UNIVERSITI PUTRA MALAYSIA

GENE CLONING, PROTEIN CHARACTERIZATION AND STRUCTURAL PREDICTION OF Amaranthus tricolor TRANSKETOLASE

ALLGENDRAN A/L RAJEENDRAN

FS 2013 24

GENE CLONING, PROTEIN CHARACTERIZATION AND STRUCTURAL PREDICTION OF *Amaranthus tricolor* TRANSKETOLASE

By

ALLGENDRAN A/L RAJEENDRAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

MAY 2013

COPYRIGHT

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

GENE CLONING, PROTEIN CHARACTERIZATION AND STRUCTURAL PREDICTION OF *Amaranthus tricolor* TRANSKETOLASE

By

ALLEGENDRAN A/L RAJEENDRAN

MAY 2013

Chairman: **Rosimah Nulit, PhD**

Faculty: **Science**

This study focused on gene cloning, protein characterization and structural prediction of Transketolase enzyme from *Amaranthus tricolor*. Oxidative Pentose Phosphate Pathway (OPPP) oxidative pentose phosphate pathway (OPPP) fulfills an essential role in metabolite intermediate of carbohydrate metabolism via the oxidation of glucose-6-phosphate in prokaryotic and eukaryotic cells. The non-oxidative branch of this pathway provides erythrose 4-phosphate for the shikimate pathway, the products of which are used for the synthesis of aromatic amino acids, flavonoids, and lignin, and ribose 5-phosphate for nucleic acid synthesis. Transketolase play important role in photosynthesis and glycolysis. Although the contribution of transketolase in plant system is important, study of this enzyme is still limited. There were only seven plants that *TKT* genes had been isolated so far, thus this leads to my first objective of my study is to isolate *TKT* gene from *A. tricolor* to compare its identity with other organisms. Unlike bacteria, fungal and all other animals, the pentose phosphate pathway is

complete in the cytosol of these living system and all enzymes of this pathway localized in the cytosol. However, in plant system, the first phase of pentose phosphate pathway is complete in the cytosol of plant but sub-localization for non-oxidative pentose phosphate pathway is still remain unclear. The identification gene of *TKT* was done by RT-PCR with gene specific primer, followed by cloning, sequencing and gene expression. The present study had isolated 1963bp transketolase from *A. tricolor.* Similarity studies using ClustalW2 and phylogenetic studied found that *A. tricolor TKT* and *Spinach sp.* transketolase has 85% identity. This may be due to classification system both plants are in the same family, Amarathaceae. Analysis by Target P 1.1 and Chloro P revealed that the *A. tricolor* is localized in the chloroplast. The complete DNA sequence of the *A. tricolor TKT* gene was showed an open reading frame of 1943 nucleotides that encodes for 646 amino acid residues. *A. tricolor TKT* was expressed in *E.coli* BL21, using pET 32(b+) as a vector. The purified recombinant *A. tricolor TKT* protein size was determined by SDS-PAGE that give 70kDa. In addition, the 3D protein structure of *A.tricolor* transketolase was analysed by using PS^2 and secondary its structure was predicted by Phyre2. As conclusion, *A. tricolor* transketolase was successfully isolated and subcellular-localization was found chloroplast in plant system. This led to conclusion that the OPPP is incomplete in the cytosol of *A. tricolor*. This study shows that *A.tricolor* transketolase has major functional role in the plastidic through the OPPP and could be an important required step for the production of secondary metabolites in plants.

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

GEN KLON, PROTEIN PENCIRI-CIRIKAN DAN KAJIAN STRUKTUR BAGI *Amaranthus tricolor* TRANSKETOLASE

Oleh

ALLEGENDRAN A/L RAJEENDRAN

Mei 2013

Pengerusi: **Rosimah Nulit, PhD**

Fakulti: **Sains**

Kajian ini memfokuskan kepada pemencilan, penciri-cirikan dan kajian struktur bagi *Amaranthus tricolor* Transketolase. Tapak jalan Pentosa Fosfat (OPPP) terdiri daripada dua fasa, iaitu, oksidatif dan bukan-oksidatif. Fungsi OPPP terutamanya untuk menjana NADPH dan prekursor untuk proses biosintetikc pelbagai kompaun sebagai blok binaan asas kehidupan. Salah satunya adalah transketolase yang memainkan peranan penting dalam fasa bukan-oksidatif di mana menghasilkan beberapa pengantara gula fosfat. Erythrosa-4-fosfat akan mamasuki tapak jalan shikimate dimana tapak jalan ini merupakan tapak jalan yang penting dalam penghasilan metabolit sekunder dalam sistem tumbuhan seperti asid amino, lignin dan flavonoid. Transketolase also memainkan peranan penting dalam proses fotosintesis dan glikolisis. Oleh sebab itu peranan transketolase dalam sistem tumbuhan adalah penting, tetapi kajian mengenai enzim ini dalam tumbuhan adalah terlalu terhad. Sehingga kini, pemencilan Transketolase gen telah dilakukan dari tujuh spesis sahaja. Dibandingkan dengan

bacteria, kulat dan haiwan dalam proses OPPP adalah lengkap. Tetapi, sistem tumbuhan, fasa pertama adalah lengkap dalam sitosol, tetapi fasa kedua masih dalam kajian. Kajian ini telah berjaya mengasingkan jujukan nukleotida transketolase dengan menguna Clustal W2 dan pilogenetik menunjukan bahawa *A. tricolor TKT* and *Spinach sp*. transketolase adalah persaman dengan 85% dan berbanding dengan tumbuhan lain. Penempatan subsellular untuk *A.tricolor* transketolase telah meramal dengan mengguna Target P 1.1 and Chloro P menunjukan bahawa dalam koloroplas. *A. tricolor TKT* gene yang dikaji telah ditunjukkan untuk mengandungi rangka bacaan dengan sebanyak 1943bp nukleotida dengan mengekod untuk 646 residu asid amino. Recombinan *A. tricolor TKT* telah disertakan dalam *E.coli* BL21, menggunakan pET 32 (a+) sebagai vector. Rekombinan saiz protein *A. tricolor* TKT telah ditentukan dengan mengguna SDS-PAGE dengan 70 kDa saiz protein. Tambahan lagi, 3D protein struktur bagi *A. Tricolor* transketolase telah menjangka dengan menguna PS ^{2} dan struktur sekunder telah meramal dengan menguna Phyre 2. Sebagai kesimpulan, jujukan nukleotida *TKT* bagi *A. tricolor* telah berjaya dipencilkan. Oleh itu, kajian ini mendapati bahawa OPPP adalah tidak lengkap dalam sitosol tumbuhan. Kajian ini, mennujukan bahawa *A. tricolor* transketolase adalah gen yang sangat penting dalam laluan OPPP dan pengeluaran kompaun sekunder dalam tumbunhan.

ACKNOWLEDGEMENTS

First of all, I wish to express my utmost thanks and deepest gratitude to the chairman of the supervisory committee, Dr. Rosimah Nulit, for her supervision, invaluable guidance and advices, patience, endless support, and encouragement throughout this study and for her critical analysis and helpful suggestions during the preparation of the thesis. I am grateful she has also given me the opportunity to pursue my Masters Degree and embark on interesting and exciting scientific research.

I would also like to express my appreciation to Dr. Christina Yong Seok Yian, who are member of the supervisory committee, for their guidance, advices and kind assistance. My heartfelt appreciations to the staffs of the Tissue Culture laboratory, Department of Biology, Faculty Science for their technical support and kind assistance throughout the course on my study.

I would like to extend my gratitude to the School of Graduate Studies, University Putra Malaysia, for providing the financial support for my study under the Graduate Research Fellowship.

I wish to thank my fellow lab mates and friends, for their friendship, help, encouragement and support. They have all helped me to overcome the challenges encountered, and indeed have been true friends. Thanks for making the long hours in the lab a pleasant and memorable one.

Finally, the most special thanks are extended to my parents, brothers and sister for their continuous support, encouragement and prayers which have inspired and motivated me throughout the course of this study. I will always be grateful for their constant love and encouragement that they gave me along the way. Most of all, I thank God for all His blessings.

I certify that a Thesis Examination Committee has met on 7 May 2013 to conduct the final examination of Allegendran A/L Rajeendran on his thesis entitled "Molecular isolation, characterization and structutal prediction of *Amaranthus Tricolor* Transketolase" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Dr. Hishamuddin b. Omar
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Prof. Madya Dr. Faridah binti Qamaruz Zaman
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Y.Bhg. Prof. Dr. Umi Kalsom bt Yusof
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Prof. Madya Dr. Fahrul Zaman Huyop
Faculty of Bioscience and Bioengineering
Universiti Teknologi Malaysia
(External Examiner)

HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
University Putra Malaysia

Date: MAY 2013

This thesis submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Rosimah Nulit, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Christina Yong Seok Yian, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
University Putra Malaysia

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 Putra Malaysia or other institutions.

_____

ALLEGENDRAN A/L RAJEENDRAN

Date: 7 MAY 2013

TABLE OF CONTENTS