論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Role of MFS Transporter QDR2 in a Fungal Pathogen Candida glabrata
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出典(和文)	学位:博士(学術), 学位授与機関:東京工業大学, 報告番号:甲第11354号, 授与年月日:2019年12月31日, 学位の種別:課程博士, 審査員:折原 芳波,山本 直之,一瀬 宏,長田 俊哉,小倉 俊一郎,梶原 将
Citation(English)	Degree:Doctor (Academic), Conferring organization: Tokyo Institute of Technology, Report number:甲第11354号, Conferred date:2019/12/31, Degree Type:Course doctor, Examiner:,,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	LIFE SCIENCE AND TECHNOLOGY 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of （ Ph.D ）
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要旨（和文 2000 字程度）
Thesis Summary (approx.2000 Japanese Characters)

INTRODUCTION

Candida glabrata represents the second-most frequent cause of candidiasis in infections of the mucosa, bloodstream, and genito-urinary tract in immunocompromised individuals. The incidence of *C. glabrata* infection has increased significantly in the last two decades, mainly due to its abilities to resist various antifungal drugs (MDR) and to form a complex cell community with extracellular matrix (ECM) called biofilm. The ability to actively extrude the drug out of the cell is mediated mainly by ATP-Binding Cassette (ABC) and MFS (Major Facilitator Superfamily) transporters. Previous study by our group had revealed that *C. glabrata QDR2*, which encodes a Drug-H^+ Antiporter1 (DHA1) of MFS transporter, was associated with biofilm formation. Further investigation is needed on Qdr2p to prevent *C. glabrata* persistent infections since *C. glabrata* biofilm formation is able to limit drug penetration. This study focused on providing insight into Qdr2p role in *C. glabrata* biofilm formation, Qdr2p characteristics, and Qdr2p role in *C. glabrata* pH homeostasis.

METHODS

The biofilm formations were compared between a *C. glabrata Δqdr2* mutant and its wild-type (WT) strain. Biofilm cells were analyzed for metabolism activity and drug susceptibility (using tetrazolium assay), morphology (via scanning electron microscopy), and adhesion activity. The evaluations of *Cg*Qdr2p characteristics were approached through sequence alignment, growth towards specified chemical reagents and antifungal drugs, and lipid analysis. The role of Qdr2p in *C. glabrata* pH homeostasis was analyzed through the mutant growth in media buffered to various pHs and intracellular pH (using flow cytometry).

RESULTS AND DISCUSSION

1. The Role of QDR2 on *C. glabrata* Biofilm Formation

One of the virulence traits of *C. glabrata* is the ability to form biofilm, when *C. glabrata* free-floating cells attach on the surface and excrete ECM, on hosts' epithelial surface as well as on implanted medical devices. In this chapter, Qdr2p existence showed a significant effect on biofilm cells' metabolic activity and drug susceptibility. Upon *qdr2* deletion, the mutant suffered from reduced metabolic activity and decreased adhesion on artificial matrix, which might influence the ECM production, biofilm morphology, and biofilm maturation of *C. glabrata*. These events may explain why *Δqdr2* biofilm was more susceptible to fluconazole. Qdr2p may play an important role in *C. glabrata*'s ability to form and maintain biofilm on implanted medical devices in human bodies. Deeper analysis through the complementation strain of *C. glabrata QDR2* would gain more comprehensive information on the gene function. However, the transformation for *CgQDR2* complement strain was more difficult than expected and might need major modifications to its transformation strategy for future trials.

2. The Characteristic of QDR2

There is limited information about the physiological and functional role of *Cg*Qdr2p in *C. glabrata* although *Cg*Qdr2p is the orthologue of *S. cerevisiae* Qdr2p, considering to be a drug/cation transporter. The identity between these two proteins is 68%; meanwhile, the identity with *C. albicans* is only 17%. The amino acid sequence alignment analysis showed that *Cg*Qdr2p conserved all the putative antiporter motifs. *Cg*Qdr2p has similar traits with *Sc*Qdr2p roles, such as in oxidative stress response and resistant phenotype towards several azoles and quinidine. *Cg*Qdr2p also showed involvement in citrate and zinc homeostasis. Besides *Sc*Qdr2p, *Cg*Qdr2p also carried out similar characteristics with *Ca*Qdr2p, despite the low identity percentage, especially in lipid profile alteration which might be related to biofilm architecture and

fundamental cell metabolism pathways in *C. glabrata*.

3. The Role of QDR2 on *C. glabrata* pH homeostasis

C. glabrata can inhabit many areas inside the host with different pH, such as the genitourinary tract (pH ±4), the gastrointestinal tract (pH range 2-8), and the bloodstream (pH 7.4). This adaptive behavior reflects *C. glabrata's* virulence trait which is able to withstand different pH conditions. As a DHA1-MFS membrane transporter, *Cg*Qdr2p function may be greatly affected by the pH changes. In this chapter, it was indicated that *Cg*Qdr2p might affect *C. glabrata* growth adaptability in neutral-alkaline media, due to its inability to maintain pHi, leading towards acidic intracellular pH (pHi). Also, *Cg*Qdr2p indicated a relationship with pH response factor *CgRIM101*. It was suspected that in the absence of *Cg*Qdr2p, other proteins could not function well due to acidic pHi, which may affect the drug resistance as well as other functions in the cell.

In summary, the analyses on *Cg*Qdr2p role have revealed that *Cg*Qdr2p is involved in the pH adaptation and the biofilm formation of *C. glabrata*. Upon loss of *QDR2*, *C. glabrata* would suffer from improper pHi regulation, leading to abnormal cell growth and defects in fundamental cell metabolism, and oxidative stress response. These events were suspected to affect *C. glabrata*'s adhesive ability, biofilm formation and maturation on artificial matrices, survival in neutral-basic conditions, and antifungal drug resistance. These findings are expected to encourage further experiments on Qdr2p, which is a potential drug target and the inhibitor of Qdr2p may provide new treatments for systemic *C. glabrata* infections worldwide.