**UNIVERSITI PUTRA MALAYSIA**

# MORPHOLOGY SWITCHING AND QUORUM SENSING IN *CANDIDA ALBICANS* PATHOGENESIS

**CRYSTALE LIM SIEW YING**

**FPSK(p) 2010 6**

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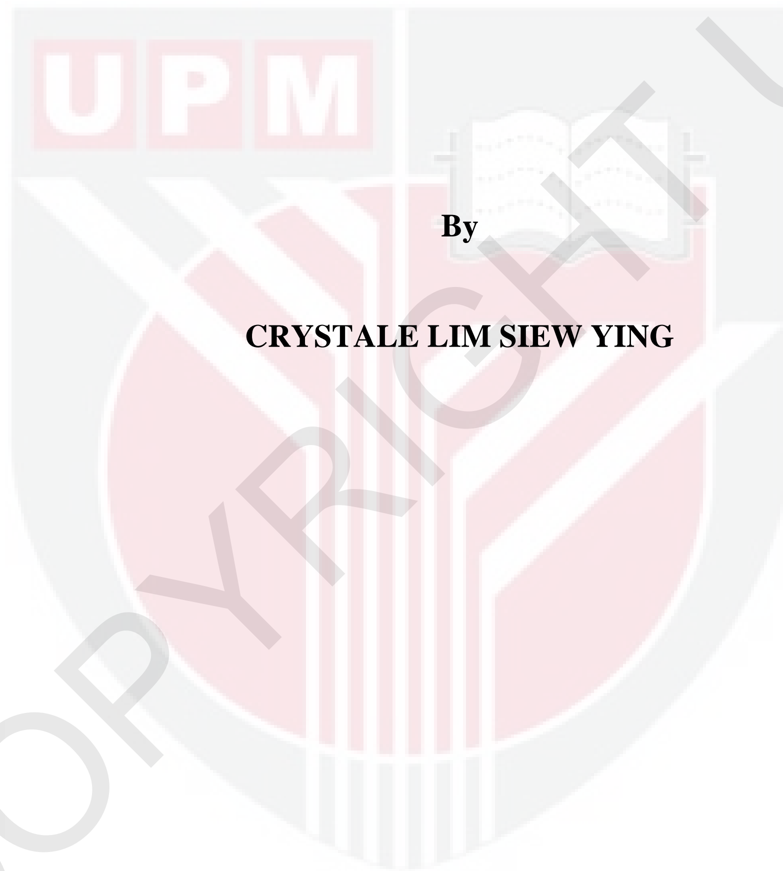


By

CRYSTALE LIM SIEW YING

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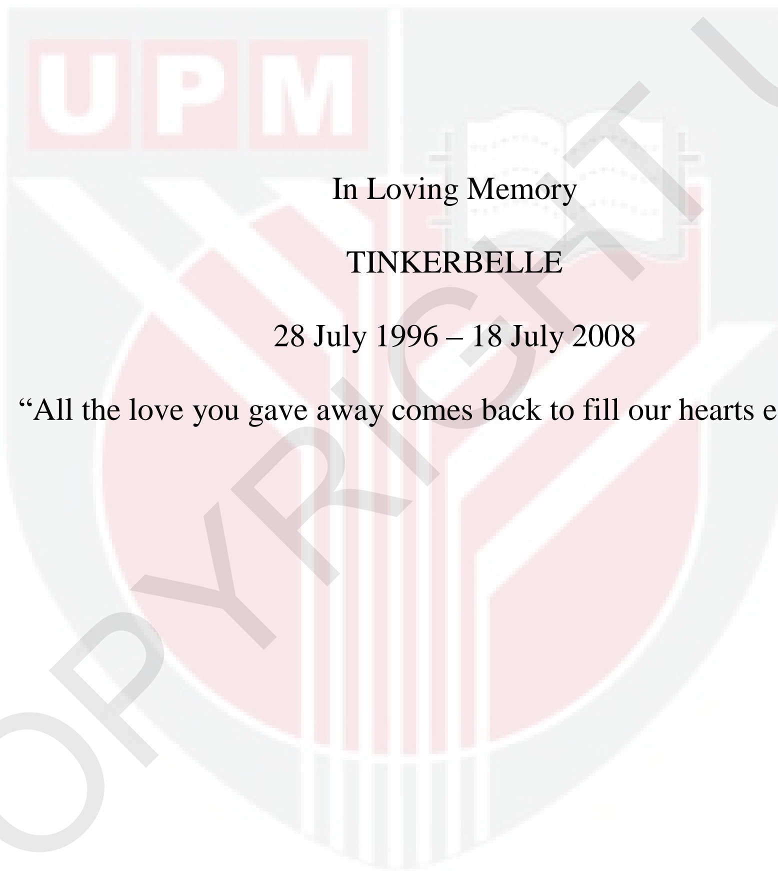

In Loving Memory

TINKERBELLE

28 July 1996 – 18 July 2008

"All the love you gave away comes back to fill our hearts each day"

Absence of evidence is not evidence of absence

- Carl E. Sagan, astronomer
(9 Nov 1934 - 20 Dec 1996)

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**MORPHOLOGY SWITCHING AND QUORUM SENSING IN *CANDIDA ALBICANS* PATHOGENESIS**

By


**CRYSTALE LIM SIEW YING**


**August 2010**


**Chairman: Associate Professor Chong Pei Pei, PhD**

**Faculty: Medicine and Health Sciences**



*Candida albicans* is the major etiological agent of invasive candidiasis. Although yeast-hyphae switching has been shown to be an important virulence factor, lately it has become evident that the phenomenon of quorum sensing, which also controls this switching, is a new and more important virulence factor. The first part of the present study was undertaken to identify novel *C. albicans* genes which may also be involved in yeast-hyphae switching. Autoradiogram data from differential display reverse transcription-polymerase chain reaction (DDRT-PCR) studies of the yeast and hyphal forms at selected time-points were recorded and analyzed. Using molecular methods and bioinformatics, 41 of these DDRT-PCR cDNA transcripts were identified as annotated genes involved in various biochemical, metabolic and physiological functions while 28 transcripts, with no significant homologies to any known *C. albicans* genes. Real-time PCR expression profiling of four out of nine selected DDRT-PCR trancripts agreed with the DDRT-PCR band intensity trends while six transcripts were found to be highly differentially expressed in biofilms. The discovery of more genes involved in the intricate pathways of *C. albicans* yeast-hyphae switching would contribute to the complete mapping of the morphology





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switching transcriptional network. The new transcripts, such as A2-5 (potential germ-tube biomarker), may also be further characterized and investigated as potential biomarkers of systemic candidiasis. The findings here as well as in recent studies suggest that *C. albicans* pathogenesis may be a consequence of environmental adaptation, as the genes described thus far to be involved in yeast-hyphae switching have been inseparable from genes involved in response to environmental signals. Hence, this study was also aimed at better understanding the phenomenon of quorum sensing (QS) as a major influence of yeast-hyphae switching. Here, 150 µM of a synthetic 12-carbon backbone molecule, 2-dodecanol, was demonstrated to prevent *C. albicans* hyphae development. Expression of *SIR2*, a gene involved in phenotypic switching, was shown in this study to be elevated during hyphae development, and this up-regulation was repressed with the presence of 2-dodecanol, thereby suggesting that *SIR2* may be involved in quorum-sensing and/or hyphae development via the Ras-cAMP-*EFG1* pathway. The final part of this study was aimed at investigating the *in vitro* host response to the three major morphological forms of *C. albicans* infections, as well as to farnesol, a QSM. Microarray transcriptome profiling of human umbilical vein endothelial cells (HUVEC) infection with *C. albicans* suggests that an initial high inoculum size of yeast cells evoked a stronger, broader range of transcriptional response than either pseudohyphae or hyphae at lower densities. In general, many of the genes which were mutually induced, such as *CSF2*, *DDIT4* and *FOS*, are involved in apoptosis and cell death. In addition, the exclusive differential expression of certain HUVEC genes in response to a particular *C. albicans* morphology was observed, suggesting that each morphological form may have different roles in the host environment. HUVEC viability was also significantly reduced after treatment with conditioned



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media from high-density *C. albicans*-HUVEC co-cultures compared to media from low-density co-cultures. This led to the postulation that the *C. albicans*-HUVEC interaction resulted in the release of an unknown soluble factor(s) which, at a certain concentration, is capable of causing HUVEC cell death and therefore may be instrumental in pathogenesis. As discovered via transcriptome profiling of HUVEC treated with farnesol, during initiation of pathogenesis farnesol may interfere with the G-protein coupled receptor (GPCR) signal transduction of host cells. In addition, HUVEC treatment with farnesol concentrations of more than 100 µM in a high-alcohol-content solvent resulted in drastic cell death. Therefore, QS, critical in *C. albicans* response dynamism and adaptation to changes in a niche environment in the host, is likely a stronger virulence factor preceding yeast-hyphae switching in invasive candidiasis. In conclusion, through molecular genetics investigations, this study has elucidated several novel *C. albicans* transcripts of unknown functions and has revealed new aspects in the relationship between *C. albicans* QS, yeast-hyphae switching, and the host response, all of which contribute to a better fundamental understanding of *C. albicans* pathogenesis.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENUKARAN MORFOLOGI DAN PENDERIAAN KORUM DALAM PATOGENESIS *CANDIDA ALBICANS***

Oleh

**CRYSTALE LIM SIEW YING**

**Ogos 2010**

**Pengerusi: Professor Madya Chong Pei Pei, PhD**

**Fakulti: Perubatan dan Sains Kesihatan**

*Candida albicans* adalah agen etiologikal utama infeksi *Candida* invasif. Walaupun penukaran yis-hifa merupakan salah satu faktor virulens, kebelakangan ini semakin jelas bahawa fenomena penderiaan korum (QS), yang juga mengawal penukaran yis-hifa, adalah satu faktor virulens baru yang lebih penting. Bahagian pertama penyelidikan ini telah dijalankan untuk mengenalpasti gen-gen *C. albicans* baru, di mana gen-gen tersebut juga mungkin terlibat dalam penukaran yis-hifa. Data autoradiogram daripada kajian tayangan perbezaan tindak balas berantai polimeras bertranskripsi terbalik (DDRT-PCR) bentuk yis dibanding dengan bentuk hifa dalam jangkamasa tertentu telah direkod dan dianalisa. Dengan menggunakan teknik molekular serta bioinformatik, empat puluh satu transkrip telah dikenalpasti sebagai gen-gen yang terlibat dalam pelbagai fungsi biokimia, metabolisma dan fisiologi, manakala 28 transkrip DDRT-PCR telahpun dimasukkan ke dbEST (GenBank) kerana transkrip-transkrip ini tidak mempunyai homologi signifikan dengan mana-mana gen *C. albicans* yang telah diketahui. Sejumlah 12 jujukan individu bakal menjadi gen-gen *C. albicans* baru. Pemprofilan PCR masa-nyata yang dijalankan ke atas empat daripada sembilan transkrip bersetuju dengan profil-profil keamatan jalur



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kepunyaan DDRT-PCR, manakala enam transkrip didapati mempunyai perbezaan pernyataan yang tinggi dalam biofilem. Penemuan lebih banyak gen-gen yang terlibat dalam penukaran yis-hifa *C. albicans* akan menyumbang kepada pemetaan rangkaian transkripsi penukaran morfologi yang lengkap. Transkrip-transkrip baru, seperti A2-5 (yang mempunyai potensi sebagai penanda tiub germa), merupakan satu calon sesuai untuk karekterisasi seterusnya. Berasaskan penemuan dalam kajian ini serta kebelakangan ini, di mana gen-gen yang terlibat dalam penukaran yis-hifa tidak dapat dipisahkan daripada gen-gen yang terlibat dalam gerak balas kepada isyarat persekitaran, maka boleh diandaikan bahawa patogenesis *C. albicans* berkemungkinan adalah akibat penyesuaian kepada persekitarannya. Justeru, kajian ini juga mengusahakan pemahaman fenomena QS sebagai pengaruh penting dalam penukaran yis-hifa. Sekurang-kurangnya 150 µM 2-dodecanol, satu molekul sintetik berasaskan tulang belakang 12-karbon, telah didapati mampu menghalang pembentukan hifa *C. albicans*. Dalam kajian ini, pernyataan *SIR2*, satu gen yang terlibat dengan penukaran fenotip, meningkat bersama pembentukan hifa, di mana peningkatan ini telah dihalang oleh 2-dodecanol. Ini mencadangkan bahawa *SIR2* mungkin terlibat dengan QS serta pembentukan hifa melalui laluan Ras-cAMP-*EFG1*. Bahagian terakhir kajian ini menumpukan perhatian kepada penyiasatan gerak balas perumah terhadap infeksi tiga bentuk morfologikal utama *C. albicans* serta kepada farnesol, satu QSM. Pemprofilan transkriptom tatasusunan mikro selepas infeksi *in vitro* sel-sel endotelium vena talipusat manusia (HUVEC) mencadangkan bahawa saiz inokulum infeksi pertama sel-sel bentuk yis yang lebih tinggi mengakibatkan tindak balas transkripsi yang lebih kuat serta luas julatnya berbanding dengan infeksi sel-sel bentuk pseudohifa mahupun hifa pada ketumpatan sel yang lebih rendah. Secara keseluruhannya, banyak gen-gen yang diinduksi



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bersama, seperti *CSF2*, *DDIT4* dan *FOS*, terlibat dalam apoptosis serta kematian sel. Tambahan pula, setiap satu morfologi *C. albicans* mengakibatkan pembezaan pernyataan ekslusif gen-gen tertentu. Ini mencadangkan bahawa setiap bentuk morfologi mungkin mempunyai peranan berbeza semasa dalam persekitaran perumah. Kebolehidupan HUVEC berkurangan secara signifikan setelah dirawat dengan supernatan dari kultur HUVEC-*C. albicans* berketumpatan tinggi berbanding dengan supernatan dari kultur HUVEC-*C. albicans* berketumpatan rendah, seperti yang ditentukan oleh ujian kebolehidupan sel. Ini telah melahirkan andaian bahawa interaksi antara *C. albicans* dan HUVEC boleh menyebabkan penghasilan faktor-faktor terlarut yang tidak diketahui, di mana pada kepekatan tertentu, molekul-molekul tersebut mengakibatkan kematian sel HUVEC lalu memainkan peranan penting dalam patogenesis. Semasa permulaan patogenesis, farnesol mungkin mengganggu transduksi isyarat reseptor protin-G berganding (GPCR) sel-sel perumah, seperti yang telah ditemui melalui memprofilan transkriptom sel HUVEC yang telah dirawat dengan farnesol. Tambahan pula, rawatan HUVEC dengan kepekatan farnesol lebih daripada 100 µM, dalam pelarut beralkohol tinggi, menyebabkan kekurangan drastik dalam kebolehidupan HUVEC. Dengan itu, QS yang kritikal dalam tindak balas dinamik serta penyesuaian *C. albicans* terhadap perubahan-perubahan dalam persekitaran nic perumah boleh diandaikan sebagai faktor virulens yang lebih kuat daripada faktor penukaran yis-hifa dalam infeksi *Candida* invasif. Kesimpulannya, kajian ini telah mendedahkan beberapa transkrip *C. albicans* baru yang belum diketahui fungsinya, serta telah menjelaskan aspek-aspek baru dalam hubungan antara QS *C. albicans*, penukaran yis-hifa dan gerak balas perumah, di mana kesemua ini menyumbang kepada pemahaman asas patogenesis *C. albicans* yang lebih baik.



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I certify that an Examination Committee has met on _________ to conduct the final examination of **Crystale Lim Siew Ying** on her **Doctor of Philosophy** thesis entitled "**Morphology switching and quorum sensing in *Candida albicans* pathogenesis"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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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 institution.

**CRYSTALE LIM SIEW YING**

**Date: 18 August 2010**

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