

中文摘要

白色念珠菌的重要性不只在它們感染的嚴重性；而且在長期或預防性治療的病人身上，容易發展出對azole等藥物有抗性的菌株。很多的生化研究因此著重在各式各樣不同的抗azole類的藥物機制。其中最常見的抗藥機制則是藥物排出幫浦 (drug efflux pumps) 基因 (例如 *CDR1* 或 *CDR2*) 的表現量增加。

近幾年來，很多研究致力於了解白色念珠菌的多重藥物轉運基因 (multi-drug transporter genes) *CDR1* 的調控機制。然而，對於 *CDR1* 的 *trans*調控因子仍然所知不多。本研究因此著重在分離鑑定 *CDR1* 的 *trans*調控因子。利用 Ym990348 *CDR1* 的啟動子與 *lacZ* 的轉譯區 (open reading frame; ORF) 建構成重組基因來當作篩選系統，且在藥物誘導下進行篩選。共有四個 ORF 被篩選分離出來，此四個 ORF 分別被命名為 *REP3*, *REP4*, *REP5*, 和 *REP6*。其中兩個 (*REP3*, *REP6*) ORF 具有 nucleic acid-binding 結構—C2H2 type 指狀區域功能基 (zinc finger domain)，因此它們可能扮演轉譯調控子 (transcription regulator) 的角色。最後根據藥物敏感性測試 (Etest) 的結果，發現 *REP3* 同型缺陷的突變株 (homozygous mutant) 對azole類藥物的感受性有提高的現象。根據以上結果推論，*REP3* 基因參與調控 *CDR1* 基因的表現，並且影響白色念珠菌對藥物的感受性。

Abstract

Candida albicans derive their importance not only from the severity of their infections but also for their ability to develop resistance against antifungals, such as azoles, in patients undergoing long-term or prophylactic treatment. Extensive biochemical studies have highlighted a significant diversity in the mechanisms conferring resistance to azoles. Up-regulation of drug extrusion pump-encoding genes belonging to the ABC (ATP binding cassette, e.g. *CDR1* and *CDR2*) superfamily represents one of the most prevalent mechanisms of *Candida* drug resistance.

In recent years, much effort has been devoted to understand the regulatory mechanisms of multi-drug transporter genes, such as *CDR1*, in *C. albicans*. Nonetheless, little is known about the *trans*-regulatory factors of *CDR1*. The study here focuses on isolation and identification of the *trans*-acting regulatory factors of *CDR1*. Using Ym00348 *CDR1* promoter in-frame fusion with *lacZ* gene as the screening system, under the presence of drugs, four candidate open reading frames (ORFs) have been isolated. These four ORFs were named *REP3*, *REP4*, *REP5*, and *REP6*. Two (*REP3* and *REP6*) of them have the C2H2 type zinc finger domain, which is one of the major nucleic acid-binding structures, indicating their potential roles as transcription regulators. According to the results of the Etest, *rep3/rep3* homozygous mutant seems to be more susceptible to azoles. It shows that *REP3* may have involved in the *CDR1* expression and may also affect the drug susceptibility of *C. albicans*.

誌謝

終於到了寫誌謝的時候了，研究所兩年的生活當中，要感謝的人真的是非常多，首先要感謝指導教授楊昀良老師和國衛院羅秀容老師，他們在實驗上的指導和許多觀念的傳授，使我對生物研究這陌生的領域有更進一步的了解和認識，也修正了我許多在思考觀念上的錯誤。也感謝他們在論文上的用心修改，並且給了我許多寶貴的意見。感謝彭慧玲老師在論文的修改及口試時給我許多建議，讓我有不一樣的思考方式，收穫良多。

感謝國衛院佳君學姊在實驗上不厭其煩的教導和生活上的鼓勵，給我許多幫忙和支持。還有孝胥在生活及電腦上的協助，銘陽、彰勳、隋哥在實驗上的幫助，以及後來加入的怡萱、家莉、筱玲、Michael 在生活上的協助，讓我得以順利的度過很多難關。也感謝在交大時的學長姐采瑜、升耀、婕琳、明皓，當我在剛進入實驗室不久，在生活及實驗上給我很大的幫忙，同學雅文也在我不能回新竹時給我很大的協助，學妹宛真平時的關心、助理阿貴及實驗室其他的成員給我許多幫助，讓我減輕很多負擔。最後要謝謝我的家人不時的支持和鼓勵，讓我無後顧之憂的完成學業，還有詠怡一路上的陪伴，彼此互相的加油打氣，使我能夠繼續走下去。再次謝謝其他曾經幫助我的人，讓我度過辛苦但充實的研究生活。

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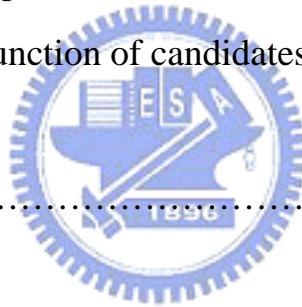
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