%) terhadap bakteria, yis dan haiwan kerana organisma-organisma ini adalah daripada alam yang berbeza. Analisa menggunakan Target P 1.1 dan Chloro P mendapati transketolase daripada pokok tebu adalah kloroplastik dimana sebanyak 85 jujukan asid amino adalah jujukan sasaran plastid tumbuhan.

Jujukan nukleotida TKT pokok tebu telah diterjemahkan kepada jujukan asid amino menggunakan perisian Expasy.org, Phyre 2, dan YASARA untuk meramal struktur sekunder, tertier dan kualiti struktur protin transketolase poko tebu. Kajian ini menunjukkan struktur protin transketolase pokok tebu adalah 100% sama seperti lipatan ikatan thiamin difosfat daripada *Zea mays*. Sebagai kesimpulan, jujukan nukleotida *TKT* pokok tebu telah berjaya dipencilkan dan gen ini adalah kloroplastik .Oleh itu, kajian ini mendapati bahawa tapak jalan pentose fosfat adalah tidak lengkap dalam sitosol tumbuhan. Kajian ini mendapati persamaan jujukan nukleotida *TKT* adalah berkait rapat dengan klasifikasi dan taksonomi tumbuhan dan organisma lain. Struktur protin transketolase adalah 100% sama dengan lipatan ikatan thiamin difosfat daripada *Zea mays*.

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I certify that a Thesis Examination Committee has met on 30 May 2013 to conduct the final examination of Nahid Kalhori on her thesis entitled "Isolation of Transketolase Gene, Subcellular Localization, and Transketolase Protein Structure Studies of Sugarcane (*Saccharum officinarum.* L)" in accordance with the Universities and Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Putra Malaysia or at any other institution.

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NAHID KALHORI

Date: 30 MAY 2013

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LIST OF ABBREVATIONS

ADP	Adenosine Diphosphate
ATP	AdenosineTriphosphate
BLAST	Basic Local Alignment Tool
BNL Buffer	Agarose gel lysis Buffer
bp	Basepair
C	Carbon
cDNA	Complementary DNA
CO_2	Carbon Dioxide
ddH_2O	Distilled water
DNA	Deoxyribonucleic acid
FAD	Flavin Adenine Dinucleotide
G	Gravitational Force
G6PDH	glucose-6-phosphate dehydrogenase
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kDa	Kilo Daltons
LB	Luria Bertani

M	Molar
Mg	Mill gram
$MgCl_2$	Magnesium chloride
ml	Mill Litter
µl	Micro Liter
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Reduced Nicotinamide Adenie Dinucleotide Phosphate
NMR	Nuclear Magnetic Resonance
OD	Optical Density
OPPP	Oxidative Pentose Phosphate pathway
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
PDF	Portable Document Format
PEP	Phosphoenolpyruvate
pH	Potentiometric Hydrogen Ion Concentration
Phyre2	Protein Homology

PPP	Pentose Phosphate Pathway
PSI-BLAST	Position-Specific Iterated BLAST
RMSD	Root Mean Square Deviation
RLC	Contains guanidine hydrochloride
RLT Buffer	Contains guanidine thiocyanate
RNase-free water	RNA treatment
RPE	Ribulose-5-phosphate-3-epimerase
RPI	Ribose-5-phosphate isomerase
PRPP	Phosphoribosyl pyrophosphate
RPPP	Reductive Pentose Phosphate Pathway
RT-PCR	Real Time Polymerase Chain Reaction
RW1	Contains ethanol
S	Seconds
S7P	Sedoheptulose-7-Phosphate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TBE	Tris hydroxyl methyl amino methane
ThDP	Thiamine diphosphate

TAL	Transaldolase
TKT	Transketolase
TPP	Thiamine Pyro Phosphate
UK	United Kingdom
UPM	Universiti Putra Malaysia
USA	United States of America
UV	Ultraviolet
V	Voltage
X	Time
X5P	Xylulose-5-Phosphate
YASARA	Yet Another Scientific Artifical Reality Application
3D	Three Dimensional
6PG	6-phosphogluconolactonase
6PGDH	6-phosphogluconate dehydrogenase
°C	Degree Celsius
%	Percentage

CHAPTER 1

INTRODUCTION

Sugarcane is type of C4 plants species in which photosynthetic rate is twice as efficient as the C3 plants species. Also sugarcane is the most important source of sucrose in world history. The oxidative pentose phosphate pathway (PPP) has crucial role in the metabolism of carbohydrates *via* the oxidation of glucose-6-phosphate. The pathway consists of oxidative and non-oxidative phase. The oxidative phase produces ribulose-5-phosphate by the oxidation of glucose-6-phosphate. The enzymes of the irreversible oxidation phase are successively glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconolactonase (6PG) and 6-phosphogluconate dehydrogenase (6PGDH). The second phase is the reversible non-oxidative phase which is series of inter conversion between 3-, 4-, 5-, 6- and 7-carbons sugars catalysed by the enzymes ribose-5-phosphate isomerase (RPI), ribulose-5-phosphate-3-epimerase (RPE) and transketolase (TKT) and transaldolase (TAL). All enzymes of the OPPP apart from TAL are also part of the reductive pentose phosphate pathway (RPPP) or Calvin cycle. The most important role of the first phase is providing 50%-60% of the required NADPH, a major by reduction of $NADP^+$.

The second phase of the OPPP produces ribose-5-phosphate and erythrose-4-phosphate. Ribose-5-phosphate synthesis the nucleotides and nucleic acids – RNA, DNA and nucleotides coenzymes such as ATP, FAD, NADH and NADPH. Erythrose-4-phosphate and phosphoenolpyrovate (PEP) from glycolysis to form the

first substrate of the shikimic pathway which is chorismate. This important substrate provides aromatic amino acids and many aromatic secondary metabolites such as flavonoids, indole acetate, UV light protectants and lignin. These all metabolism have crucial role in the interaction of plant with environment (Nulit 2010).

1.1 Problem Statements

Unlike bacteria, fungi and animal, the number of *TKT* gene had been isolated from plant species is small, only seven *TKT* genes had been isolated from plants which are *Zea mays* (AY148193), *Solanum tuberosum* (Z50099), *Spinacia oleracea* (L76554), *Capsicum annum* (Y15781), *Arabidopsis thaliana* (AY091094), *Persicaria tinctoria* (AB066206), *Craterostigma plantagineum* (Z46646), *Platanus acerifolia* (AJ427413). In addition, the subcellular localization of PPP in bacteria, fungi and animal are in the cytosol. However, in plants, the subcellular localization is not clear. Therefore, another approach to resolve the compartmentation of the PPP genes in plants is molecular approach. According to Garnier *et al.* (2003) as more genome can be sequenced and published, the understanding of the genetic capacity of plants system to catalyse the reactions involved in the PPP could be obtained. In addition, the increasing availability of whole-genome transcript profiles under different physiological and developmental conditions will allow us to determine the relationships between the expressions of the specific PPP isoenzymes, and to establish the contribution of such isoenzymes to particular physiological processes. Therefore, a question regarding a complete PPP in the cytosol and plastid of plant could be answered.

1.2 Objectives of Study

Thus, the objectives of my study are:

- To isolate and clone transketolase gene from sugarcane (*Saccharum officinarum*) and compare DNA sequence of sugarcane transketolase gene with other plants, bacteria, fungi, animal and human being.
- To determine the subcellular localisation of transketolase gene in sugarcane
- To predict the structure of sugarcane transketolase protein

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