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Proteomic analysis of *Lawsonia intracellularis* reveals expression of outer membrane proteins during infection

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ABSTRACT

Lawsonia intracellularis is the aetiological agent of the commercially significant porcine disease, proliferative enteropathy. Current understanding of host–pathogen interaction is limited due to the fastidious microaerophilic obligate intracellular nature of the bacterium. In the present study, expression of bacterial proteins during infection was investigated using a mass spectrometry approach. LC-ESI-MS/MS analysis of two isolates of *L. intracellularis* from heavily-infected epithelial cell cultures and database mining using fully annotated *L. intracellularis* genome sequences identified 19 proteins. According to the Clusters of Orthologous Groups (COG) functional classification, proteins were identified with roles in cell metabolism, protein synthesis and oxidative stress protection; seven proteins with putative or unknown function were also identified. Detailed bioinformatic analyses of five uncharacterised proteins, which were expressed by both isolates, identified domains and motifs common to other outer membrane-associated proteins with important roles in pathogenesis including adherence and invasion. Analysis of recombinant proteins on Western blots using immune sera from *L. intracellularis*-infected pigs identified two proteins, LI0841 and LI0902 as antigenic. The detection of five outer membrane proteins expressed during infection, including two antigenic proteins, demonstrates the potential of this approach to interrogate *L. intracellularis* host–pathogen interactions and identify novel targets which may be exploited in disease control.

1. Introduction

Lawsonia intracellularis is the aetiological agent of proliferative enteropathy (PE), or ileitis, which affects swine populations worldwide with considerable economic impact (Jacobson et al., 2009). *L. intracellularis* is a Gram-negative, microaerophilic obligate intracellular bacterium which replicates in the cytoplasm of infected cells. The bacterium primarily infects the immature enterocytes of intestinal crypts where it induces proliferation and

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subsequently hyperplasia (Smith and Lawson, 2001). A variety of clinical manifestations are presented; acute cases are associated with bloody diarrhoea and sudden death whereas chronic infection, more common in younger pigs, is typified by wasting and loss of condition which may be accompanied by mild diarrhoea. This results in a significant decrease in financial returns due to the effects on pig production compounded by the cost of prophylactic and therapeutic antibiotics. PE has been reported in a wide variety of other domestic and wild animals including the hamster, rabbit, rat, guinea pig, ferret, deer, dog, wolf, fox, ostrich, emu and rhesus macaque (Lawson and Gebhart, 2000) and equine PE is an emerging problem with infection primarily reported in post-weaning foals (Frazer, 2008).

In the laboratory the bacterium is extremely fastidious, due to its microaerophilic obligate intracellular lifestyle. As a result, conventional laboratory approaches, including the use of genetic manipulation, which can be used to interrogate the host–pathogen interaction, are restricted. Genetic dissimilarity between *L. intracellularis* and other enteric pathogens has also hampered characterisation. The closest known relatives of *L. intracellularis* are *Bilophila wadsworthia* and *Desulfovibrio desulfuricans* although the niches occupied by these free-living bacteria are distinct from *L. intracellularis* and their relevance as models for *L. intracellularis* pathogenesis is limited. Although there is a growing body of literature describing expression of *L. intracellularis* gene transcripts (Vannucci et al., 2012), few expressed bacterial determinants have been reported at the protein level, including mainly uncharacterised immunogens (Guedes and Gebhart, 2003) and more recently, major components of a type III secretion system (Alberdi et al., 2009). Shotgun proteomic analysis of complex protein mixtures has proved to be a successful approach for investigating the proteomes of obligate intracellular bacteria where technical difficulties in obtaining preparations of organisms free from extraneous host cell material has previously hampered protein identification. To date, such methods have been extensively employed to analyse proteomes and sub-proteomes of a number of obligate intracellular bacteria including *Rickettsia typhi, Anaplasma phagocytophilum*, *Neorickettsia sennetsu*, and *Ehrlichia chaffeensis*, (Lin et al., 2011; Sears et al., 2012; Troese et al., 2011).

We recently described the detection of a *L. intracellularis* immunogenic autotransporter protein expressed during host cell infection using liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS) (Watson et al., 2011). In the present study we applied these same methodologies to perform a shotgun proteomic analysis of bacterial cells and report a number of *L. intracellularis* proteins that are expressed during the interaction with host cells *in vitro*. Bioinformatic analyses were used to tentatively assign function to consistently expressed proteins and several outer membrane proteins were identified. Further immunological investigation was facilitated by recombinant fusion proteins and a panel of sera from naturally infected and uninfected pigs, which identified two proteins as potential immunogens.

2. Materials and methods

2.1. Bacterial strains, plasmids and growth conditions

Bacterial isolates and plasmids used in this study are listed in Table 1. The *Lawsonia intracellularis* isolates LR189/5/83 and N343, were co-cultured in IEC-18 or INT-407 epithelial cells respectively, essentially as previously described (Lawson et al., 1993) at 37 °C under microaerophilic conditions (8.0% CO_2; 8.8% O_2). Recombinant plasmids were maintained in the *Escherichia coli* TOP10 strain which was routinely cultured under aerobic conditions on LB medium containing 50 μg/ml ampicillin. The *E. coli* BL21(DE3)pLysS strain was used for expression of recombinant fusion proteins and was cultured on LB medium containing ampicillin (50 μg/ml) and chloramphenicol (35 μg/ml).

2.2. L. intracellularis sample preparation

LR189/5/83 isolate was prepared by extracting approximately 5 ml of cell culture medium from heavily infected

Table 1
Bacterial isolates, plasmids and oligonucleotide primers used in this study.

Isolate	Description	Source
LR189/5/83	*L. intracellularis* isolate	UK isolate
N343	*L. intracellularis* reference strain	ATCC 55672
TOP10	*E. coli* cloning isolate	Invitrogen, Paisley, UK
BL21(DE3)pLysS	*E. coli* cloning isolate	Invitrogen, Paisley, UK
Plasmid	Description	Source
pRSETA	Expression vector	Invitrogen, Paisley, UK
pEWSF1	pRSETA::LI0841	This study
pEWSF2	pRSETA::LI0902	This study
pEWSF3	pRSETA::LI0691	This study
Primer	Sequence (5′-3′)	Restriction sites
LI0841F	CGGGTACCGCATGAGAAAATTATGGATTTT	KpnI
LI0841R	GCGAATTCTTAGAATGTTAAACGTGCACTT	EcoRI
LI0902F	CGGGTACCGCTTTGCTGTTACATTATTAAC	KpnI
LI0902R	GCAAGCTTCTAATCAAAAAAGACAAGTTCT	HindIII
LI0691F	CGGGTACCGCTTAGTAGTATTAAGTGCAGG	KpnI
LI0691R	GCGAATTCTTACTTGGCAATAATACGAAAA	EcoRI