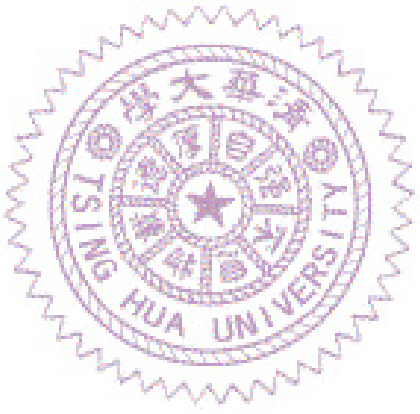
摘要

胃幽門螺旋桿菌 (*Helicobacter pylori*) 是一種呈現螺旋狀並帶有鞭毛的微好氧革蘭氏陰性菌，世界上有超過一半的人口都曾經被此菌所感染，並造成慢性胃部疾病。過去有報導指出，革蘭氏陰性菌在它生長的過程中會不斷從外膜釋放出外膜囊泡 (outer membrane vesicles, OMVs)，其功能有幫助傳送致病因子、調控宿主免疫反應與面對壓力之反應。脂多醣 (lipopolysaccharide, LPS) 是胃幽門螺旋桿菌重要毒素之一，同時脂多醣的長度也被認為在外膜囊泡中的蛋白質分選過程中扮演重要的角色。脂多醣的結構有三個部分所組成：包含脂質 A (lipid A)、核寡糖 (core oligosaccharide) 和 O 抗原 (O-antigen)。先前我們實驗室已建構出不同脂多醣結構截斷的突變株，包含在 inner-core，outer-core，O-antigen，七碳醣生合成路徑(heptose biosynthetic pathway)，與第四型分泌系統(*cag* type Ⅳ secretion system) 產生缺陷。

在本篇研究報告中，我們利用銀染檢視各種突變株的外膜囊泡上不同的脂多醣圖譜 (LPS profile)，而這些突變株會根據所帶有的不同長度的脂多醣釋放出不同蛋白總量的外膜囊泡。接著我們利用動態光散射粒徑分析儀 (dynamic light scattering) 分析不同突變株釋放的外膜囊泡之粒徑大小，實驗結果發現，從七碳醣生合成路徑突變株釋放較大直徑的外膜囊泡。我們利用西方墨點法去偵測不同突變株釋放的外膜囊泡中所帶有的毒素因子 (CagA 和 VacA) 與貼附因子 (BabA/B 和 AlpA/B)，實驗結果顯示主要釋放 CagA 到外膜囊泡並不是藉由第四型分泌系統，因為在第四型分泌系統中的主要的 *cagL* 缺失時，並不會影響 CagA 在外膜囊泡中的存在。此外，七碳醣生合成路徑突變株所釋放的外膜囊泡所含有毒素與貼附因子也會隨之減少，並且推測脂多醣的結構也會影響貼附因子的醣化作用。最後，我們將不同突變株分離出的外膜囊泡與胃腺癌細胞 (AGS cells) 一同培養，發現七碳醣生合成路徑突變株分離的外膜囊泡所感染的胃腺癌細胞當中的毒素蛋白 CagA 和 VacA 含量明顯減少。綜合以上的實驗結果，胃幽門螺旋

桿菌上的脂多醣結構不僅以影響外膜囊泡的形成，也在蛋白質分選到外膜囊泡過程中扮演重要的角色。



Abstract

Helicobacter pylori is a microaerophilic Gram-negative, spiral-shaped and flagellated bacterium that colonizes more than half of the world's population and frequently causes chronic infection. Gram-negative bacteria have been reported to release outer membrane vesicles (OMVs) from the outer membrane during their growth. The functions of OMVs include the delivery of virulence factors, modulation of the host's immune system and stress response. LPS is considered as a key virulence factor of *H. pylori* and may contribute to sorting of proteins into OMVs. It is composed of lipid A, core oligosaccharide and O-antigen. Previously, our laboratory had constructed various *H. pylori* LPS truncated mutants, including those having defects in the inner-core, outer-core, O-antigen, heptose biosynthetic pathway, and the *cag* type Ⅳ secretion system. In this study, we examined the different LPS profiles of these mutants on OMVs by sliver staining. The amounts of OMVs produced were found to be related to the length (or the structure) of LPS. Using dynamic light scattering analysis, we found that the average size of OMVs derived from the heptose biosynthetic pathway knockout mutants were larger than others. In addition, we observed the presence of key virulence factors CagA, VacA and various adhesins on OMVs by immunoblotting. The results also suggested that the major route of releasing CagA into OMVs is not through the type Ⅳ secretion system because the disruption of this system by knocking out *cagL*, an important component of the type Ⅳ secretion system, did not affect the presence of CagA in OMVs. In addition, CagA and the tested adhesins were significantly reduced in OMV samples collected from the heptose biosynthetic pathway knockout mutants. Furthermore, the change of LPS structure also altered the glycosylation status of the adhesins on OMVs. Moreover, the amount of CagA and VacA present in the whole cell extract of AGS cells were

significantly decreased after treating with OMVs derived from the heptose biosynthetic pathway knockout mutants while compared to those treated with OMVs from the wild-type strain. In conclusion, we reported that LPS structure plays an important role not only in the formation of OMVs, but also in the sorting of proteins into OMVs.