Identification of virulence factors of Streptococcus suis

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Publication date
2002

Citation for published version (APA):
de Greeff, A. (2002). Identification of virulence factors of Streptococcus suis. [, Universiteit van Amsterdam].
Chapter 7

Response regulator important in pathogenesis of *Streptococcus suis* serotype 2

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Accepted for publication in Microbial Pathogenesis.
Abstract

In this study, we describe the first response regulator of *S. suis* serotype 2, designated RevS. RevS was cloned and the sequence was determined. No histidine kinase was found in the vicinity of *revS*, therefore RevS was considered to be an orphan response regulator. An isogenic knock-out mutant of RevS was shown to be attenuated in colonizing the *S. suis* specific organs in a competitive assay in four piglets, indicating that RevS plays a role in the pathogenesis of *S. suis* infections. We analyzed the protein expression profiles of various fractions of the wild-type strain 10 and the mutant strain 10ΔRevS. The expression of known virulence factors of *S. suis* by wild-type and the mutant strain, was not different. However, one protein in the protoplast fraction with unknown identity was shown to be repressed by *revS* in the exponential growth phase of the mutant.
Introduction

*Streptococcus suis* is an important pig pathogen, that causes meningitis, arthritis and septicemia in piglets. The piglets often do not survive the disease (Vecht *et al.*, 1985). Occasionally, *S. suis* causes meningitis in humans too (Arends and Zanen, 1988). Thirty-five capsular serotypes of *S. suis* have been described (Staats *et al.*, 1997), of which serotypes 2 and 9 are most frequently isolated from diseased pigs in The Netherlands (Wisselink *et al.*, 2000). Control of the disease is hampered by the lack of sensitive diagnostic tools as well as protective vaccines. More insight into the proteins involved in the pathogenic process is necessary to overcome these shortages. Recently, we described a successful complementation strategy for the assessment of important factors that determine the difference in virulence between a pathogenic *S. suis* serotype 2 strain and a weakly pathogenic strain of the same serotype (Smith *et al.*, 2001b). Complementation with this factor derived from a pathogenic strain, could transform a weakly virulent strain into a highly virulent strain. However, many important virulence factors are environmentally regulated and induced at specific stages of the infection process (Mahan *et al.*, 1993). Therefore, we previously selected environmentally regulated genes of *S. suis* serotype 2 to identify more putative virulence factors (Smith *et al.*, 2001a). Two of these environmentally regulated genes are known virulence factors of *S. suis* serotype 2: the *epf* gene, encoding EF (Vecht *et al.*, 1992), and *fbps*, encoding a fibrinogen- and fibronectin-binding protein (de Greeff *et al.*, 2002).

Here, we describe the analysis of a gene that was selected *in vivo*, and *in vitro* under iron restricted conditions. This gene showed similarity in the database to AgrA of *Staphylococcus aureus* (Projan and Novick, 1997), SapR of *Streptococcus mutans* (GenBank acc. no. X65164), and several other response regulators. Response regulators are often part of two-component signal transduction systems. Pathogenic bacteria use these systems among other things for regulation of expression of virulence factors that are required for survival inside the host (Dziejman and Mekalanos, 1995).

The role of this selected response regulator (RevS) in the pathogenesis of an *S. suis* infection was also studied. In an experimental infection in piglets we found that an isogenic knock-out mutant of *revS* was attenuated compared to the wild-type strain indicating that RevS was indeed involved in the pathogenesis of *S. suis* infections in piglets. The protein expression patterns of the RevS mutant and the wild-type strain were rather similar. The *in vitro* expression of MRP, EF, FBPS and suilysin, the known virulence factors of *S. suis* was
unaffected in the RevS mutant, but another protein was found to be repressed by RevS in the exponential growth phase of the mutant.

Materials and Methods

Bacterial strains, plasmids and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *S. suis* strains were grown in Todd-Hewitt broth (Biotrading, Mijdrecht, The Netherlands) and plated on Columbia blood base agar (Biotrading), containing 6% (vol/vol) horse blood. *Escherichia coli* strains were grown in Luria Broth (Miller, 1972) and plated on Luria Broth containing 1.5% (wt/vol) agar. If required, antibiotics were added in the following concentrations: 50 μg/ml of spectinomycin (Sigma, St. Louis, Mo.) and 100 μg/ml of ampicillin (Boehringer, Mannheim, Germany) for *E. coli* and 100 μg/ml of ampicillin for *S. suis*. For the growth experiments using whole blood, heparin-blood from 2-3 week old piglets was used.

Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Relevant characteristics</th>
<th>Source/reference</th>
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<tr>
<td><strong>Strains</strong></td>
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<td><em>E. coli</em></td>
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<td>XL2-Blue</td>
<td>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F’proAB lacZΔM15 Tn10 (Tet') Amy Cam']&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Stratagene</td>
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<td><em>S. suis</em></td>
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<td>10</td>
<td>Virulent serotype 2 strain</td>
<td>Vecht et al., 1989</td>
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<tr>
<td>10ΔRevS</td>
<td>Isogenic revS mutant of strain 10</td>
<td>This work</td>
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<td><strong>Plasmids</strong></td>
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<tr>
<td>pGEM5Zf(+)</td>
<td>Replication functions pUC, Amp&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Promega Corp.</td>
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<td>pDL282</td>
<td>Replication functions of pBR322 and pVT736-1, Amp&lt;sup&gt;R&lt;/sup&gt;, Spe&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Sreenivasan et al., 1991</td>
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<tr>
<td>pIC-spc</td>
<td>pIC19R containing Spe&lt;sup&gt;R&lt;/sup&gt; gene of pDL282</td>
<td>Lab collection</td>
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<tr>
<td>pE194</td>
<td>Em&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Horinouchi and Weisblum, 1982</td>
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<tr>
<td>pIVS-E</td>
<td>Replication functions of pWVO1, Spe&lt;sup&gt;R&lt;/sup&gt;, promoterless erm gene of pE194</td>
<td>Smith et al., 2001a</td>
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<td>pIVS-25</td>
<td>pIVS-E containing 800 bp showing homology to <em>Streptococcus mutans sapR</em>/<em>Staphylococcus aureus AgrA</em></td>
<td>Smith et al., 2001a</td>
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<td>pRevS-1</td>
<td>pGEM5Zf(+) containing 2.3 kb <em>Neol</em>-NcoI fragment of RevS</td>
<td>This work</td>
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<td>pRevS-2</td>
<td>pRevS-1 in which a Spe&lt;sup&gt;R&lt;/sup&gt;- was inserted into the unique XhoI site</td>
<td>This work</td>
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<sup>a</sup>Tet<sup>R</sup>, tetracyclin resistant; Cam<sup>R</sup>, chloramphenicol resistant; Amp<sup>R</sup>, ampicillin resistant; Spe<sup>R</sup>, spectinomycin resistant; Em<sup>R</sup>, erythromycin resistant; RevS Regulator of virulence of *S. suis*.
Preparation of *S. suis* fractions. *S. suis* cells grown in Todd Hewitt broth (100 ml) were centrifuged for 30 min at 2,500 x g and the supernatant was collected. To prepare protoplast supernatant, *S. suis* cells grown in Todd Hewitt broth (100 ml) were centrifuged for 30 min at 2,500 x g and washed in 100 ml of phosphate buffered saline (PBS) (0.1 M NaCl, 33 mM Na₂HPO₄, 17 mM NaH₂PO₄, 2 H₂O; pH 7.4). The pellet was resuspended in 10 ml of protoplast buffer (30 mM Tris, 3 mM MgCl₂, 25% sucrose, 30 μg/ml of lysozym [Boehringer, Mannheim, Germany]), incubated for 1 h at 37°C, and centrifuged for 10 min at 11,600 x g. Both the supernatant and the pellet were collected. To prepare whole lysates, *S. suis* cells grown in Todd Hewitt broth (15 ml) were centrifuged for 30 min at 2,500 x g. The pellet was resuspended in 2 ml of TES-buffer (10 mM EDTA, 10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 20% sucrose) containing 10 mg/ml of lysozym, and incubated for 30 min at 37°C. Subsequently, 500 μl of 10% sodium-dodecyl sulphate was added, and the mixture was centrifuged for 10 min at 11,600 x g. The supernatant was collected.

**DNA manipulations and sequence analysis.** Routine DNA manipulations were performed as described by Sambrook *et al.* (1989). DNA sequences were determined on a 373A DNA Sequencing System (Applied Biosystems, Warrington, Great Britain). Samples were prepared by use of an ABI Prism dye terminator cycle sequencing ready reaction kit (Applied Biosystems). Sequencing data were assembled and analyzed using the Lasergene program (DNASTAR). The BLAST software package was used to search for protein sequences homologous to the deduced amino acid sequences in GenBank/EMBL database.

**Construction of a revS-knock-out mutant.** In order to construct the mutant strain 10ΔRevS, the pathogenic *S. suis* serotype 2 strain 10 (Vecht *et al.*, 1989) was electrotransformed (Smith *et al.*, 1995) with the plasmid pRevS-2 (Figure 1). In this plasmid the revS gene was inactivated by the insertion of a resistance cassette, consisting of the spectinomycin resistance gene from pDL282 (Sreenivasan *et al.*, 1991). To obtain pRevS-2, pRevS-1 (Figure 1) was digested with *XhoI*, made blunt, and ligated to the 1,200 bp Smal/EcoRV fragment from pIC-spc containing the spectinomycin-resistance cassette. The ligation mixture was transformed to *E. coli* XL-2 blue cells, and ampicillin and spectinomycin resistant colonies were selected on Luria Broth containing 6% horse blood and 1.5% (wt/vol) agar, 100 μg/ml of ampicillin and 50 μg/ml of spectinomycin. After electrotransformation of *S. suis* strain 10 with pRevS-2, spectinomycin resistant colonies were selected on Columbia agar plates containing 100 μg/ml of spectinomycin. Southern blotting and hybridization experiments using DNA probes of the revS and spc genes, and of pGEM5Zf(+), were used to select for double crossover integration events (data not shown).

**Growth rate of *S. suis* strains.** The growth rate of wild-type *S. suis* serotype 2 strain 10 and the mutant strain 10ΔRevS in a mixed culture in Todd Hewitt was determined. One individual colony of each strain derived from a fresh Columbia agar plate was inoculated in 90 ml of Todd Hewitt broth and grown for 16 h. Subsequently, both cultures were 30-fold diluted in Todd Hewitt broth and mixed in a 1 : 1 ratio. As a function of time samples were withdrawn from the mixed culture and serial dilutions were plated on Columbia agar plates containing 6% horse blood and 100 μg/ml of spectinomycin to quantify the number of wild-type and mutant bacteria. The number of mutant strain 10ΔRevS cells was determined by counting the number of grown on the selective plates inoculated in appropriate serial dilutions. The number of wild-type strain 10 cells was determined by counting the number of cfu grown on the selective plates. The growth rate assay of wild-type strain 10 and mutant strain 10ΔRevS in whole blood of piglets was determined in the same way.
**Figure 1.** Schematic representation of pRevS-1 and pRevS-2. A 2.3 kb Ncol-Ncol fragment from *S. suis* that hybridized with the selected environmentally regulated clones *ivs*-25, *iri*-1, and *iri*-3 was cloned in the Ncol-site of pGEMZf(+) , yielding pRevS-1. To create pRevS-2, the 1.2 kb spectinomycin resistance gene from pIC-Spc was inserted in the unique *XhoI*-site of revS, to construct an insertional knock-out mutant of RevS.

**Competitive infection assay in piglets.** To determine whether the virulence of wild-type strain 10 and mutant strain 10ΔRevS differed, LD₅₀ values of both strains had to be determined in series of experiments for which a large number of piglets is required. For ethical reasons this is not acceptable. To circumvent this problem the competitive infection assay in piglets as described by de Greeff *et al.* (2002) was used to compare the virulence of the isogenic mutant strain 10ΔRevS and the wild-type strain. Duration of the experiment was maximally 14 days, or until the piglets developed specific *S. suis* symptoms such as meningitis, arthritis or mortally illness. All animal experiments were approved by the ethical committee of the Institute for Animal Science and Health, in accordance with the Dutch law on animal experiments.

**Electrophoresis and protein staining.** Proteins were separated by sodium dodecyl sulphate (SDS)-poly-acrylamide gel electrophoresis by standard procedures, using 6% poly-acrylamide gels (Sambrook *et al.*, 1989). After electrophoresis, the proteins were stained with silver (Morissey *et al.*, 1981).

**Dotblot analysis.** Proteins were spotted onto nitrocellulose using a Bio Dot Apparatus (Bio-Rad Laboratories, Richmond, Calif.) according to the manufacturer’s recommendations. The nitrocellulose membrane
was put in the Bio Dot Apparatus and washed three times with 100 μl of TBS (150 mM NaCl; 50 mM Tris-HCl, pH 7.5). The protein samples were diluted 1 : 1 in SDS-loading buffer (4% SDS; 0.2% bromophenol blue; 20% glycerol; 100 mM Tris-HCl, pH 6.8). Ten μl of this mixture was applied and the membrane was washed three times with 100 μl of TBS. The blots were blocked in TBS containing 4% skimmed milk and 0.05% Tween-20 (Blotto) for 1 h. To detect specific proteins, the membrane was incubated for at least 1 h with either a polyclonal antibody against FBPS (de Greeff et al., 2002), or a monoclonal antibody against suilysin (a kind gift of dr. A. Jacobs). Detection was done with a secondary antibody conjugated with alkaline phosphatase. As a substrate, we used Nitro Blue Tetrazolium (Merck, Darmstadt, Germany)-bromochloroindolyl phosphate (Sigma, St. Louis, Mo.). All washing steps were performed in Blotto-TBS.

Nucleotide sequence accession number. The nucleotide sequence data of revS have been submitted to GenBank, in which the sequence is listed under accession no. AY125957.

Results

Cloning of a virulence associated regulator. Previously, environmentally regulated genes of S. suis were selected under different selection conditions. One of the selected genes was selected as in vivo expressed (ivs-25), and induced under iron restriction (iri-1 and iri-3), and showed similarity in the database to several response regulators (Smith et al., 2001a). Since response regulators were shown to be involved in virulence for other organisms (Dziejman and Mekalanos, 1995, Throup et al., 2000), we studied this response regulator of S. suis in more detail. A 32P labeled probe of ivs-25/iri-1 was used to identify the flanking regions from chromosomal DNA of S. suis strain 10. A 2.3 kb NcoI - NcoI fragment was detected and cloned into the unique Ncol-site of pGEM5Zf(+) yielding pRevS-1 (Figure 1). Sequence analysis of the complete insert of pRevS-1, revealed the presence of one complete ORF of 173 aa, designated RevS and an incomplete ORF of 137 aa (Figure 1). The complete ORF was preceded by a putative RBS, and by two putative -35 and -10 promoter sequences. Downstream of the complete ORF no other ORFs nor a putative transcription terminator were found. The complete ORF showed similarity in the database to sapR of Streptococcus mutans (49%), agrA of Staphylococcus aureus (51%), but the highest similarity was found to a response regulator of Streptococcus pneumoniae (61%) (Smith et al., 2001a). Compared to the homologous regulators, RevS is truncated at its C-terminal end, due to a frameshift mutation, but did contain the consensus sequence for the active site (Lukat et al., 1990, Lange et al, 1999). Upstream of RevS, an incomplete ORF was found (Figure 1) that was similar to a conserved hypothetical protein of several organisms including Streptococcus pneumoniae (AE008517), and Streptococcus pyogenes (AE006621).
Role of RevS in the pathogenesis of \textit{S. suis} serotype 2 infection. Two-component signal transduction systems have been implicated in the control of virulence in several different micro-organisms (Dziejman and Mekalanos, 1995), including \textit{S. pneumoniae} (Throup \textit{et al.}, 2000). Therefore, we tested the role of RevS in the pathogenesis of \textit{S. suis} infection. An isogenic knock-out mutant of RevS was constructed by inserting a spectinomycin resistance cassette into \textit{revS}. The growth rates of the wild-type and mutant strain in Todd Hewitt broth were compared. Figure 2 clearly shows that both strains had essentially identical growth rates. Subsequently, the virulence of the RevS mutant strain was tested in an infection experiment in piglets by comparing the virulence of the isogenic mutant strain 10ΔRevS and the wild-type strain 10 in a competitive infection assay in piglets. Four piglets (no. 74, 75, 76, and 77) were inoculated with a mixture of wild-type and mutant bacteria at an actual ratio of 2.6 : 1 (1.9 x 10^6 cfu of strain 10 and 7.2 x 10^5 cfu of strain 10ΔRevS). During the experiment, piglets developing specific \textit{S. suis} symptoms (meningitis, arthritis, or mortally illness) were sacrificed for analysis. Piglets that did not develop these symptoms were sacrificed 14 days after infection. From all piglets the ratio of wild-type and mutant bacteria in various organs was determined. Piglets no. 74 and 75 developed a severe meningitis and arthritis in at least one joint. Piglet no. 78 developed a severe arthritis with a
Figure 3. Efficacy of colonization of wild-type and mutant bacteria on various organs of infected pigs. Panel A depicts colonization of wild-type strain 10 and mutant strain 10ΔRevS on the tonsils. Panel A: • tonsil piglet no. 74; ■ tonsil piglet no 75; ▲ tonsil piglet no 76; ● tonsil piglet no 77. Panel B depicts colonization of the specific organs. ◇ joints piglet no 74; ■ CNS piglet no 74; ▲ joint piglet 75; ● CNS piglet 75; * joint piglet 76; × pus joint 77; ○ CNS piglet no 77. Each dot represents the numbers of wild-type or mutant bacteria isolated from one particular organ, from one piglet.

purulent exsudate, and at the post-mortem section a starting meningitis was diagnosed. Piglet no. 77 survived until the end of the experiment, but at the post-mortem section, an arthritis was observed. As shown in Figure 3A, wild-type and mutant bacteria were reisolated from the
tonsils in a ratio of 1.6 : 1 (wild-type : mutant). This is very similar to the input ratio of 2.6 : 1, indicating that both strains colonized the tonsil with the same efficiency. Figure 3B depicts the colonization ratio for both strains in the specific organs. The wild-type strain colonized specific organs at a higher degree than the mutant strain. In addition, the numbers of wild-type bacteria isolated from organs were considerably higher than the numbers of mutant bacteria. This finding shows that the mutant strain 10ΔRevS was less capable of infecting the specific organs than the wild-type strain 10, indicating that the mutant strain was attenuated in colonizing the organs specific for an S. suis infection.

**Whole blood growth assay.** To determine whether the attenuation of the RevS mutant was caused by decreased multiplication in blood, we compared the growth of the mutant and the wild-type bacteria as mixed cultures in whole blood. Both *in vitro* grown strains as well as strains reisolated from *S. suis* specific organs after animal passage were grown in heparin blood obtained from 2-3 weeks old SPF-piglets. The numbers of wild-type and mutant bacteria were determined as a function of time. For 8 hours growth rates of wild-type and mutant bacteria, irrespective of the origin of the strains, were not different (data not shown).

**Protein expression profiles.** Pathogenic bacteria use two-component signal transduction systems to regulate expression of essential virulence factors (Dziejman and Mekalanos, 1995). Therefore, we determined whether RevS was involved in the regulation of the known virulence factors of *S. suis*: MRP, EF, FBPS, and suilysin. Various protein fractions of both strains were tested: whole lysates, culture supernatants, protoplast supernatants, and protoplasts were included. All fractions were isolated in triplicate, both from stationary-phase cells and from exponential-phase cells. The levels of expression of MRP, EF, suilysin, and FBPS of both strains were similar (data not shown).

For *Staphylococcus epidermidis* it was shown that protein expression patterns of an *agr* deletion mutant were strongly altered (Vuong *et al.*, 2000). To test the effect of RevS on protein expression profiles of *S. suis*, profiles of strain 10ΔRevS were compared to the profiles of wild-type strain 10 on silver-stained poly-acrylamide gels. In general, no differences were found (data not shown). However, one protein in the protoplast fraction of exponentially-phase cells of the mutant strain was not present in exponential-phase cells of the wild-type (data not shown). In the stationary-phase, this protein was abundantly present in the protoplast fractions of both strains. This difference in expression of this protein was also observed in the whole lysate fraction (data not shown). These data indicate that this protein is repressed by *revS* in the exponential growth phase. The identity of this protein is yet unknown.
Discussion

We here described the cloning and characterization of a response regulator of *S. suis* serotype 2, designated RevS. Compared to other response regulators, RevS of *S. suis* was truncated at its C-terminus due to a frameshift mutation. In *vivo* co-colonization experiments in piglets demonstrated that an isogenic knock-out mutant of RevS, was attenuated in colonizing *S. suis* specific organs. This *in vivo* attenuation demonstrated that, RevS was functional in *S. suis*, and that RevS plays an important role in the pathogenesis of *S. suis* infections.

Response regulators are almost invariably part of a two-component signal transduction system, consisting of a response regulator and a histidine protein kinase. In *S. suis* neither upstream nor downstream of RevS, a histidine protein kinase was identified. Recently, for *S. pneumoniae* a response regulator, without a flanking histidine protein kinase was identified and shown to be involved in virulence (Throup *et al.*, 2000), illustrating that orphan response regulators can be functional. Sequence analysis of RevS revealed that compared to similar response regulators, RevS was truncated at its C-terminal end. The active site of a similar response regulator of *Salmonella typhimurium* consists of an acidic pocket formed by the residues D-12, D-13, and D-57 where D-57 was shown to be the phosphorylation site (Lukat *et al.*, 1990). In other organisms the position of D-12 is often replaced by a glutamic acid residue (Lange *et al.*, 1999). In RevS of *S. suis* the conserved acid pocket is formed by E-8, D-9 and D-60, where D-60 forms the tentative phosphorylation site. Two other residues that were described to be important for functionality (Lukat *et al.*, 1990, Lange *et al.*, 1999), were predicted to be present in *S. suis* at positions T-90 and K-112. These two residues correspond to K-109 and T-87 of *S. typhimurium*, and are involved in an activation event subsequent to phosphorylation (Lukat *et al.*, 1990), and crucial for proper function of the regulator in general (Lange *et al.*, 1999) respectively. Based on these results we suggest that RevS could be active.

The functional activity of RevS was confirmed by the finding that the revS mutant was attenuated *in vivo* in an experimental infection in piglets, compared to the wild-type strain. The virulence was determined in a competitive co-colonization experiment with the wild-type strain in piglets. Ideally, virulence of mutants is studied in an LD$_{50}$ test. For ethical reasons, we were unable to do this kind of experiments in piglets. Previous studies have shown that co-colonization experiments form a good alternative to study the virulence of strains (de Greeff *et al.*, 2002, Fuller *et al.*, 2000). The data showed that the RevS mutant colonized the tonsils...
of the piglets very efficiently, indicating RevS is not involved in tonsil colonization. The mutant strain infected the specific organs less effectively than the wild-type strain; less organs were infected, and lower amounts of mutant bacteria were reisolated from these organs. Therefore the revS mutant is attenuated in colonizing the S. suis specific organs. One possible mechanism for this attenuation could be the inability of the mutant to multiply efficiently in the blood-stream. Unfortunately, we were unable to detect differences in growth between wild-type and revS mutant strain in whole blood in vitro. Therefore, it seems likely the attenuation of the mutant is caused at the stage of colonization of the specific organs. It has been described that response regulators are often involved in the regulation of expression of virulence factors (Dziejman and Mekalanos, 1995). Since RevS is a response regulator, the effect on virulence is most probably caused by an effect of RevS on the regulation of expression of virulence factors. In S. suis, we observed no differences in level of expression of the known virulence factors, MRP, EF, suilysin and fibronectin- and fibrinogen binding protein, between the wild-type strain and the RevS mutant. For S. pneumoniae response regulators were suggested to be involved in key processes of pathogenesis, such as adherence, autolysis, and cell-cell signaling (Throup et al., 2000). RevS could regulate similar processes in S. suis.

When overall protein profiles of mutant and wild-type strain were compared, one protein was found to be repressed by RevS. In the wild-type strain, the protein was absent in fractions of the exponential growth phase, and present in fractions of the stationary growth phase. In the mutant strain, this repression was switched off, and the protein was present in both growth phases. The identity of this protein is unknown. Two possible explanations can be envisaged for the lack of induction/repression of protein expression. Firstly, since RevS was selected under in vivo and iron-restricted conditions (Smith et al., 2001a), it is possible that the effects of RevS on protein expression profiles can only be detected under these conditions. Secondly, we may have missed these proteins since we analyzed the protein profiles after separation on 1D gels. Using this system, only a limited number of proteins can be discriminated. Therefore, in future experiments, 2D analysis of protein fractions of mutant and wild-type strains, preferably grown under in vivo (-like) conditions, seems to be required to obtain more information about the response of RevS. This method could also be used to study the repressed protein in more detail.
Acknowledgements

We thank the people from the Animal and Laboratory Services and the people from Pathobiology of the ID-Lelystad for their assistance during the animal experiments.

References


