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Study of an Exported Protein of *Mycobacterium avium subspecies paratuberculosis*

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Abstract

Johne's disease is a chronic, progressive enteric disease of ruminants caused by infection with *Mycobacterium avium subspecies paratuberculosis* (*M. ptb*) from the MAIS complex (*M. avium*, *M. ptb*, *M. intracellulare* and *M. scrofulaceum*). The lack of specific and sensitive diagnostic tests often leads to *M. ptb* infected animals being diagnosed with bovine tuberculosis, a member of the MTB complex (*M. tb*, *M. bovis*, *M. bovis* BCG, *M africanum*, *M. microti* and *M. canetti*). Secreted proteins from pathogenic mycobacteria have been found to be important for the development of protective immunity, namely a cell mediated immune response (CMI). The development of reliable differential diagnostic tests will require the use of species-specific secreted protein antigens and the CMI response.

Due to the taxonomic distance between the MAIS and MTB complexes our hypothesis was that the *M. ptb* genome may encode for secreted proteins that are absent from members of the MTB complex. If such proteins can stimulate an immune response they may be suitable for use as antigens in a differential diagnostic test for Johne's disease. To this end, the secreted protein library clone pJEM11-*M. ptb*281 was examined and its insert found to contain the 5' region of the hypothetical *M. ptb*281 ORF fused in frame with *phoA*. The entire ORF was determined using *M. avium* and *M. ptb* database sequences then cloned into *E. coli* and mycobacterial expression systems. These systems incorporate 6x histidine (His_6) affinity tags into recombinant proteins allowing them to be semi-purified by Ni-NTA affinity chromatography. Semi-purified recombinant proteins tested positive by western blot analysis to highly specific anti-His_6-tag antibodies. Amino acid sequencing to confirm the identity of these recombinant proteins and screening for their ability to stimulate an immune response were prevented by time constraints.

Homologs to *M. ptb*281 were absent from *M. tb*, *M. bovis* and *M. bovis* BCG but present in the MAIS complex, making this protein unsuitable for use as an antigen to differentiate between MAIS complex species in a diagnostic test. *M. ptb*281 homologs found in the genomes of two members of the Acetomycetes order corresponded to hypothetical proteins predicted by computer software programs trained to identify genes, which may indicate that the hypothetical *M. ptb*281 ORF may encode a functional protein.

Abbreviations and Definitions

ADC	albumin-dextrose-catalase
Amp^r	gene conferring ampicillin resistance
AP	alkaline phosphatase
ATCC	American type culture collection
BCG	*M. bovis* Bacilli Calmette-Guerin
BCIP	5-bromo-4-chloro-3-indoyl phosphate disodium
bp	base pair
CDP^{star}	Disodium 2-chloro-5-(4-methoxyspiro {1,2-dioxetane-3,2´(5´-chloro) tricyclo ($3.3.1.1^{3.7}$) decan}-4-yl)-1-phenyl phosphate
CSPD	Disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro) tricyclo [$3.3.1.1^{3.7}$] decan}-4-yl) phenyl phosphate
C-terminal	carboxyl terminal
DIG	Digoxigenin
DIG-11-dUTP	Digoxigenin-11-2'-deoxy-uridine-5'-triphosphate
DMSO	dimethyl-sulphoxide
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
E. coli	*Escherichia coli*
hr	hour
IPTG	isopropyl β-D-thiogalactoside
kan	kanamycin sulphate
Kan^r	gene conferring kanamycin resistance
kb	kilo base
kDa	kilodalton
LB	Luria-Bertani
MAIS complex	*M. avium, M. ptb, M. intracellulare, M. scrofulaceum* complex

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Abbreviations and Definitions continued

M. ptb	*M. paratuberculosis*
M. tb	*M. tuberculosis*
M-MPTB281	pMIP MPTB281 recombinant protein
MPTB281	hypothetical MPTB281 protein
mRNA	messenger ribonucleic acid
MTB	*Mycobacterium tuberculosis* complex (*M. tb*, *M. bovis*, *M. bovis* BCG, *M africanum, M. microti* and *M. canetti*).
NBT	Nitro blue tetrazolium chloride
Ni-NTA	nickel nitrilo-tri-acetic acid
N-terminal	amino terminal
OADC	oleic acid-albumin-dextrose-catalase
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
pers. comm.	personal communication
phoA	Truncated alkaline phosphatase gene
PhoA	Truncated alkaline phosphatase protein
$PhoA^+$	Alkaline phosphatase phenotype
phoA-281-DIG	*phoA-M. ptb*281
pI	iso-electric point
SDS	sodiumdodecyl sulphate
Sec	secretion
soln.	solution
sp.	species
TAE	tris acetate EDTA
Taq	*Thermus aquaticus* DNA polymerase
TEMED	N, N', N, N-tetramethyl ethylene diamine
UV	ultra violet light
xg	multiplied by gravity
X-MPTB281	pPROEX MPTB281 recombinant protein

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