影響豬霍亂沙門氏菌第一型線毛培養條件的探討

Characterization of the culture conditions that may affect the expression of the type 1 fimbriae in Salmonella enterica serotype Choleraesuis

中文摘要

線毛 (fimbriae) 為類似毛髮的蛋白質結構，存在於許多腸內寄生菌的外膜上，使細菌會吸附宿主細胞，而細菌吸附於宿主細胞被認為是入侵的首要條件。豬霍亂沙門氏菌 (Salmonella enterica serotype Choleraesuis) 是一株對於豬隻及人類有高侵入性的 Salmonella 血清型，常常造成養豬產業巨大損失，沙門氏菌的散播也造成公共衛生上的隱憂。在大部分 Salmonella 血清型中第一型線毛是其最常見的線毛種類，而 S. Choleraesuis 中有 15 種不同線毛的基因組，但是對於其線毛的表現特性仍未釐清。因此設計了不同的生長環境探討 S. Choleraesuis 第一型線毛的表現。實驗結果發現在適合第一型線毛的靜置液態培養環境之下從豬隻分離出的 S. Choleraesuis 卻將近 70 % (64/98) 不表現，而從人類分離出的六株 S. Choleraesuis 在所有測試的環境中皆不表現第一型線毛。培養在室溫以及 42 ℃皆會抑制 S. Choleraesuis 第一型線毛的表現。pH 6 和 pH 8 的環境下，S. Choleraesuis 第一型線毛將近八成會表現，但是隨著 pH 值的升高與降低，第一型線毛的表現會完全被抑制。加入 1 % 葡萄糖的環境以及震盪的環境同樣也會抑制第一型線毛的表現，甚至產生其他類型的線毛。降低固態培養基洋菜膠的濃度也不會使第一型線毛產生。RT-PCR 觀察 fimA mRNA 發現無論線毛是否在菌體表面有表現，將近九成都偵測到 mRNA 表現。以西方墨點法偵測也可以觀察到菌株是會產生 FimA 蛋白質。在 S. Choleraesuis 中 fim gene cluster 的轉錄與轉譯的功能都是沒有缺損的，但是卻無法在菌體外膜形成第一型線毛，也許是因為 posttranslational modification 所造成。S. Choleraesuis 感染宿主後會遇見不同的環境，細菌若有能力隨著環境變化，調控第一型線毛的表現，應該會比失去此能力的菌株，容易躲避宿主免疫系統的攻擊而存活。

英文摘要

Adherence is a prerequisite step for most of the bacteria to establish infection both in human and animals. The adherent event is mediated by the surface appendages called fimbriae. Fimbriae are hair-like protein structures on the outer membrane of the cell. They are produced by many members of the family Enterobacteriaceae including Salmonella. Salmonella enterica serotype Choleraesuis is an invasive serotype and can infect both human and swine. There are 15 different fimbrial operons that have the potential to produce fimbriae in S. Choleraesuis. Previous observation indicated that the most commonly found fimbrial type in the Salmonella serotypes is the type 1 fimbriae. Nevertheless, the type 1 fimbriae of S. Choleraesuis had not been characterized well. The objective of the present study is to investigate the type 1 fimbriae of S. Choleraesuis in more detail. Our result indicated that almost 70 % (64/98) of the S. Choleraesuis isolated from swine and 6 isolates from human did not produce type 1 fimbriae in the static broth culture condition which usually favors the type 1 fimbriae to express in another serotype S. Typhimurium. In both acidic and alkaline conditions changes the expression of type 1 fimbriae. The production of type 1 fimbriae in S. Choleraesuis was only observed among certain osmolarity range (0.06 M to 0.8 M NaCl). Room temperature and 42 ℃ inhibited the type 1 fimbrial expression. Decreasing the concentration of agar to 0.6 % still did not promote S. Choleraesuis to exhibit type 1 fimbriae. The mRNA of fimA was detected by reverse-transcriptase PCR in most of the strains that did not produce type 1 fimbriae. Western blot data was also detected FimA , the result indicated that maybe posttranslational modification of FimA may occur. The fim gene clusters containing the DNA fragments of the S. Choleraesuis isolate from swine (fimbriated) and human (non-fimbriated) were subjected to sequencing. Comparison of these two sequences is 99 % consistent. The absence of type 1 fimbriae may be beneficial for S. Choleraesuis to spread systemically and escape from the immune attack of the host.