Title: IDENTIFICATION OF TRANSCRIBED GENES BY *Pasteurella multocida* IN PORCINE LUNGS THROUGH THE SELECTIVE CAPTURE OF TRANSCRIBED SEQUENCES (SCOTS)

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Abstract:

Pasteurella multocida can cause disease in a wide range of animals and is the causative agent of numerous, economically important disease, including avian fowl cholera, bovine haemorrhagic septicemia, snuffles in rabbit, enzoonotic pneumonia and in swine it can cause pneumonia and atrophic rhinitis. The pathogenicity of *P. multocida* is complex and several virulence factors have been identified previously, to identify genes involved in the pathogenesis of *P.multocida* several genetic technologies have emerged as powerful tools. In this study we report the use of Selective Capture of Transcribed Sequence (SCOTS) approach to screen the *P.multocida* genes expressed in the lung of swine with acute pneumonia (3 days post infection). Total RNA was isolated from two conditions: bacterial pellets (BAC) and infected lungs (LUNG). The samples BAC and LUNG were reverse transcribed with primer SCOTS-N6-01 (5´-GCCGGTCGACTGCAGAATTC-3´) or SCOTS-N6-02 (5´-CTACGCATGCTCGAGGTACC-3´), respectively. Each cDNA population (BAC and LUNG) was subjected to three rounds of normalization to reduce sequences transcribed in abundance (rRNA sequences in particular) which would otherwise lead to false positives and/or loss of rare transcripts. For each round of SCOTS 3µg cDNA samples were denatured at 98°C for 3 min and normalized by self-hybridization, and hybridized subsequently at 65°C for 18h with 0.6µg photobiotinylated genomic DNA. The cDNAs were captured by streptavidin-coupled magnetic beads, were re-amplified by PCR and the fragments were cloned. A total of 172 possible clones were obtained, 151 lung-specifc and 21 bacteria. The positive cDNA clones from the library were amplified by PCR using M13 primers and sequenced. A total of 25 genes were identified, of which 21 encoded enzymes for metabolism, cell surface, cell wall, proteins for regulatory adaptive responses or transport. Four were unknown, novel genes. Five genes representing four categories were chosen to verify the expression by real-time reverse transcriptase-polymerase chain reaction.

Keywords: pasteurelosis, pneumonia, swine, transcriptome

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