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論 文 要 旨
THESIS SUMMARY

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学生氏名： Student's Name	Tenagy		

申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Philosophy)
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Malassezia spp. are common habitant yeast in human or homothermic animals' skin. All species of this yeast does not poesses fatty acid synthase causing the dependence on exogenous lipids from the host for their growth and proliferation. In immunocompromised individuals, this yeast can cause various skin diseases such as seborrheic dermatitis, ptyriasis versicolor, dog's otitis, and dandruff. One of the pathogenicity treats of *Malassezia* spp. is the lipase secretion which degrade host's lipid into fatty acids. Some of the fatty acids can penetrate through the epidermal barrier of the skin causing inflammation. The other fatty acids will be consumed by the yeast for its growth. However, how a fatty acid is utilized by *Malassezia* spp. and how is it related to the pathogenicity remain to be clarified.

1. Identification of acyl-CoA synthetase (ACS) involved in fatty acid utilization in *Malassezia* spp.

Fatty acids are important building blocks of lipid molecules that constitute cell membranes. They are also used as energy sources, precursors of hormones, protein modification and signal transduction. Before a fatty acid can enter any of the metabolism pathways, it needs to be activated to fatty acyl-CoA by ACS (fatty acid:CoA ligase, E.C. 6.2.1.3). This enzyme catalyzes the thioseterification of fatty acid and CoA using ATP. Six genes encoding ACS in *Saccharomyces cerevisiae* have been well studied with *ScFAA1* and *ScFAA4* as the main fatty acid activators. Deletion of these two genes resulted in the inability of *S. cerevisiae* to grow in medium containing fatty acids and fatty acid synthase inhibitor cerulenin.

In this study, orthologs of *S. cerevisiae FAA1* and *FAA2* were identified in *M. pachydermatis, M. globosa,* and *M. sympodialis.* Complementation of genes encoding *FAA1* of *M. pachydermatis* (Malapachy_0054, *MpFAA1*), *M. globosa* (MGL_3626, *MgFAA1*), and *M. sympodialis* (MSYG_3835, *MsFAA1*) into *S. cerevisiae faa1*Δ *faa4*Δ restored the growth of *S. cerevisiae* mutant in minimal medium containing cerulenin and long-chain fatty acid C14:0 (myrstic acid), C16:0 (palmitic acid), and C18:1 (oleic acid). Unexpectedly, *S. cerevisiae faa1*Δ *faa4*Δ harboring *MpFAA1* and *MgFAA1* could grow in fatty acid C12:0 (lauric acid), which is usually lethal for *S. cerevisiae* wild-type if it is used as a sole carbon source. On the other hand, the complementation of genes encoding *FAA2* of *M. pachydermatis* (Malapachy_0976, *MpFAA2*), *M. globosa* (MGL_0129, *MgFAA2*), and *M. sympodialis* (MSY001_0514, *MsFAA1*) into *S. cerevisiae faa1*Δ *faa4*Δ could not restore the growth of *S. cerevisiae faa1*Δ *faa4*Δ in medium containing any long-chain fatty acids proving the different function of *Malassezia FAA2* which is possibly function in fatty acid import into the peroxisome because peroxisome targeting signal 1 (PTS1) sequence were found in C-terminal of these *Malasseia* Faa2 proteins.

Gene expression level analysis by qRT-PCR suggested that *MpFAA1* was induced in the presence of palmitic acid and oleic acid, while *MpFAA2* was induced only in palmitic acid.

2. Effect of ACS inhibition and its potential as new drug targets for *Malassezia* treatment.

Drugs for diseases caused by *Malassezia* are usually from the azole groups like ketoconazole, fluconazole, and itraconazole. However, recently some azole-resistant *Malassezia* strains have been isolated causing the need for a new drug discovery. ACSs are assumed to be potential as new drug targets because FAS is absent in all *Malassezia* spp. Inhibition of activation of exogenous fatty acids can disrupt the fatty acid metabolism in this yeast and probably cause the cell death.

Triacsin C, a potent ACS inhibitor, hampered the growth of *M. pachydermatis, M. globosa,* and *M. sympodialis* in rich medium mDixon, with *M. pachydermatis* as the most senstive species among the three. Supplemetation of this inhibitor in minimal medium containing cerulenin and fatty acids also block the restore of *MpFAA1, MgFAA1,* and *MsFAA1* in *S. cerevisiae faa1*Δ *faa4*Δ. However, the *S. cerevisae* WT could still grow in the presence of Triacsin C, showing that *Malassezia FAA1* is the target of Triacsin C but not that of *S. cerevisiae.* This inhibitor also affected the lipid droplet formation (smaller size) in *Malassezia* and interfere the lipid synthesis for the cell wall formation, causing the weaker cell membrane. Addition of Triacsin C also reduced the acyl-CoA products and increased FFAs were detected. Thus, *Malassezia* Faa1p is indeed potential as a new drug target for *Malassezia* treatment.

3. Purification and Characterization of *Malassezia* ACS protein.

Purification of *Malassezia* ACS proteins, especially Faa1p, was carried out to understand more about the characteristics of these enzymes. *MpFAA1* and *MgFAA1* ORF which have no introns were tagged with 6xhis-tag at their N-terminal and successfully cloned into BL21 (DE3) *E. coli* strain for Faa1 protein purification. The purification also resulted the clear band for the target protein with the correct size. Even though few impurities still appeared, this is the first attemp (to our knowledge) that demonstrate the purification of *Malassezia* proteins using *E. coli.*