Applied and Environmental Microbiology, July 2007, p. 4359–4364 Vol. 73, No. 13
0099-2240/07/$08.00+0 doi:10.1128/AEM.00258-07
Copyright © 2007, American Society for Microbiology. All Rights Reserved.

Use of Selective Capture of Transcribed Sequences To Identify Genes Preferentially Expressed by *Streptococcus suis* upon Interaction with Porcine Brain Microvascular Endothelial Cells[▽]

Nahuel Fittipaldi,[1,2] Marcelo Gottschalk,[1,2] Ghyslaine Vanier,[1,2] France Daigle,[1,3] and Josée Harel[1,2]*

Groupe de Recherche sur les Maladies Infectieuses du Porc and Centre de Recherche en Infectiologie Porcine[1] and Faculté de Médecine Vétérinaire,[2] Université de Montréal, St-Hyacinthe, Quebec J2S 7C6, Canada, and Département de Microbiologie et Immunologie, Université de Montréal, Montreal, Quebec H3C 3J7, Canada[3]

Received 31 January 2007/Accepted 29 April 2007

By using the selective capture of transcribed sequences (SCOTS) approach, we identified 28 genes preferentially expressed by the major swine pathogen and zoonotic agent *Streptococcus suis* upon interaction with porcine brain microvascular endothelial cells. Several of these genes may be considered new *S. suis* candidate virulence factors. Results from this study demonstrate the suitability of SCOTS for the elucidation of gene expression in streptococcal species and may contribute to a better understanding of the pathogenesis of *S. suis* infections.

Streptococcus suis is a gram-positive bacterium responsible for, among other diseases, meningitis and septicemia in swine (14). *S. suis* is also a zoonotic agent. Many cases of human *S. suis* infection have been reported in different Asian and European countries, as well as in New Zealand, Australia, Argentina, and Canada (25). Very recently, the first case of human meningitis caused by *S. suis* was recorded in the United States (43). Indeed, *S. suis* is increasingly becoming a public health concern. For instance, during a recent outbreak in China more than 200 cases of human *S. suis* infection were reported, 39 of which resulted in death (33, 45). Despite increasing research in recent years, knowledge of the pathogenesis of *S. suis* infection remains limited (11, 14). Only the capsular polysaccharide and a recently described serum opacity-like factor have been shown to play a critical role in the pathogenesis of the infection (3, 14). Proposed putative virulence factors such as the suilysin, the extracellular protein factor, and the muramidase-released protein, although associated with virulence, have been found to be nonessential factors (6, 14). Other determinants, such as a fibronectin/fibrinogen-binding protein, were found to be partially involved in virulence (6, 14), while the actual roles of some other virulence candidates (e.g., the cell wall and several putative adhesins and proteases) in the pathogenesis of *S. suis* infection remain to be verified (11, 14).

S. suis needs to attain the central nervous system (CNS) in order to cause meningitis in swine. It has been suggested that this pathogen might reach the CNS by crossing the porcine blood-brain barrier (BBB) by transcytosis through porcine brain microvascular endothelial cells (PBMEC) and/or porcine choroid plexus epithelial cells, as well as by disruption of the barrier caused by toxic effects on BBB-forming cells (11, 36). Support for these mechanisms has been provided by recent studies showing that *S. suis* is able to affect the viability of porcine choroid plexus epithelial cells through necrotic and apoptotic mechanisms (37) and to adhere to and invade in vitro-cultured PBMEC (38). However, little is known about the molecular means by which *S. suis* accomplishes these processes.

Selective capture of transcribed sequences (SCOTS) is a PCR-based RNA analysis method that offers several advantages in comparison to other genomic approaches, such as in vivo expression technology (IVET) or signature-tagged mutagenesis (29). In fact, SCOTS directly identifies bacterial genes rather than promoter regions and is not confounded by polar effects when genes are arranged in polycistronic operons (29). The SCOTS approach has been used with success in many gram-negative bacteria, as well as in *Mycobacterium tuberculosis* and *Listeria monocytogenes* (5, 10, 13, 22). In this work, we used the SCOTS approach to identify genes preferentially expressed by *S. suis* during its interactions with cells of the BBB, a process that might be essential for the pathogenesis of the meningitis caused by this pathogen.

Experimental model and bacterial transcriptome recovery. *S. suis* serotype 2 highly virulent strain 31533 (38) and the PBMEC line PBMEC/C1-2 (34) were used in this study. PBMEC were grown in Primaria six-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ) with IF culture medium (a 1:1 mixture of Iscove's modified Dulbecco's and Ham's F-12 media; Invitrogen, Burlington, Ontario, Canada) as previously described (38). *S. suis* was grown in Todd-Hewitt broth (Becton Dickinson, Sparks, MD) for 16 h at 37°C, harvested by centrifugation, washed twice in phosphate-buffered saline (pH 7.3), and resuspended in fresh IF culture medium at 10^6 CFU/ml. Confluent monolayers of PBMEC (at 3×10^6 cells per well) were inoculated with 3 ml of this bacterial suspension (multiplicity of infection = 1). Plates were centrifuged at $800 \times g$ for 10 min and incubated for 4 h at 37°C with 5% CO_2. After incubation, actual *S. suis* adhesion to and invasion of PBMEC were verified in selected wells and found to be in agreement with reported values (38; data not shown). For identification of

* Corresponding author. Mailing address: GREMIP, Faculté de Médecine Vétérinaire, Université de Montréal, CP 5000, St-Hyacinthe, Quebec J2S 7C6, Canada. Phone: (450) 773-8521, ext. 1-8233. Fax: (450) 778-8108. E-mail: josee.harel@umontreal.ca.
▽ Published ahead of print on 4 May 2007.

TABLE 1. Oligonucleotide primers used in this study

Primer	Sequence (5′–3′)
AROA-F	AACGTGACCTACCTCCGTTG
AROA-R	CGGTCATCGTAGAATTCGAGT
CELL-RNA	ACACTCTCGAGACATCACCGGTACCN NNNNNNNN
MOCK-RNA	CTTAGCCACTACGTGCGGATCCAGAC NNNNNNNNN
RDNA-F	GGCTCAGGACGAACGCTG
RDNA-R	GCTAAGCGACTACCGTATCT
MOCK-PCR	CTTAGCCACTACGTGCGGATCCAGAC
CELL-PCR	GACACTCTCGAGACATCACCGGTACC
RPOD-F	TCTTTCAAATACATGCGGACTG
RPOD-R	ATTCCATTTACGCTTGATGCTG
SSU0424-F	AATCAAAGATTGGACGAGCC
SSU0424-R	CAATCCATCCCAATTCAGACAG
SSU0870-F	GGTATCATGAATACGGACGAAG
SSU0870-R	GAATGGATGGGCAATGAGAG
SSU0067-F	ATCAATCATCAAGGGATGCG
SSU0067-R	GATAGCCACCTCTTTTTCCAC
SSU1448-FQ	TTCTCTCTGTACTTGCTCCC
SSU1448-RQ	GGTCGCTCTAACCTTTGATG
SSU0457-F	ACCCAGATAGCCACTATTCC
SSU0457-R	CTGATCATAAGTGAAGTCGCC
SSU0597-F	TGCGTCTGGTTAAGACTTTG
SSU0597-R	GTTCTTGCCCAGCTTTTTTTC

the genes transcribed during interaction, total RNA from *S. suis*-infected PBMEC cells was prepared from 24 independent P6 wells with RNAwiz (Ambion, Austin, TX) according to the manufacturer's instructions. Total RNA from *S. suis* grown under the same conditions but without cells (mock infection) was prepared from five P6 wells. Samples were treated with TURBO DNase (Ambion), and absence of contaminating DNA was verified by PCR with primers AROA-F and BA9 (Table 1), which target the *aroA* gene. RNA was quantified by measurement of absorbance at 260 nm, and its integrity was verified by visualization on 1% denaturing agarose gels.

Selective capture of transcribed sequences. Five micrograms of total RNA prepared from both infected and mock-infected samples was reverse transcribed by random priming with SuperScript II (Invitrogen). Primer CELL-RNA or MOCK-RNA, with a defined terminal sequence at the 5′ end and a random nonamer at the 3′ end, was used (Table 1). Thereafter, cDNA sequences corresponding to bacterial mRNAs were selectively captured from the mixture of total PBMEC-*S. suis* RNAs or total *S. suis* RNAs by performing three rounds of SCOTS as previously described (5). Briefly, samples were normalized by self-hybridization and then hybridized overnight at 68°C to biotinylated genomic *S. suis* 31533 DNA that had previously been blocked with PCR-generated DNA representing 16S and 23S *S. suis* rRNA sequences (primers RDNA-F and RDNA-R, Table 1). Bacterial cDNAs were then separated with streptavidin-coupled magnetic beads. After elution, cDNAs were PCR reamplified with primer CELL-PCR or MOCK PCR (Table 1), which corresponds to the defined sequence added during reverse transcription and is specific to each condition. Sequences preferentially transcribed by *S. suis* upon interaction with PBMEC were obtained after three rounds of enrichment carried out as previously described (5). Briefly, cDNAs obtained during PBMEC interaction were subjected to the procedure outlined above, but this time the biotinylated genomic *S. suis* DNA used for capture had previously been prehybridized with DNA sequences corresponding to 16S and 23S *S. suis* rRNAs and cDNAs from the mock infection. The resulting interaction-specific cDNAs were cloned into vector pCR4 (TOPO TA cloning kit; Invitrogen) and sequenced. DNA sequences were determined at the DNA Sequencing Facility of the University of Maine (Orono) on a 373A DNA Sequencing System (Applied Biosystems, Foster City, CA).

Identification of preferentially expressed genes. The BLAST software package was used to determine sequence homologies in the GenBank databases (http://www.ncbi.nlm.nih.gov/BLAST/). Sequence comparison was also performed against sequence data produced by the *S. suis* Sequencing Group at the Sanger Institute (http://www.sanger.ac.uk/Projects/S_suis) for European strain P1/7 and at the Joint Genome Institute Microbial Genomics website (http://genome.jgi-psf.org/cgi-bin/runAlignment?db=strsu&advanced=1) for North American strain 89-1591. We report here the identification, by SCOTS, of 28 genes as being preferentially expressed by *S. suis* upon interaction with PBMEC. These genes can be divided into the following eight functional groups: metabolism/housekeeping, cell envelope, secreted proteases, cell division/replication, regulatory, protein sorting- and transport/binding-related genes, and genes with unknown function. To the best of our knowledge, none of the identified genes has ever before been associated with the pathogenesis of *S. suis* infection. Similar to other studies of host-pathogen interaction (2, 5, 31), most of the genes identified by SCOTS in this study are putatively involved in metabolic/housekeeping functions and do not encode "genuine" virulence factors. However, identification of these genes may be of importance, since new information about the metabolism of *S. suis* is rendered that may eventually prove useful for vaccine development. On the other hand, some genes identified by SCOTS in this study are known to be important for the virulence of other gram-positive bacteria (including at least three different streptococcal species). The relevance of these genes will be discussed below. For all of the other genes identified in this study, putative functions and references to publications describing their in vivo expression and/or involvement in virulence in other organisms are listed in Table 2.

Validation of SCOTS results by q-PCR. The SCOTS approach, as used in this study, should result in the identification of genes that are upregulated by *S. suis* upon interaction with PBMEC (5). Therefore, to validate our SCOTS results, we used quantitative PCR (q-PCR) to measure the level of expression of random selected genes on a new series of infection replicates. Infection of PBMEC, mock infections, and RNA extraction from both samples were performed as described above. cDNAs were synthesized in triplicate by using SuperScript II with random hexamers (Roche, Laval, Quebec, Canada). q-PCR was performed by using the QuantiTect SybrGreen PCR kit (QIAGEN, Mississauga, Ontario, Canada) according to the manufacturer's instructions. For each sample, a no-reverse transcription reaction was run as a control. The primers used are described in Table 1. For each q-PCR run, the calculated threshold cycle (C_T) was normalized to the C_T of the internal control *rpoD* gene amplified from the corresponding sample, and the *n*-fold change was calculated by the $2^{-\Delta\Delta C_T}$ method as previously described (23). Results of q-PCR analysis

TABLE 2. Genes identified by SCOTS that are differentially expressed by *S. suis* upon interaction with PBMEC

Function and clone	Gene[a]	Putative function (organism)	GenBank identification	Reference(s)
Metabolism/housekeeping				
D3C3	ssu0707	Putative exonuclease RexB (*S. suis* 89/1591)	ZP_00874950	
2H6	ssu0767	1-Phosphofructokinase (*S. suis* 89/1591)	ZP_00875124	24
2G7	ssu1411	Aminotransferase, classes I and II (*S. suis* 89/1591)	ZP_00875661	
D3G2	ssu1527	Aminodeoxychorismate lyase-like protein (*S. suis* 89/1591)	ZP_00876086	
1B7	ssu1444	Uridine kinase (*S. thermophilus* CNRZ1066)	AAV_62804	
D1B7	ssu0844	Haloacid dehalogenase-like hydrolase (*S. suis* 89/1591)	ZP_00874652	
1G10	ssu1044	Ribonucleoside diphosphate reductase (*S. suis* 89/1591)	ZP_00875241	
D1H10	ssu1159	Ribosome recycling factor (*S. suis* 89/1591)	ZP_00875081	
1C7	ssu0870	Nucleotidyltransferase (*S. suis* 89/1591)	ZP_00874394	12
2F10	ssu0871	Glucose-1-phosphate adenylyltransferase (*S. suis* 89/1591)	ZP_00874395	
1A11	ssu0764	tRNA (guanine-N1-)-methyltransferase (*S. suis* 89/1591)	ZP_00875121	
Cell envelope				
2A11	ssu0597	Membrane-bound O-acyl transferase, DltB (*S. suis* 89/1591)	ZP_00875261	12
2A8	ssu1184	D-Alanine-D-alanine ligase (*S. suis* 89/1591)	ZP_00875052	18
1H9	ssu1448	Peptidoglycan polysaccharide deacetylase PgdA (*S. suis* 89/1591)	ZP_00876135	40, 41
1E9	ssu1487	VanZ-like protein (*S. suis* 89/1591)	ZP_00875572	12
D1G11	ssu1114	Glycosyltransferase, group 1 (*S. suis* 89/1591)	ZP_00875224	
Regulatory				
D2D4	ssu0869	Putative transcriptional regulator, LysR family (*S. pneumoniae* TIGR4)	AAK74821	
Protein sorting				
1C11	ssu0424	Signal peptidases S24, S26A, and S26B (*S. suis* 89/1591)	ZP_00875273	
1B8	ssu0453	Sortase-like protein SrtE (*S. suis*)	BAB83972	29
Secreted protease				
D1E9	ssu0457	Collagenase-peptidase U32 (*S. suis*)	BAB83975	
Cell division/replication				
1G5	ssu0007	DNA polymerase III, β chain (*S. suis* 89/1591)	ZP_00875475	
2F4	ssu0432	Cell division protein FtsQ/DivIB (*S. suis* 89/1591)	ZP_00876117	12
Transport/binding				
D1H3	ssu1787	Multidrug ABC transporter, ATP-binding protein (*B. cereus* E33L)	AAU18528	
D3G1	ssu1023	Putative permease (*S. suis* 89/1591)	ZP_00874974	
Unknown function				
D1G1	ssu0067	Protein of unknown function DUF925 (*S. suis* 89/1591)	ZP_00876271	
2F6	ssu1424	Hypothetical protein (*S. suis* 89/1591)	ZP_00875489	
D1H2	ssu0858	Protein of unknown function UPF0153 (*S. suis* 89/1591)	ZP_00875788	
D1A3	ssu1792	Conserved hypothetical protein (*S. suis* 89/1591)	ZP_00876058	

[a] Genes are named in accordance with the *S. suis* strain P1/7 sequencing project nomenclature.

for these selected genes showed that they were indeed upregulated by *S. suis* upon interaction with PBMEC (Fig. 1), with changes ranging from 2.18- to 10-fold. The gene *aroA*, which is known to be expressed in equal amounts under both conditions (our unpublished results), was also used.

Genes involved in cell envelope modification. As stated above, some genes identified by SCOTS might be considered, on the basis of their functions in other organisms, potential *S. suis* candidate virulence factors. For instance, the ssu0597 gene (*dltB*) belongs to an operon comprising four genes, *dltA*, *dltB*, *dltC*, and *dltD*, which is present in all of the genomes of low-G+C bacteria determined so far (28). In all of the species where this operon has been studied, all four of the genes are required to catalyze the incorporation of D-alanine residues into the lipoteichoic acids (LTAs). D-Alanylation of LTAs allows gram-positive bacteria to modulate their surface charge, to regulate ligand binding, and to control the electromechanical properties of the cell wall (28). In addition, formation of D-alanyl-LTAs is required to resist the action of antimicrobial peptides in *L. monocytogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* (group A *Streptococcus*), and *Streptococcus agalactiae* (group B *Streptococcus* [GBS]) (1, 20, 21, 30, 42). Besides, the virulence of mutants deficient in D-alanylation of LTAs of GBS, *L. monocytogenes*, or *S. aureus* was severely impaired in the murine or rabbit model of infection (1, 30, 42). The D-alanylation of *S. suis* LTAs has not been documented. However, it is known that wild-type *S. suis* LTAs are important for adhesion of this bacterium to PBMEC. Indeed, inhibition of the adhesion of *S. suis* to this cell type can be obtained by preincubation of PBMEC with purified LTA (39). From our SCOTS results, it might be hypothesized that *S. suis* might be able to modulate the degree of D-alanylation of its LTAs by upregulation of its *dlt* operon upon interaction with PBMEC. Further studies focusing on

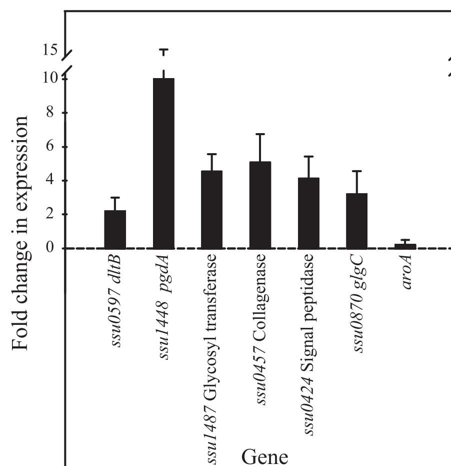

FIG. 1. *n*-Fold changes in the expression of selected *S. suis* genes identified by SCOTS as measured by q-PCR upon interaction of the bacterium with PBMEC. See the text for details.

this operon of *S. suis* are under way to evaluate this hypothesis. However, it is interesting that in some gram-positive pathogens it has been shown that D-alanyl-LTAs contribute to adhesion to and invasion of various cell lines and that these steps may depend on a high ratio of D-alanine to glycerol/ribitol phosphate in their LTAs (1, 21, 30, 42).

The main clinical feature of *S. suis* is meningitis, and this bacterium is often isolated from the cerebrospinal fluid of animals or patients with meningitis (14). On the other hand, it has been reported that patients suffering from meningitis present increased titers of lysozyme in their cerebrospinal fluid (19). As shown in this study, *S. suis* differentially expresses a gene (ssu1448) highly homologous to *S. pneumoniae pgdA*, which encodes a peptidoglycan *N*-acetylglucosamine deacetylase. Peptidoglycan is an essential component of the bacterial cell wall and an important target for the innate immune system. Peptidoglycan modification by deacetylation seems to be important for gram-positive pathogens. Indeed, pneumococci in which *pgdA* was inactivated became hypersensitive to the action of lysozyme (41) and showed reduced virulence in a murine model of infection (40). In addition, it has very recently been reported that a *pgdA* mutant strain of *L. monocytogenes* was impaired in the ability to induce disease in the murine model of infection and that the *pgdA* gene was required by this species to resist the host innate immune response mediated by lysozyme (4). In this regard, it may be of interest to further evaluate the hypothesis that *S. suis*, through the action of the *pgdA* gene product, has the ability to modify its peptidoglycan by deacetylation and therefore resist a host innate response mediated by this enzyme. On the other hand, it is intriguing that in our in vitro model, where the immune response of the host would not be as relevant as in the in vivo situation, the *pgdA* gene was found to be highly upregulated. However, it has been proposed that, in vivo, *S. suis* might gain access to the CNS by transcytosis across PBMEC (38). It might therefore be plausible that during its interaction with PBMEC, in addition to genes required for adhesion to and invasion of these cells, *S. suis* also upregulates genes required for the steps immediately following the BBB crossing. Further studies are required to evaluate this hypothesis.

Identification of a putative pilus island in *S. suis*. Pili in several gram-positive bacteria have recently been described, and it has been proposed that they may play an important role in virulence (35). For instance, in GBS, pili participate in adhesion to human epithelial cells (7) and their role in adhesion to extracellular matrix (ECM) proteins has been suggested (27). In this work, we identified a gene (ssu0424) putatively encoding a signal peptidase homologous to the LepB-type signal peptidases of gram-negative bacteria. A homologous LepB-type signal peptidase is the first gene in GBS pilus island 2b (PI-2b), one of the three identified pilus islands in this species (35). GBS PI-2b contains five other downstream genes, encoding two LPXTG proteins (suggested to be an ancillary protein and the main pilus subunit), a class C sortase, a third LPXTG protein (ancillary protein), and a second class C sortase (35) (Fig. 2). The presence of thin, pilus-like structures on the surface of *S. suis* has been revealed by electron microscopy (15). Interestingly, analysis of data from the two *S. suis* sequencing projects strongly suggests that *S. suis* possesses a truncated version of this pilus island. In fact, in sequenced *S.*

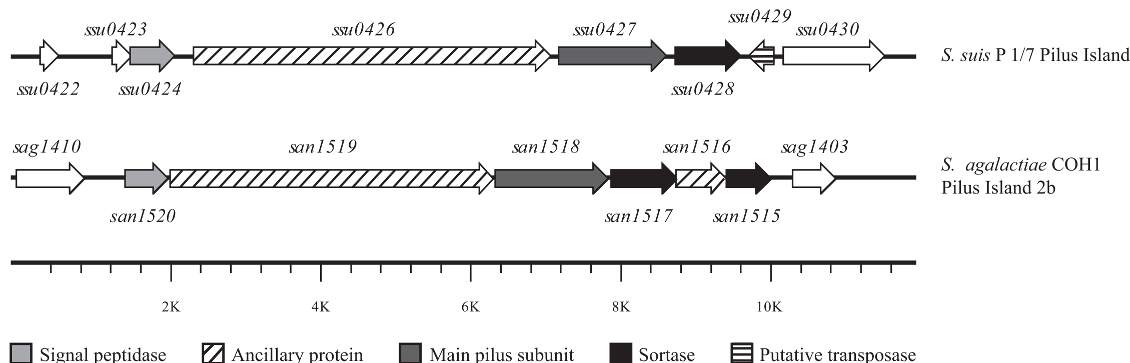

FIG. 2. Putative pilus island in *S. suis* (top) and its counterpart, PI-2b, found in *S. agalactiae* strain COH1 (bottom). In *S. suis*, the first gene of the locus is a LepB-type signal peptidase (identified by SCOTS and q-PCR as upregulated upon interaction with PBMEC), which is followed downstream by the genes for a putative ancillary protein and a main pilus subunit. A previously undescribed class C sortase is encoded by the last gene of the island. *S. agalactiae* PI-2b, which is organized in a similar way, comprises an additional ancillary pilus subunit and a second class C sortase (GenBank entry NZ_AAJR01000022).

suis strains P1/7 and 89-1591, two genes encoding LPXTG proteins (highly homologous to the ancillary and main pilus subunits of GBS, respectively) and a gene encoding an undescribed putative class C sortase-like protein are found downstream of the LepB signal peptidase that was identified by SCOTS (Fig. 2). Although the *S. suis* putative pilus island lacks the last two genes in comparison to that of GBS, the similarity in genetic organization, the strong homology showed by the LPXTG proteins to the main and ancillary pilus proteins of the latter species, and the current proposed mechanism for pilus formation in gram-positive bacteria (7, 27) suggest that a pilus might be formed by the gene products of this island. In addition, we speculate that this pilus might participate in *S. suis* adhesion to or invasion of PBMEC. In fact, pili have been very recently shown to be important for GBS adhesion to and invasion of human BMEC (26). Although functional analysis of this *S. suis* putative pilus island is needed to fully evaluate this hypothesis, it is interesting that the LepB signal peptidase was found to be highly upregulated by q-PCR (fourfold change) upon interaction of *S. suis* with PBMEC (Fig. 1).

Additionally, in this study we identified the ssu0453 gene, which was previously named *srtE* and encodes one of the four class C sortases already described in *S. suis* (8, 29). In GBS and group A *Streptococcus* models of pilus assembly, class C sortases have been proposed to catalyze the covalent polymerization of the pilin subunits encoded by genes within the pilus island bearing the class C sortases (7, 27). However, previous work with *S. suis* (29), as well as analysis of sequenced strains P1/7 and 89-1591, indicates that the *S. suis srtE* gene is not flanked by genes encoding LPXTG proteins and thus does not seem to be part of a pilus island. Therefore, the putative participation of *S. suis* ssu0453/*srtE* in pilus formation following the proposed model is unlikely. However, it might be interesting to evaluate whether this sortase is required for, or contributes to, the assembly of pilin subunits encoded by the island described in this work or by other, as yet unidentified, islands.

Interaction of *S. suis* with ECM proteins. It has been shown that *S. suis* is able to interact with ECM proteins (9). *S. suis* also has the ability to degrade ECM proteins through the upregulation of metalloproteinase 9 production by human macrophages (16), which may result in tissue destruction and BBB disruption. However, the ability of *S. suis* to degrade ECM proteins directly has not been demonstrated. Interestingly, one of the genes identified by SCOTS (ssu0457) encodes a putative collagenase which, in sequenced strains P1/7 and 89-1591, is located upstream of a gene that putatively encodes a second collagenase, in an operon-like organization. It has been suggested that the impairment of BBB function during infection with different *S. suis* strains may depend on proteases produced by this pathogen (17). It is thus tempting to speculate, even if we lack evidence regarding its exact function, that upregulating the expression of the collagenase identified in this work upon interaction with PBMEC might, in vivo, be useful to increase the permeability of the BBB and therefore contribute to the migration of *S. suis* to the CNS.

Suitability of the SCOTS approach for elucidation of gene expression in *S. suis*. The SCOTS approach has been used successfully with several bacterial species (5, 10, 13, 22). To the best of our knowledge, this is the first report of its use with a streptococcal species. Results presented here clearly demonstrate that SCOTS is also suitable for the elucidation of gene expression in streptococci and particularly in organisms like *S. suis*, for which very few molecular tools exist. Indeed, with the exception of the present study, only one genomic approach has been used to study this pathogen (32). In that work, an adapted IVET approach identified several *S. suis* iron-induced and/or in vivo (porcine infection model)-expressed genes (32). However, all of the genes identified in that study were also expressed in vitro under standard laboratory growth conditions. These results can be explained by the absence of promoter sequences exclusively expressed under the conditions tested (32). However, since a plasmid-based system was used instead of an integrative promoter trap system, the results obtained might also be explained by the inability of that system to detect in vitro silent genes because of gene dose effects (32). On the other hand, with SCOTS we clearly showed condition-specific differences in *S. suis* gene expression. In fact, SCOTS may be considered the only approach available for the direct study of global differential gene expression in *S. suis*. Despite the fact that IVET and SCOTS have identified the same genes in some cases (31), there were no overlapping genes in the IVET and SCOTS *S. suis* studies. This was not surprising, however, since only a small number of genes were identified in either study and, more importantly, the experimental conditions used were essentially different. Therefore, in this study, the use of the SCOTS approach resulted in original insights into the molecular mechanisms that this pathogen might use to cross the BBB. Indeed, the identification of the 28 genes preferentially expressed upon interaction of *S. suis* with PBMEC, several of which show great potential as virulence factors, may result in a better understanding of how this pathogen causes meningitis. In addition, extending the SCOTS analysis to identify transcriptional differences at different in vivo locations (i.e., brain, heart, tonsils), as well as at different stages of infection, may lead to comprehension of the mechanisms of disease progression and provide clues to prevention.

Addendum. While this work was under revision, an article was published (44) describing the use of the signature-tagged mutagenesis approach to study genes important for the virulence of *S. suis* in a pig model of infection. The ssu0457 gene reported in the present article was also found in that study.

We are grateful to T. Sekizaki for critical reading of the manuscript. We thank P. Friedl for kindly providing the PBMEC/C1-2 cell line. We also thank D. Tremblay, S. Faucher, and M. Lamarche for helpful suggestions and M. Esgleas for assistance.

This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) grant 0680154280 and by the Canadian Research Network on Swine Infectious Diseases. N.F. and G.V. are holders of NSERC postgraduate scholarships.

REFERENCES

1. **Abachin, E., C. Poyart, E. Pellegrini, E. Milohanic, F. Fiedler, P. Berche, and P. Trieu-Cuot.** 2002. Formation of D-alanyl-lipoteichoic acid is required for adhesion and virulence of *Listeria monocytogenes*. Mol. Microbiol. **43:**1–14.
2. **Autret, N., and A. Charbit.** 2005. Lessons from signature-tagged mutagenesis on the infectious mechanisms of pathogenic bacteria. FEMS Microbiol. Rev. **29:**703–717.
3. **Baums, C. G., U. Kaim, M. Fulde, G. Ramachandran, R. Goethe, and P. Valentin-Weigand.** 2006. Identification of a novel virulence determinant with serum opacification activity in *Streptococcus suis*. Infect. Immun. **74:**6154–6162.

4. **Boneca, I. G., O. Dussurget, D. Cabanes, M.-A. Nahori, S. Sousa, M. Lecuit, E. Psylinakis, V. Bouriotis, J.-P. Hugot, M. Giovannini, A. Coyle, J. Bertin, A. Namane, J.-C. Rousselle, N. Cayet, M.-C. Prevost, V. Balloy, M. Chignard, D. J. Philpott, P. Cossart, and S. E. Girardin.** 2007. A critical role for peptidoglycan N-deacetylation in *Listeria* evasion from the host innate immune system. Proc. Natl. Acad. Sci. USA **104:**997–1002.
5. **Daigle, F., J. Y. Hou, and J. E. Clark-Curtiss.** 2002. Microbial gene expression elucidated by selective capture of transcribed sequences (SCOTS). Methods Enzymol. **358:**108–122.
6. **de Greeff, A., H. Buys, R. Verhaar, J. Dijkstra, L. van Alphen, and H. E. Smith.** 2002. Contribution of fibronectin-binding protein to pathogenesis of *Streptococcus suis* serotype 2. Infect. Immun. **70:**1319–1325.
7. **Dramsi, S., E. Caliot, I. Bonne, S. Guadagnini, M. C. Prevost, M. Kojadinovic, L. Lalioui, C. Poyart, and P. Trieu-Cuot.** 2006. Assembly and role of pili in group B streptococci. Mol. Microbiol. **60:**1401–1413.
8. **Dramsi, S., P. Trieu-Cuot, and H. Bierne.** 2005. Sorting sortases: a nomenclature proposal for the various sortases of gram-positive bacteria. Res. Microbiol. **156:**289–297.
9. **Esgleas, M., S. Lacouture, and M. Gottschalk.** 2005. *Streptococcus suis* serotype 2 binding to extracellular matrix proteins. FEMS Microbiol. Lett. **244:**33–40.
10. **Faucher, S. P., S. Porwollik, C. M. Dozois, M. McClelland, and F. Daigle.** 2006. Transcriptome of *Salmonella enterica* serovar Typhi within macrophages revealed through the selective capture of transcribed sequences. Proc. Natl. Acad. Sci. USA **103:**1906–1911.
11. **Gottschalk, M., and M. Segura.** 2000. The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. Vet. Microbiol. **76:**259–272.
12. **Hava, D. L., and A. Camilli.** 2002. Large-scale identification of serotype 4 *Streptococcus pneumoniae* virulence factors. Mol. Microbiol. **45:**1389–1406.
13. **Haydel, S. E., and J. E. Clark-Curtiss.** 2006. The *Mycobacterium tuberculosis* TrcR response regulator represses transcription of the intracellularly expressed Rv1057 gene, encoding a seven-bladed β-propeller. J. Bacteriol. **188:**150–159.
14. **Higgins, R., and M. Gottschalk.** 2005. Streptococcal diseases, p. 769–783. *In* B. E. Straw, S. D'Allaire, W. L. Mengeling, and D. J. Taylor (ed.), Diseases of swine. Iowa State University Press, Ames.
15. **Jacques, M., M. Gottschalk, B. Foiry, and R. Higgins.** 1990. Ultrastructural study of surface components of *Streptococcus suis*. J. Bacteriol. **172:**2833–2838.
16. **Jobin, M.-C., M. Gottschalk, and D. Grenier.** 2006. Upregulation of prostaglandin E2 and matrix metalloproteinase 9 production by human macrophage-like cells: synergistic effect of capsular material and cell wall from *Streptococcus suis*. Microb. Pathog. **40:**29–34.
17. **Jobin, M. C., and D. Grenier.** 2003. Identification and characterization of four proteases produced by *Streptococcus suis*. FEMS Microbiol. Lett. **220:**113–119.
18. **Jones, A. L., K. M. Knoll, and C. E. Rubens.** 2000. Identification of *Streptococcus agalactiae* virulence genes in the neonatal rat sepsis model using signature-tagged mutagenesis. Mol. Microbiol. **37:**1444–1455.
19. **Klockars, M., S. Reitamo, T. Weber, and Y. Kerttula.** 1978. Cerebrospinal fluid lysozyme in bacterial and viral meningitis. Acta Med. Scand. **203:**71–74.
20. **Kovács, M., A. Halfmann, I. Fedtke, M. Heintz, A. Peschel, W. Vollmer, R. Hakenbeck, and R. Bruckner.** 2006. A Functional *dlt* operon, encoding proteins required for incorporation of D-alanine in teichoic acids in gram-positive bacteria, confers resistance to cationic antimicrobial peptides in *Streptococcus pneumoniae*. J. Bacteriol. **188:**5797–5805.
21. **Kristian, S. A., V. Datta, C. Weidenmaier, R. Kansal, I. Fedtke, A. Peschel, R. L. Gallo, and V. Nizet.** 2005. D-Alanylation of teichoic acids promotes group A *Streptococcus* antimicrobial peptide resistance, neutrophil survival, and epithelial cell invasion. J. Bacteriol. **187:**6719–6725.
22. **Liu, S., J. E. Graham, L. Bigelow, P. D. Morse II, and B. J. Wilkinson.** 2002. Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. Appl. Environ. Microbiol. **68:**1697–1705.
23. **Livak, K. J., and T. D. Schmittgen.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta}C_T$ method. Methods **25:**402.
24. **Loo, C. Y., K. Mitrakul, I. B. Voss, C. V. Hughes, and N. Ganeshkumar.** 2003. Involvement of an inducible fructose phosphotransferase operon in *Streptococcus gordonii* biofilm formation. J. Bacteriol. **185:**6241–6254.
25. **Lun, Z. R., Q. P. Wang, X. G. Chen, A. X. Li, and X. Q. Zhu.** 2007. *Streptococcus suis*: an emerging zoonotic pathogen. Lancet Infect. Dis. **7:**201–209.
26. **Maisey, H. C., M. Hensler, V. Nizet, and K. S. Doran.** 2007. Group B streptococcal pilus proteins contribute to adherence to and invasion of brain microvascular endothelial cells. J. Bacteriol. **189:**1464–1467.
27. **Mora, M., G. Bensi, S. Capo, F. Falugi, C. Zingaretti, A. G. Manetti, T. Maggi, A. R. Taddei, G. Grandi, and J. L. Telford.** 2005. Group A *Streptococcus* produce pilus-like structures containing protective antigens and Lancefield T antigens. Proc. Natl. Acad. Sci. USA **102:**15641–15646.
28. **Neuhaus, F. C., and J. Baddiley.** 2003. A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. Microbiol. Mol. Biol. Rev. **67:**686–723.
29. **Osaki, M., D. Takamatsu, Y. Shimoji, and T. Sekizaki.** 2002. Characterization of *Streptococcus suis* genes encoding proteins homologous to sortase of gram-positive bacteria. J. Bacteriol. **184:**971–982.
30. **Poyart, C., E. Pellegrini, M. Marceau, M. Baptista, F. Jaubert, M.-C. Lamy, and P. Trieu-Cuot.** 2003. Attenuated virulence of *Streptococcus agalactiae* deficient in D-alanyl-lipoteichoic acid is due to an increased susceptibility to defensins and phagocytic cells. Mol. Microbiol. **49:**1615–1625.
31. **Rediers, H., P. B. Rainey, J. Vanderleyden, and R. De Mot.** 2005. Unraveling the secret lives of bacteria: use of in vivo expression technology and differential fluorescence induction promoter traps as tools for exploring niche-specific gene expression. Microbiol. Mol. Biol. Rev. **69:**217–261.
32. **Smith, H. E., H. Buijs, R. de Vries, H. J. Wisselink, N. Stockhofe-Zurwieden, and M. A. Smits.** 2001. Environmentally regulated genes of *Streptococcus suis*: identification by the use of iron-restricted conditions in vitro and by experimental infection of piglets. Microbiology **147:**271–280.
33. **Sriskandan, S., and J. D. Slater.** 2006. Invasive disease and toxic shock due to zoonotic *Streptococcus suis*: an emerging infection in the East? PLoS Med. **3:**e187.
34. **Teifel, M., and P. Friedl.** 1996. Establishment of the permanent microvascular endothelial cell line PBMEC/C1-2 from porcine brains. Exp. Cell Res. **228:**50.
35. **Telford, J. L., M. l. A. Barocchi, I. Margarit, R. Rappuoli, and G. Grandi.** 2006. Pili in gram-positive pathogens. Nat. Rev. Microbiol. **4:**509–519.
36. **Tenenbaum, T., R. Adam, I. Eggelnpohler, D. Matalon, A. Seibt, K. N. GE, H. J. Galla, and H. Schroten.** 2005. Strain-dependent disruption of blood-cerebrospinal fluid barrier by *Streptococcus suis* in vitro. FEMS Immunol. Med. Microbiol. **44:**25–34.
37. **Tenenbaum, T., F. Essmann, R. Adam, A. Seibt, R. U. Janicke, G. E. Novotny, H. J. Galla, and H. Schroten.** 2006. Cell death, caspase activation, and HMGB1 release of porcine choroid plexus epithelial cells during *Streptococcus suis* infection in vitro. Brain Res. **1100:**1–12.
38. **Vanier, G., M. Segura, P. Friedl, S. Lacouture, and M. Gottschalk.** 2004. Invasion of porcine brain microvascular endothelial cells by *Streptococcus suis* serotype 2. Infect. Immun. **72:**1441–1449.
39. **Vanier, G., M. Segura, and M. Gottschalk.** 2007. Characterization of the invasion of porcine endothelial cells by *Streptococcus suis* serotype 2. Can. J. Vet. Res. **71:**81–89.
40. **Vollmer, W., and A. Tomasz.** 2002. Peptidoglycan *N*-acetylglucosamine deacetylase, a putative virulence factor in *Streptococcus pneumoniae*. Infect. Immun. **70:**7176–7178.
41. **Vollmer, W., and A. Tomasz.** 2000. The *pgdA* gene encodes for a peptidoglycan N-acetylglucosamine deacetylase in *Streptococcus pneumoniae*. J. Biol. Chem. **275:**20496–20501.
42. **Weidenmaier, C., A. Peschel, V. A. J. Kempf, N. Lucindo, M. R. Yeaman, and A. S. Bayer.** 2005. DltABCD- and MprF-mediated cell envelope modifications of *Staphylococcus aureus* confer resistance to platelet microbicidal proteins and contribute to virulence in a rabbit endocarditis model. Infect. Immun. **73:**8033–8038.
43. **Willenburg, K. S., D. E. Sentochnik, and R. N. Zadoks.** 2006. Human *Streptococcus suis* meningitis in the United States. N. Engl. J. Med. **354:**1325.
44. **Wilson, T. L., J. Jeffers, V. J. Rapp-Gabrielson, S. Martin, L. K. Klein, D. E. Lowery, and T. E. Fuller.** 2007. A novel signature-tagged mutagenesis system for *Streptococcus suis* serotype 2. Vet. Microbiol. **122:**135–145.
45. **Yu, H., H. Jing, Z. Chen, H. Zheng, X. Zhu, H. Wang, S. Wang, L. Liu, R. Zu, L. Luo, N. Xiang, H. Liu, X. Liu, Y. Shu, S. S. Lee, S. K. Chuang, Y. Wang, J. Xu, and W. Yang.** 2006. Human *Streptococcus suis* outbreak, Sichuan, China. Emerg. Infect. Dis. **12:**914–920.