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Expression by *Lawsonia intracellularis* of type III secretion system components during infection

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ABSTRACT

Contact-dependent secretion systems, such as the type III secretion system (T3SS), have been shown to play significant roles in the pathogenicity of many Gram-negative bacterial pathogens. *Lawsonia intracellularis* is a novel, obligate intracellular Gram-negative bacterium, which has been identified as the etiological agent of proliferative enteropathies in numerous animal species. Analysis of the genome sequence of the *L. intracellularis* strain PHE/MN1-00 has revealed the presence of a T3SS secretion system in this bacterium. In this study we aimed to determine whether this important virulence mechanism is also present in *L. intracellularis* strain LR189/5/83. Using a PCR-based approach, we verified the presence of a genomic region encoding a T3SS. Specifically, a gene highly homologous to the *yscN* energiser component of the prototypic T3SS of *Yersinia* spp. was identified and termed *lscN*. Two further open reading frames (ORFs) contiguous with *lscN* were also identified: *lscO* and *lscQ*, which are also homologues of ORFs within the T3SS of *Yersinia* spp. To establish whether this T3SS may be functional, expression was monitored directly by RT-PCR and indirectly by detection of serological responses in vaccinated and infected animals. Transcripts for *lscN* and *lscQ* were detected and purified rLscQ was recognized by antiserum from infected pigs, indicating expression *in vivo* during infection. By analogy to other bacteria, this T3SS may be crucial for intracellular development and is likely to play a significant role in the virulence of this unusual pathogen.

1. Introduction

Lawsonia intracellularis is an obligate intracellular Gram-negative bacterium which causes proliferative enteropathy (PE), a highly important disease in pigs. The disease is characterised by proliferation of the small intestinal epithelium leading to hyperplasia thereby causing poor feed conversion, weight loss and diarrhoea with fatalities occurring in some animals (Smith and Lawson, 2001). Only rudimentary understanding of the molecular basis of *L. intracellularis* physiology and pathogenicity has been gained to date due to its genetic intractability.

Sequencing of the *L. intracellularis* genome has identified a T3SS with similarity to that of *Yersinia* species as well as flagellar type III secretion system, which is considered ancestral to T3SS (Hueck, 1998). Contact-dependent secretion systems such as the type III system (T3SS) are

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found in many Gram-negative including enteroinvasive pathogens such as *Shigella*, *Salmonella* and *Yersinia* (Hueck, 1998; Coburn et al., 2007) as well as the other obligate intracellular bacteria such as *Chlamydia*/*Chlamydophila* (Peters et al., 2007). This is a common secretion system through which bacterial proteins (effectors) are translocated into the host cell thereby dysregulating various cellular processes (Espinosa and Alfano, 2004; Cornelis, 2006). In all of these pathogens, T3SS contribute essential functions to pathogenicity including roles in cellular invasion, promotion or prohibition of apoptosis, and suppression of innate defences. Consequently, we postulate that secretion systems such as T3SS could play a significant role in the pathogenicity of *L. intracellularis*.

In this study we have detected for the first time that T3SS components of *L. intracellularis* are expressed during infection. This is a very important development towards achieving better understanding of the molecular mechanisms underlying the pathogenicity and to further develop effective control measures for *L. intracellularis*.

2. Materials and methods

2.1. Bacterial isolates and growth conditions

Bacterial isolates and plasmids used in this study are listed in Table 1. *L. intracellularis* isolates LR189/5/83, LI916/91 and LI963/93 were co-cultured as previously described (Lawson et al., 1993) in IEC 18 cells at 37 °C under microaerophilic conditions (8.8% CO_2; 8.0% O_2). *Escherichia coli* TOP10 (Invitrogen) and AAEC189 strains (Blomfield et al., 1991), used for maintaining recombinant plasmids, were routinely cultured on Luria–Bertani (LB) medium containing ampicillin (100 μg/ml). *E. coli* BL21

Table 1
Oligonucleotide primers, bacterial isolates and plasmids used in this study.

Target	Primer	Sequence
L. intracellularis lscN	yli1	5′-CCTTGAGGTGAGTGAATTGAG
	yli1R	5′-TAGCATCATCCGCAGCAGGATCAT
	YscNF	5′-TCTTGGAGCAGAAGGTTTAA
	YscNR1	5′-TGCTGCAGCTAACTTCCTTG
	YscNR2	5′-ATACAACAAGTACAGACGCT
	YscNR3	5′-TGCTGTATAAGCTGATTTAA
	TSPF1	5′-AGCGAGCAGGAAACTCAGAT
	TSPF2	5′-GACAGAGCCAATTGCTGACGAAA
	TSPF3	5′-CAAGGAAGTTAGCTGCAGCAAATC
	TSPR1	5′-CCTATTTCACGTAATGCACGA
	TSPR2	5′-GTCGCTCCATAGAAGAACGTCAG
	TSPR3	5′-CTCCTGCTCCCAAGATCATGCTC
	[a]DW-ACP1™	5′-ACP-AGGTC
	[a]DW-ACP2™	5′-ACP-TGGTC
	[a]DW-ACP3™	5′-ACP-GGGTC
	[a]DW-ACP4™	5′-ACP-CGGTC
	[a]DW-ACPN	5′-ACPN-GGTC
	[a]Uni-primer	5′-TCACAGAAGTATGCCAAGCGA
	lscNF	5′-TGAAATAGGCTTGCATCTGGAG
	lscNNF	5′-CATCTAGGCCCTGCTGTAAAAA
	lscNR	5′-GTTTCCACCTTCTGTCTTTTCT
	lscNNR	5′-ATTTCCCTGCATTTTCTTTAT
L. intracellularis lscQ	yli3	5′-GAGCTATTAAGAATCTTACAGAAT
	yli3R	5′-AGTCCCGTATATGATTTATTCTCT
	TSPF4	5′-TCCAGCTTTATCTTCTGTAAGAT
	TSPF5	5′-TGCCCCATTAAAGCATAAGTTACAGA
	TSPF6	5′-GAACCATTAGTAGATTATCAGTTTGGATG
	TSPR4	5′-GCTAAAATACAAACATCAGCAGTTGTG
	TSPR5	5′-TTCTCCAAGTCCATTTAATACACGAC
L. intracellularis 16S rRNA	16SF	5′-CGCCGCGTGAGGGATGAA
	16SR	5′-CACGGCACGAGCTGACGACA
	16SNF	5′-ACGGTACCCCCAGAGGAAGAACAC
	16SNR	5′-AGCGTACGGCACCGAAGATAACTC
pRSETA	lscQK	5′-CGGGTACCAGATGTCTAATCTGGGG
	lscQE	5′-CGGAATTCTTAGTCCCGTATATGATT

Isolate or plasmid	Description	Source or reference
pRSETA		Invitrogen
pMPAV6	pRSETA::lscQ	This study
TOP10	*E. coli* cloning isolate	Invitrogen
AAEC189	*E. coli* cloning isolate, Δ*lac recA endA*	(3)
LR189/83	*L. intracellularis* isolate	UK isolate
LI916/91	*L. intracellularis* isolate	UK isolate
LI963/93	*L. intracellularis* isolate	UK isolate

[a] Primers used for DOP-PCR analysis.