

論文 / 著書情報  
Article / Book Information

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(論文博士)  
(Dissertation Doctorate)

論 文 要 旨 ( 英 文 )

(800語程度)

Dissertation Summary (approx. 800 words in English)

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| 報告番号<br>For<br>administrative<br>use only                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 乙 第<br>号 | 氏 名<br>Name | Tee Chui Boon |
| <p>( 要 旨 )<br/>(Summary)</p> <p><i>Malassezia</i> spp. are opportunistic pathogens associated with a number of skin diseases in animals and humans. The lipophilic characteristic of <i>Malassezia</i> spp. requires an external lipid source such as olive oil to support their growth <i>in vitro</i>. In addition, <i>Malassezia</i> spp are frequently found at sebum rich areas of skin such as scalp, chest, back and trunk, indicating the importance of a sebum rich environment in <i>Malassezia</i> growth. The abundant hydrolases are necessary for <i>Malassezia</i> to utilise external lipid source, which accords with their adaption on sebum rich but carbohydrate deficient skin areas. On the other hand, the pathogenesis of <i>Malassezia</i> in skin disease development remains poorly understood. Haemolysis, the ability to degrade red blood cells (RBCs) often has been associated with pathogenicity and virulence in bacteria. Recently, haemolytic activity has also been observed on <i>Malassezia</i> spp. However, the role of haemolytic activity and its relationship in <i>Malassezia</i> pathogenesis remain unknown.</p> <p>Hydrolytic activities such as lipase and phospholipase activities are widely recognised as some of the most important factors for the growth of <i>Malassezia</i> spp. due to their lipid-dependent manner and due to free fatty acids released through these lipase and phospholipase activities, which trigger inflammation in the host. Hence, in this study, the relationship between these secreted hydrolytic activities and the haemolytic activity in isolated <i>Malassezia</i> clinical strains was investigated. This characterisation was expected to elucidate pathogenicity of this fungus. There were 49 clinical strains identified as <i>Malassezia</i> spp. via 26S rDNA restriction fragment length</p> |          |             |               |

polymorphism analysis (26S rDNA RLPF), which were later confirmed at molecular level by internal transcribed spacer (ITS 1) sequencing. Among the 48 identified clinical strains, 35 survived the -80°C storage due to the very fastidious characteristic of *Malassezia* fungus. All of 35 clinical strains (except for one *M. dermatis* isolate) showed detectable lipase and phospholipase activities against 4-nitrophenyl palmitate (p-NPP) and L- $\alpha$ -phosphatidylcholine, dipalmitoyl (L-DPPC), respectively. Most of the *M. globosa* isolates showed higher lipase activities than isolates of other *Malassezia* species. In terms of phospholipase activity, no significant difference was observed among species of *Malassezia*, although one isolate of *M. globosa* showed considerably higher phospholipase activity than the others. All tested strains also exhibited haemolytic activity, both as determined using 5 % (v/v) sheep blood agar (halo assay) and by quantitative assay. Although all tested strains showed detectable haemolytic activity, there was no obvious correlation observed between the secreted lipase and phospholipase activities and haemolytic activity. Hence, combining with a previous report, the haemolytic activity of *Malassezia* spp. is seemingly mediated by non-protein components present in the secreted samples. The considerable haemolytic activity found in all clinical strains, suggests a haemolytic factor may be played an important role in *Malassezia* pathogenesis. Thus, the factor can be a biomarker to identify pathogenic *Malassezia*. It may also to use to control and suppress the growth of *Malassezia* to normal level on human skin.

The haemolytic factor/compound is thought to be secreted out from cells. Ethyl acetate was used to extract the secreted haemolytic compound from agar that *M. globosa* grew on. The crude extract from *M. globosa* showed high polarity and readily dissolved in methanol. Gradient column chromatography using the combination of chloroform and methanol was used to separate the crude extract. A total of 89 fractions were collected and analysed by thin layer chromatography (TLC). The major spot (combined fraction 57-58) showed haemolytic activities. It was selected for further structural analysis by time-of-flight mass spectrometry (ESI-TOF-MS) and,  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR). The compound in this spot presented in combined fraction 57-58 exhibited a molecular ion at  $m/z$  408 in ESI-TOF-MS. A search of database MFSearcher indicated

that this molecule is cholic acid ( $C_{24}H_{40}O_5$ ), which may be the secreted haemolytic compound from *M. globosa*. Indeed, superimposed signals of NMR results, measured with both  $^1H$  and  $^{13}C$ , showed significant similarities between the isolated compound and commercial cholic acid. The discrepant signals in both  $^1H$  and  $^{13}C$  NMR requires further purification steps as future work. Bile acids have strong surface activity and can solubilise membrane proteins and lipids. Cholic acid may be causing the surface tension change in the membrane of red blood cells and then lead to the lysis of red blood cells. On the other hand, bile acids are strongly cytotoxic and have been associated carcinogenesis of some tissues. Cholic acid is a primary bile acid in mammals. This suggests that cholic acid may be also toxic to organisms.

Cholic acid has been reported in cholesterol metabolism. *Malassezia* possesses high lipid content in the cell wall, which suggests that cholic acid may also have a role in lipid membranes in the cell wall. For example, it may have a role in triggering hyphal formation of *Malassezia* and lead to a phase switching from commensal to pathogenic fungus.

Eventually, the continual study of *Malassezia* spp. pathogenesis will help us to better understand this intriguing skin fungus and modulate the effects which contribute to the development of skin diseases.

備考：論文要旨は、和文2000字と英文300語を1部ずつ提出するか、もしくは英文800語を1部提出してください。

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