UNIVERSITI PUTRA MALAYSIA

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By

NOR AZIZAH BINTI PARMIN

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

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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 Science

EXPRESSION OF TRUNCATED CHALCONE SYNTHASE FROM *Boesenbergia rotunda L.* MANSF. IN *Escherichia coli*

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March 2013

Chairman: **Prof. Raja Noor Zaliha Raja Abd. Rahman, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

Chalcone synthase (CHS) is an entrance enzyme in the biosynthetic pathway of different flavonoids compound and become a critical regulatory enzyme. Truncated CHS was isolated from *Boesenbergia rotunda* (*B. rotunda)*, also known as Chinese ginger, which is a medicinal and culinary herb from China and Southeast Asia. The popularity of its medicinal usage has drawn the attention of scientists to investigate on its medicinal properties. This research is a fundamentals works to study on the mechanism of truncated CHS from *B. rotunda* in *E. coli*. This study is new for this plant but CHS has been studied by many researchers in other plants. Optimization of *B. rotunda* specific CTAB RNA extraction method developed in this study was able to produce high yield of RNA with minimum polysaccharide contamination. The value of RNA yield for optimization of *B. rotunda* specific CTAB RNA extraction method was 2.0 µg/g. A reverse transcriptase-polymerase chain reaction (RT-PCR) based approach was used to

identify and isolate a partial-length cDNA coding for CHS gene. The gene encoding truncated CHS from *B. rotunda* was cloned into pTrcHis2 expression vector and placed under the control of the *trc* promoter for high level expression in *E. coli*. The effect of inducer concentration and induction time on CHS production from inducible system was evaluated. The recombinant truncated CHS protein was characterized and optimized for higher expression. The optimization of the recombinant truncated CHS production of inducible system in 50 ml culture was done for the best clone truncated CHS from *E. coli* TOP10. The effect of IPTG concentration and induction time on truncated CHS production from inducible system was evaluated. A time course profile of recombinant truncated CHS production with the optimized condition was performed and produced after 16 h of induction time from recombinant truncated CHS5, while optimal IPTG concentration was 0.5 mM.

The recombinant truncated CHS was purified by using affinity chromatography and Ion-Exchange Chromatography (IEX). The expressed CHS protein had a molecular weight of about 21 kDa, a size that matched with the prediction by bioinformatic analysis. Truncated CHS was highly active at 40^{o}C, pH 8.0 with a half-life of 1 h 10 min at 30^{o}C. 3D structure of truncated CHS from *B. rotunda* was predicted using CHS from alfalfa (*Medicago sativa*) as a template with sequence identity 74.87%. Tyr-42, His-130 and Asp-163 were assigned as catalytic triad residues. Truncated CHS was evaluated with Ramachandran plot, Verify 3D and ERRAT to validate the structure. This study may provide useful information on this enzyme and its function in the flavonoids biosynthetic

pathway in *B. rotunda that* offers significant pharmaceutical properties for human health.

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

PENGEKSPRESSAN SEPARA CHALCONE SYNTHASE DARIPADA *Boesenbergia rotunda* L. MANSF. DI DALAM *Escherichia coli*

Oleh

NOR AZIZAH BINTI PARMIN

Mac 2013

Pengerusi: **Prof. Raja Noor Zaliha Raja Abd. Rahman, PhD**

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Chalcone synthase (CHS) merupakan enzim pintu masuk ke dalam tapak jalan biosintesis sebatian flavonoid berbeza menjadi enzim pengawalatur yang kritikal. CHS separa diasingkan daripada *Boesenbergia rotunda* (*B. rotunda*), juga dikenali sebagai halia Cina yang merupakan herba perubatan dan masakan dari China dan Asia Tenggara. Popularitи nilai perubatan yang terkandung di dalam *B. rotunda* telah menarik minat para saintis untuk mengkaji nilai perubatan yang tersembunyi di dalamnya. Penyelidikan ini bertujuan untuk mengetahui mekanisme CHS separa daripada *B. rotunda* di dalam sistem *E. coli*. Kaedah penyelidikan ke atas enzim separa CHS adalah baru untuk tumbuhan *B. rotunda* tetapi CHS daripada tumbuh-tumbuhan yang lain telah banyak dibuat kajian. Bagi mengeksploitasikan potensi *B. rotunda* sebagai herba perubatan ke arah kajian saintifik, kaedah pengekstrakan CTAB RNA spesifik *B. rotunda* yang dioptimumkan dilakukan dalam kajian ini telah dapat menghasilkan RNA yang tinggi dengan polisakarida yang minima. Hasil RNA untuk pengoptimuman kaedah

pengekstrakan CTAB RNA spesifik ialah 2.0 µg/g. Enzim penjujukan berbalik tindakbalas rantai berpolimer (RT-PCR) yang telah digunakan untuk mengenal pasti dan mengasingkan cDNA separa panjang bagi gen CHS. Gen yang membawa CHS separa daripada *B. rotunda* diklonkan ke dalam vektor pengekspressan pTrcHis2 vektor dan diletakkan di bawah kawalan promoter *trc* untuk pengekspressan yang tinggi dalam *E. coli*. Kesan kepekatan pengaruh dan masa induksi bagi penghasilan CHS daripada sistem perangsangan telah dinilai. Protein rekombinan CHS separa dicirikan dan dioptimumkan untuk penghasilannya yag lebih tinggi. Pengoptimuman pengeluaran rekombinan CHS separa daripada sistem aruhan dalam 50 ml kultur telah dilakukan untuk klon separa CHS5D dari *E. coli* TOP10. Kesan kepekatan IPTG dan masa induksi untuk penghasilan separa CHS dari sistem aruhan telah dinilai. Profil masa penghasilan rekombinan CHS separa keadaan optimum telah dijalankan dan dihasilkan selepas 16 jam daripada masa induksi manakala kepekatan pengaruh terbaik IPTG adalah pada 0.5 mM.

Rekombinan CHS separa rekombinan telah ditulenkan dengan menggunakan kromatografi afiniti dan IEX. Protein CHS separa yang mempunyai berat molekul lebih kurang 21 kDa, saiz yang sepadan dengan ramalan oleh analisis bioinformatik. CHS separa adalah sangat aktif pada 40^{o}C dan pH 8.0 dengan separa hayatnya daripada 1 j 10 minit pada 30^{o}C. Struktur 3D CHS separa daripada *B. rotunda* telah diramalkan dengan menggunakan CHS daripada alfalfa (*Medicago sativa*) sebagai templat dengan urutan identiti 74.87%. Tyr42, His130 dan Asp163 telah bertindak sebagai tapak pemangkin enzim. CHS separa dinilai dengan plot Ramachandran dan 3D ERRAT untuk

mengesahkan strukturnya. Kajian ini boleh memberikan maklumat berguna untuk enzim ini dan fungsinya dalam tapak jalan biosintesis flavonoid dalam *B. rotunda* yang mempunyai banyak ciri-ciri farmaseutikal untuk kesihatan manusia.

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I certify that an Examination Committee has met to date of viva to conduct the final examination of Nor Azizah bt Parmin on her degree of Masters of Science thesis entitled, "Expression of truncated chalcone synthase from *Boesenbergia rotunda* (L.) Mansf. in *Escherichia coli*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Degree of Masters of Science.

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

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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 Universiti Putra Malaysia or at any other institutions.

NOR AZIZAH BINTI PARMIN

Date: 14 March 2013

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