DETECTION OF FIMBRIAL GENE IN LIVE AND LIVE-ATTENUATED *Pasteurella multocida* B:2 USING PCR

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By

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Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:
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LIST OF ABBREVIATIONS

µM	micromolar
ATP	adenosine triphosphate
bp	base pair
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dH_20	distilled water
DNA	deoxyribonucleic acid
dNTPs	deoxynucleoside triphosphates
dTTP	deoxythymidine triphosphate
EDTA	ethylenediaminetetraacetate
H_2S	hydrogen sulphide
HS	Haemorrhagic septicaemia
Kb	kilo base
kDa	kilo Dalton
LPS	Lipopolysaccharide
mer	oligomer
$MgCl_2$	magnesium chloride
NaCl	sodium chloride
ng	nanogram

P. multocida	*Pasteurella multocida*
PCR	polymerase chain reaction
rpm	rotation per minute
TAE	Tris-Acetate EDTA
U	Unit(s)
V	Volt(s)

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ABSTRACT

Gram-negative bacteria use various adhesion strategies to colonize host tissue and type 4 fimbriae are one of them. Their presence has been associated with pathogenesis of several bacterial species. The type 4 fimbriae have been identified on the surfaces of *Pasteurella multocida* serotype B:2, the causative agent of Haemorrhagic Septicaemia (HS) in Asia. These structures have been shown to mediate colonization of mucosal surfaces. The present study was conducted to detect the fimbrial gene in live and attenuated *P. multocida* strain M16 (serotype B:2) following the PCR amplification. The results showed the existence of fimbrial gene in both live *P. multocida* strain M16 and also the attenuated strains derived from the live strain after passaging the latter in decreasing concentration of blood supplement from 7% to 0% of blood in the agar from for a period of two months. The results demonstrated that the gene is an important survival factor of the organism as it still remains in the genome of the bacteria even after multiple passages in suppressed environment that have been reduced from 7% of blood supplement to 0% of total absent of blood in the growth medium. The results also verified that the type 4 fimbriae are one of the important virulence determinants in the *P. multocida* type B:2 that is crucial for host invasion upon their prime role to first colonize the mucosal site of infection. The findings from this study are important to initiate further research concerning the role of fimbrial protein in the development of disease and vaccine.

PENGENALPASTIAN GEN FIMBRIA MELALUI TEKNIK PCR DALAM *P. multocida* B:2 YANG HIDUP DAN YANG DILEMAHKAN

ABSTRAK

Bakteria Gram-negatif menggunakan pelbagai strategi pelekatan untuk mengkoloni tisu hos dan fimbria kelas 4 merupakan salah satu daripada strategi tersebut. Fimbria kelas 4 ini wujud pada lapisan permukaan *Pasteurella multocida* B:2 yang merupakan agen penyebab penyakit Haemorrhagic Septicaemia (HS) di Asia. Struktur fimbria ini dapat mengkoloni permukaan mukosa dan mempercepatkan infeksi bakteria terhadap hos. Kajian ini telah dijalankan untuk mengenalpasti gen fimbria melalui teknik PCR dalam *P. multocida* B:2 yang hidup dan yang telah dilemahkan berikutan pengsubkulturan yang berulang selama dua bulan di dalam media agar yang mengandungi kepekatan darah yang semakin berkurang iaitu dari 7% hingga 0%. Keputusan kajian ini menunjukkan bahawa gen fimbria adalah satu faktor kemandirian yang penting pada *P. multocida* B:2 kerana gen ini kekal di dalam genom dan tidak hilang walaupun dilemahkan dalam keadaan yang tertekan, yakni kekurangan nutrien darah dalam media. Hasil kajian ini juga memutuskan bahawa gen fimbria merupakan faktur virulen yang penting dalam *P. multocida* B:2 di mana ia menyerang tisu hos sebaik sahaja bakteria mengkoloni permukaan mukosa. Keputusan daripada kajian ini dapat digunakan untuk menginisiasi kajian lanjutan mengenai peranan protein fimbria dalam jangkitan penyakit dan penemuan vaksin.