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The development of non antibiotic resistant recombinant vaccines against mycoplasma hyopneumoniae

Jake Matic
University of Wollongong

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The development of non antibiotic resistant recombinant vaccines against *Mycoplasma hyopneumoniae*

Jake Matic
PhD candidate
University of Wollongong
2008
Declaration of Authenticity

This thesis is submitted in accordance with the regulations of the University of Wollongong in fulfillment of the degree of Doctor of Philosophy. It does not include any material previously published by another person except where due reference is made in the text. The experimental work described in this thesis is original, and has not been submitted for a degree to any other university.

Jake Nicholas Matic
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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ATP</td>
<td>Ribosome binding site</td>
</tr>
<tr>
<td>BALF</td>
<td>Bronchoalveolar lavage fluid</td>
</tr>
<tr>
<td>BALT</td>
<td>Bronchus associated lymphoid tissue</td>
</tr>
<tr>
<td>CBE</td>
<td>Chromosomal based expression</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>DIG</td>
<td>Digoxigenin</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>Interlukin</td>
</tr>
<tr>
<td>kb</td>
<td>Kilo basepairs</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo Daltons</td>
</tr>
<tr>
<td>Km&lt;sup&gt;r&lt;/sup&gt;</td>
<td>Kanamycin resistant</td>
</tr>
<tr>
<td>LB</td>
<td>Luria-Bertini</td>
</tr>
<tr>
<td>ORF</td>
<td>Open reading frame</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PBE</td>
<td>Plasmid based expression</td>
</tr>
<tr>
<td>PCV2</td>
<td>Porcine circovirus type 2</td>
</tr>
<tr>
<td>PDH</td>
<td>Pyruvate dehydrogenase</td>
</tr>
<tr>
<td>PE</td>
<td><em>Pseudomonas</em> exotoxin A</td>
</tr>
<tr>
<td>PEP</td>
<td>Porcine enzootic pneumonia</td>
</tr>
<tr>
<td>PGE2</td>
<td>Prostaglandin E2</td>
</tr>
<tr>
<td>p.i.</td>
<td>Post infection</td>
</tr>
<tr>
<td>PRDC</td>
<td>Porcine respiratory disease complex</td>
</tr>
<tr>
<td>PRRSV</td>
<td>Porcine reproductive respiratory syndrome virus</td>
</tr>
<tr>
<td>PRV</td>
<td>Pseudorabies virus</td>
</tr>
<tr>
<td>RNR</td>
<td>Ribonucleotide reductase</td>
</tr>
<tr>
<td>RR1</td>
<td>Repeat region 1</td>
</tr>
<tr>
<td>RR2</td>
<td>Repeat region 2</td>
</tr>
<tr>
<td>rmB&lt;sub&gt;to&lt;/sub&gt;</td>
<td>E. coli ribosomal RNA terminator</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SIV</td>
<td>Swine influenza virus</td>
</tr>
<tr>
<td>SPF</td>
<td>Specific pathogen free</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris buffered saline</td>
</tr>
<tr>
<td>Th1</td>
<td>T helper cell type 1</td>
</tr>
<tr>
<td>Th2</td>
<td>T helper cell type 2</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tissue necrosis factor alpha</td>
</tr>
<tr>
<td>xg</td>
<td>Times gravity</td>
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ABSTRACT

*Mycoplasma hyopneumoniae* is a respiratory pathogen of pigs that has substantial global economic impact and currently there is no vaccine available that prevents colonisation. This study focuses on the characterisation of novel *M. hyopneumoniae* vaccine candidates and the development of a live, attenuated vaccine expressing heterologous *M. hyopneumoniae* antigens. The putative *M. hyopneumoniae* vaccine antigen, the lipoyl binding domain of the dihydrolipoyl dehydrogenase subunit (PdhD) of the pyruvate dehydrogenase complex, was strongly recognised by porcine hyperimmune sera. Western blot analysis with PdhD antisera detected the protein in geographically diverse *M. hyopneumoniae* isolates. However PdhD was only weakly recognised by convalescent swine sera indicating it is not likely to contribute significantly to the protective convalescent response. Therefore it was not used in further vaccination experiments. Live vaccine delivery systems expressing two antigens from *M. hyopneumoniae*, adhesin (Adh) and ribonucleotide reductase (NrdF), were constructed using either plasmid-based expression (PBE) or chromosomally-based expression (CBE) systems. The PBE system was formed by cloning both antigen genes into pJLA507 to create an operon downstream of temperature-inducible promoters. Constitutive CBE was achieved using a promoter trapping technique whereby the promoterless operon was stably integrated into the chromosome of *Salmonella enterica* serovar Typhimurium *aroA* (STM-1) and the expression of antigens assessed. The chromosomal position of the operon was mapped in four clones. Inducible CBE was obtained using the *in vivo* induced *sspA* promoter and recombining the expression construct into *aroD*. Dual expression of the antigens was
detected in all systems with PBE producing much higher quantities of both antigens. The stability of antigen expression was higher in the CBE system with 60-100% of individual cells still expressing antigen after 60 generations without selection. PBE and CBE strains were selected for comparison in a vaccination trial. The vaccine strains were delivered orally into mice and significant systemic IgM and IgG responses against both antigens amongst all CBE groups were detected. No significant immune response against either antigen was detected using PBE strains. Expression of recombinant antigens in *S. enterica* serovar Typhimurium *aroA* from chromosomally-located strong promoters without the use of antibiotic resistance markers is a reliable and effective method of inducing a significant immune response.