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Structure, Regulation, and Putative Function of the Arginine Deiminase System of *Streptococcus suis*

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Streptococcus suis **is an important cause of infectious diseases in young pigs. Little is known about the virulence factors or protective antigens of *S. suis*. Recently, we have identified two proteins of the arginine deiminase system (ADS) of *S. suis*, which were temperature induced and expressed on the streptococcal surface (N. Winterhoff, R. Goethe, P. Gruening, M. Rohde, H. Kalisz, H. E. Smith, and P. Valentin-Weigand, J. Bacteriol. 184:6768-6776, 2002). In the present study, we analyzed the complete ADS of *S. suis*. Due to their homologies to the recently published *S. gordonii* ADS genes, the genes for arginine deiminase, ornithine carbamoyl-transferase, and carbamate kinase, which were previously designated *adiS*, *octS*, and *ckS*, respectively, were renamed *arcA*, *arcB*, and *arcC*, respectively. Our data revealed that *arcA*, *arcB*, and *arcC* of the *S. suis* ADS are transcribed from an operon (*arcABC* operon). Additionally, putative ADS-associated genes were cloned and sequenced which, however, did not belong to the *arcABC* operon. These were the *flpS* gene upstream of the *arcABC* operon with homology to the *flp* transcription regulator of *S. gordonii* and the *arcD*, *arcT*, *arcH*, and *argR* genes downstream of the *arcABC* operon with high homologies to a putative arginine-ornithine antiporter, a putative dipeptidase of *S. gordonii*, a putative β-*N*-acetylhexosaminidase of *S. pneumoniae*, and a putative arginine repressor of *S. gordonii*, respectively. The transcriptional start point of the *arcABC* operon was determined, and promoter analysis provided evidence that multiple factors contribute to the regulation of the ADS. Thus, a putative binding site for a transcription regulator of the Crp/Fnr family, an ArgR-binding site, and two *cis*-acting catabolite response elements were identified in the promoter-operator region of the operon. Consistent with this, we could demonstrate that the ADS of *S. suis* is inducible by arginine and reduced O_2 tension and subject to carbon catabolite repression. Furthermore, comparing an *arcA* knockout mutant in which expression of the three operon-encoded proteins was abolished with the parental wild-type strain showed that the *arcABC* operon of *S. suis* contributes to survival under acidic conditions.**

Streptococcus suis is a major cause of meningitis, septicemia, bronchopneumonia, and sudden death in young pigs. As a zoonotic agent, *S. suis* infects humans, causing meningitis and septicemia (2, 9, 13). Very little is known about the pathogenesis, virulence factors, and protective antigens of *S. suis*. The serotype 2 polysaccharide capsule of *S. suis* is the only virulence factor proven so far (10). However, in *S. suis* 35 different capsular serotypes have been described, and virulence differs among the serotypes and strains of the same serotype. Serotype 2 *S. suis* is considered the most prevalent capsular type in diseased pigs (1, 25), and the capsule of *S. suis* serotype 2 has been shown to play an essential role in pathogenesis (46). The muramidase-released protein (52), the extracellular protein factor (52), suilysin (26, 30), adhesins (49), and the fibronectin- and fibrinogen-binding protein (17) have been described as virulence-associated factors. However, virulent strains lacking virulence factors have been isolated from diseased pigs (25), indicating that as-yet-unknown virulence factors exist.

We have previously identified two cell wall-associated proteins of *S. suis* that were induced by a temperature shift from 32°C or 37°C to 42°C (53). Amino-terminal sequence analysis of the two proteins indicated homologies to an ornithine carbamoyl-transferase (OCT) and the streptococcal acid glycoprotein from *S. pyogenes*, an arginine deiminase (AD) recently proposed as a putative virulence factor (15, 16). Cloning and sequencing of the respective genes of *S. suis*, *adiS* (80.2% homology to the *sagP* gene), and *octS* (81.2% homology to an OCT of *S. pyogenes*) and their adjacent upstream and downstream regions revealed that they were clustered together with two additional open reading frames (ORFs). The first ORF (*orf-2*) showed 59.8% homology to a gene encoding a hypothetical cytosolic protein, and the second ORF (*ckS*) showed 70.1% homology to a carbamate kinase of *S. pyogenes*. Thus, we concluded that the genes *adiS*, *octS*, and *ckS* make up the AD system (ADS) of *S. suis* (53).

The ADS catalyzes the conversion of arginine to ornithine, ammonia, and carbon dioxide and concomitantly generates 1 mol of ATP per mol of arginine consumed. The ADS is widely distributed among prokaryotic organisms, including halobacteria, *Pseudomonas* spp., *Bacillus* spp., lactic acid bacteria, and oral streptococci (7, 14, 22, 36, 43, 55). It comprises three major enzymes, the AD, the OCT, and the carbamate kinase. The genes encoding the ADS are commonly organized as an operon. In addition to the genes encoding the catalytic activities, a number of genes can be found associated with the ADS gene cluster. These include regulatory genes belonging to different families of transcriptional regulators, not-yet-characterized genes, and genes encoding putative transport proteins.

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The gene arrangement and regulation and the biological role of the ADS differ among species (3, 7, 18, 19, 22, 35, 57). In some bacteria, such as *Pseudomonas aeruginosa* and *Bacillus licheniformis*, the ADS permits growth under anaerobic conditions (18, 36). In others, such as oral streptococci (20) and *Lactobacillus sakei* (55), expression of the ADS is under the control of carbon catabolite repression (CCR) and its expression is increased in the presence of arginine (19, 55). In oral streptococci and *S. pyogenes*, the ADS seems to provide protection against acidic stress by the production of ammonia (7, 15). Furthermore, it has been shown that the AD of *S. pyogenes* is involved in adhesion to and invasion of epithelial cells (15, 37). Little is known about the regulation of the ADS in *S. pyogenes* and other pathogenic bacteria.

In this study, we characterized the ADS of *S. suis* with respect to its genetic organization and regulation and putative functions.

MATERIALS AND METHODS

Materials, bacterial strains, and growth conditions. If not stated otherwise, all chemicals were purchased from Sigma (Munich, Germany). Previously described *S. suis* strains I9841/1 (1) and 10 (46) were grown in Todd-Hewitt broth (THB; Oxoid, Wesel, Germany) and subcultured on Columbia blood agar base (Oxoid) containing 6% (vol/vol) sheep blood overnight at 37°C. For microaerophilic and anaerobic growth conditions, bacteria were inoculated into freshly autoclaved THB and cultured in jars with respective gas delivery envelopes (CampyGen or AnaeroGen; Oxoid, Wesel, Germany) overnight at 37°C. The influence of different glucose concentrations on AD activity was analyzed with bacteria grown in Trypticase soy broth (TSB; Difco) supplemented with different glucose concentrations (0.125 to 1%). To examine the influence of glucose, galactose, and arginine on AD activity, the bacteria were grown in a low-carbohydrate tryptone-vitamin (TV)-based minimal medium (5) supplemented with the respective sugar at 0.2 or 2%, with or without 1% arginine. For temperature stress experiments, *S. suis* was cultured as described previously (53).

Escherichia coli strains were grown in Luria-Bertani broth and subcultured on Luria-Bertani agar plates. If required, antibiotics were added at the following concentrations: spectinomycin at 100 μg/ml (*S. suis*) and 50 μg/ml (*E. coli*), ampicillin at 100 μg/ml (*E. coli*), and kanamycin at 25 μg/ml (*E. coli*).

DNA techniques and sequence analysis. Chromosomal *S. suis* DNA was prepared according to standard procedures as described by Sambrook and Russell (44). Plasmid preparations were performed with Plasmid Kits from Macherey and Nagel (Dueren, Germany) according to the manufacturer's instructions.

The up- and downstream regions of the *arcABC* operon were cloned by PCR and sequenced (Seqlab, Goettingen, Germany). The specific primer sequences are available on request. Sequence analyses were performed with the HUSAR server (Heidelberg Unix Sequence Analysis Resource).

RNA preparation. Total RNA was prepared by the method of Chomczynski and Sacchi (12), with some modifications. Briefly, *S. suis* cultures were stress treated for 1 h at 42°C (53) or incubated at 37°C to late exponential growth phase. Ten milliliters of each culture was chilled on ice and then centrifuged for 10 min at 14,000 × *g* and 4°C. The bacterial pellet was suspended in 600 μl denaturation buffer (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7, 0.5% Sarkosyl, 100 mM 2-mercaptoethanol) with 400 mg zirconium beads. The cells were disrupted by bead beating for 2 × 3 min with intermediate cooling on ice. Cell debris and zirconium beads were removed by centrifugation, and the supernatant was transferred into a new 2-ml polypropylene tube. The preparation was then continued exactly as described by Chomczynski and Sacchi (12). RNA was treated with DNase I (Roche, Mannheim, Germany) for 30 min at 37°C, followed by phenol extraction and precipitation.

Primer extension. The transcriptional start point of the AD operon was determined by primer extension analysis as described by Sambrook and Russell (44). Briefly, 20 μg DNase I-treated RNA from temperature-induced *S. suis* cultures was incubated with 100,000 cpm of [γ-^{32}P]dATP-labeled primer PE (5′-CTGCCTCTCATTTCCTCTTTACAT-3′) for 15 min at 58°C and reverse transcribed at 42°C for 1 h. The extension product was analyzed on a denaturing 6% polyacrylamide gel containing 6 M urea. Sequencing was performed with the Reader Sequencing Kit (Fermentas, St. Leon-Rot, Germany) according to the manufacturer's instructions with plasmid pBAD and primer PE.

cDNA synthesis and PCR. For cDNA synthesis, 2 μg of DNase I-treated total RNA from *S. suis* grown at 37°C was reverse transcribed with primers specific for *flpS* (5′-AAACATGGCTAACCGTTTCC-3′), *arcA* (5′-GTAGCAAATGGATC CCGTGT-3′), *arcC* (5′-TTTGCATGCAGAGTCATCAAT-3′), and *arcD* (5′-G CAAGATGATGGCTACACCA-3′) and 100 U Superscript II (Invitrogen, Groningen, The Netherlands) as described by the manufacturer. PCRs for gene-specific and intragenic products were performed with 2 U *Taq* polymerase (Invitrogen) and specific oligonucleotide primer pairs according to the manufacturer's instructions. Primer pair 1 (P1 [5′-GGAGTTTTGATGATAAGCAAC G-3′] and P2 [5′-AAACATGGCTAACCGTTTCC-3′]) and primer pair 2 (P3 [5′-TGGTTCGAGAGGACCAGTTT-3′] and P2) were used in combination with the *flpS* cDNA. PCRs with the *arcA* cDNA were performed with primer pair 3 (P4 [5′-ATGAGAGGAGGAAAGCGTA-3′] and P5 [5′-TCATGCTCTTTCT GTGCATCTT-3′]) and primer pair 4 (P6 [5′-GCAACTGAAGCAGGATGG-3′] and P7 [5′-CCTGCCTCTCATTTCCTCTTT-3′]). Primer pairs 4, 5 (P8 [5′-TTTGCTCAAGCTCTTCGTGA-3′] and P9 [5′-ACCTTCAGCCAAGGCAAC TA-3′]), and 6 (P10 [5′-GTACTCGTGCTGCCTTCACA-3′] and P11 [5′-CTG CCAAACATTGAGAAGCA-3′]) were used in the PCR with the *arcC* cDNA, and primer pairs 7 (P12 [5′-AAATATTATAGGAGGTTTTTGCGATG-3′] and P13 [5′-GCAAGATGATGGCTACACCA-3′]) and 8 (P13 and P14 [5′-TCATT GCAAAGGCTAAGCAG-3′]) were used in combination with the *arcD* cDNA. PCR conditions consisted of 2 min of initial denaturation at 94°C; 30 cycles of 1 min at 94°C, 1 min at 57 to 60°C, and 1 min at 72°C; and a final elongation for 10 min at 72°C.

Production and purification of recombinant proteins. Recombinant ArcA, ArcB, and ArcC were produced as His tag fusions in *E. coli* with the QIAexpress paired pREP4-pQE plasmid system (QIAGEN, Hilden, Germany). Genes were amplified with deleted translation initiation codons by PCR from chromosomal DNA with respective specific oligonucleotide primers containing restriction sites (*arcA*KpnI [5′-GGGGTACCTCAAACCATCCAATTCAT-3′], *arcA*HindIII [5′-CCCAAGCTTTTAGATGTCTTCACGTTC-3′], *arcB*KpnI [5′-GGGGTACCA CAAACGTATTTAAAGGT-3′], *arcB*HindIII [5′-CCCAAGCTTTACACACG TGGAACAAAT-3′], *arcC*BamHI [5′-CGGGATCCTGTAAGCGATTACTAT TT-3′], and *arcC*HindIII [5′-CCCAAGCTTCAACGATCATACAGCTGC-3′]). PCR products were cloned first into the pCR2.1 TA cloning vector (Invitrogen). The KpnI-HindIII *arcA* and *arcB* fragments and the BamHI-HindIII *arcC* fragments were subsequently subcloned into correspondingly digested pQE vectors and verified by sequencing (Seqlab). Resulting plasmids pQE32*arcA*, pQE30*arcB*, and pQE30*arcC* were introduced into *E. coli* strain M15(pREP4) (QIAGEN). After isopropyl-β-D-thiogalactopyranoside (IPTG) induction, proteins were overexpressed as N-terminal hexahistidyl derivates (His_6-ArcA, His_6-ArcB, and His_6-ArcC) and isolated by Ni^{2+}-nitrilotriacetic acid affinity chromatography according to the manufacturer's instructions. Polyclonal rabbit antisera against the purified proteins were obtained from Seqlab.

Construction of gene-specific knockout mutants. Inactivation of the *arcABC* operon was achieved by insertion of a spectinomycin resistance cassette into the *arcA* gene. A 2,840-nucleotide (nt) PCR fragment (positions −965 to +1875 according to the transcriptional start point) comprising the *arcA* gene and adjacent regions was amplified by PCR from chromosomal DNA of *S. suis* strain I9841/1 with oligonucleotide primers 5′-TGGTTCGAGAGGACCAGTTT-3′ and 5′-TTGTCAATGTCTGGCACCAT-3′. The PCR was performed in a total volume of 50 μl containing 100 ng chromosomal DNA of *S. suis* and 2 U *Taq* polymerase (Invitrogen) as described by the manufacturer. PCR conditions were an initial 2 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 57°C, and 3 min at 72°C; and a final elongation for 10 min at 72°C. The PCR product was cloned into the pGEM-T Easy vector (Promega, Mannheim, Germany), resulting in plasmid pGEMAD. The DNA insert was then subcloned as an ApaI-SacI fragment into the pBluescriptII SK^+ vector (Stratagene, Heidelberg, Germany), resulting in plasmid pBAD. The internal ClaI-EcoRV fragment (positions +35 to +341) was replaced with the ClaI-SmaI fragment of pICspc (39, 46) containing the spectinomycin resistance gene, resulting in pBADspc. The EcoRI fragment from plasmid pBADspc, containing the inserted spectinomycin resistance cassette and the *arcA*-adjacent regions, was inserted into the pIC vector to gain plasmid pICADspc. Electrotransformation of *S. suis* was done as described by Smith et al. (47), with 1 μg DNA of plasmid pICADspc and a Bio-Rad Gene Pulser apparatus (Bio-Rad, Munich, Germany). Pulses were performed with settings of 25 μF, 2.5 kV, and 200 Ω and a time constant of 4.5 to 4.6 ms. After electroporation, 1 ml THB–0.3 M sucrose was added to the cells. Bacteria were incubated for 2 h at 37°C and plated on agar plates containing selective antibiotics. The *arcABC* mutant strain was grown under aerobic conditions in THB–1% yeast extract (THY) and subcultured on Columbia blood agar base containing 6% (vol/vol) horse blood and spectinomycin (100 μg/ml) overnight at 37°C. Control of the *arcABC* mutant strain was performed by PCR with oligo-

nucleotide primers for *arcB* (5′-GGGGTACCACAAACGTATTTAAAGGT-3′ and 5′-CCCAAGCTTTACACACGTGGAACAAAT-3′), *arcC* (5′-CGGGATC CTGTAAGCGATTACTATTT-3′ and 5′-CCCAAGCTTCAACGATCATACA GCTGC-3′), and *spc* (5′-TGGTACCGTGGAATCATCCT-3′ and 5′-GGAGA AGATTCAGCCACTGC-3′) and primers adjacent to the insertion site of the *spc* resistance gene cassette (5′-AGCGAGAGCAGTTTGCTACC-3′ and 5′-TCAT GCTCTTTCTGTGCATCTT). The PCR was performed as described above. Southern analyses were performed with BamHI-cleaved genomic DNA according to standard protocols (44).

Immunoblot analysis. Immunoblot analysis of whole-cell lysates was performed as previously described (53), with specific anti-ArcA, anti-ArcB, and anti-ArcC antisera at a final dilution of 1:200.

Determination of AD activity. AD activity from whole-cell lysates was assayed as described previously (53). Results were expressed as nanomoles of citrulline produced per hour per milligram of streptococcal protein.

Survival of *S. suis* under acidic conditions. Experiments were performed as described previously (4). Briefly, *S. suis* cultures were grown overnight in TSB medium and harvested by centrifugation. Cells were suspended in 20 mM Na_2HPO_4–1 mM $MgCl_2$–25 mM arginine-HCl adjusted to pH 4, 5, 6, or 7. Controls were incubated at pH 4 without arginine-HCl. Suspensions were incubated for up to 4 h at 37°C. The number of viable bacteria was quantified by plating on THY. Results were expressed as percent survival compared to the CFU of the initial inoculum.

RESULTS

Genetic organization and characterization of the ADS gene cluster. While our first report on the ADS of *S. suis* was in press (53), the ADS of *S. gordonii* was published by Dong et al. (20). Subsequent sequence analysis of the *S. suis* ADS genes revealed high homologies to their analogues in *S. gordonii*. Hence, the genes for AD, ornithine carbamoyl-transferase, and carbamate kinase, previously designated *adiS*, *octS*, and *ckS*, respectively, were renamed *arcA*, *arcB*, and *arcC*, respectively. Further sequence analysis of ORF2 revealed similarities to an acetyltransferases of *S. pyogenes* and *S. agalactiae*. However, due to a weak Shine-Dalgarno box and a start codon within the AD coding sequence, we assume that this ORF is not expressed in *S. suis*. Therefore, this ORF was not assigned a specific name.

The genes of the ADS are generally organized in an operon-like structure (56). Many ADS operons are associated with genes encoding an arginine-ornithine antiporter and a transcription regulator of the Crp/Fnr or ArgR/AhrC family (3, 15, 22, 33, 35, 36) (see also Fig. 6). Therefore, we cloned and sequenced the up- and downstream flanking regions of the *arcA*, *arcB*, and *arcC* gene cluster of *S. suis*. Sequence analysis confirmed an additional ORF of 696 nt upstream of the *arcA* gene. The predicted protein sequence shared 43% identity with a transcription regulator of *S. pyogenes* (AE014160) belonging to the family of Crp/Fnr transcription regulators and also a homology of 36% to the ArcR protein of *B. licheniformis* (CAB95946). Alignment with the partial genome of *S. gordonii* (www.tigr.org) revealed the highest sequence identity to *S. gordonii flp* (64%). Analysis of the predicted amino acid sequence revealed a putative CAP effector domain in the N-terminal region of the protein and a helix-turn-helix motif in the C-terminal region, which might be responsible for DNA binding. Due to the homology to the Flp regulator protein of *S. gordonii*, this ORF was named *flpS*. Upstream of the *flpS* gene, two additional ORFs were identified; both putative proteins showed homologies of up to 78% to conserved and hypothetical proteins SP1565 (AAK756562) and spr1422 (E98049), respectively, of *S. pneumoniae* that have not been

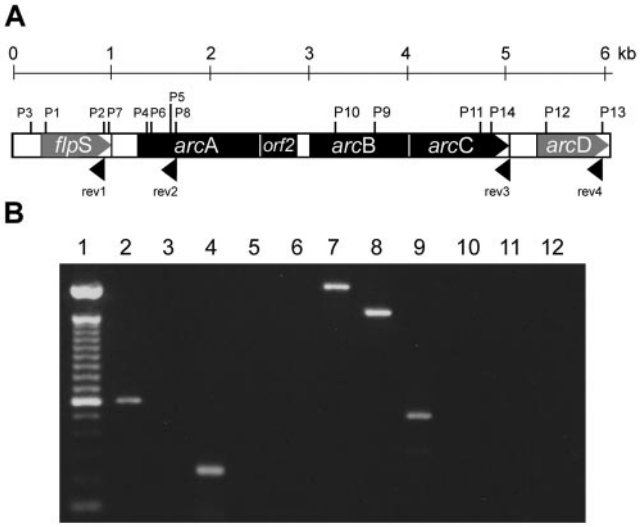

FIG. 1. The *arcABC* operon of *S. suis* consists of the *arcA*, *arcB*, and *arcC* genes. (A) Schematic representation of the positions of the primer sequences used for RT (rev1 to rev3) and the primers used for PCR (P1 to P14) as described in Materials and Methods. (B) Ethidium bromide-stained gel from the RT-PCR analysis of mRNA of *S. suis* grown in THB at 37°C. Lane 1, molecular weight marker; lane 2, *flpS* intragenic PCR fragment (603 bp, primer pair 1); lane 3, *flpS* upstream region 3′-end *flpS* PCR fragment (primer pair 2); lane 4, *arcA* intragenic PCR fragment (229 bp, primer pair 3); lanes 5 and 6, *flpS-arcA* gene intergenic PCR fragment (primer pair 4 each); lane 7, *arcA-arcB* intergenic PCR fragment (2,132 bp, primer pair 5); lane 8, *arcB-arcC* intergenic PCR fragment (1,621 bp, primer pair 6); lane 9, *arcD* intragenic PCR fragment (527 bp, primer pair 7); lane 10, *arcC-arcD* intergenic PCR fragment (primer pair 8); lanes 11 and 12, control reactions with mRNA without RT and double-distilled H_2O, respectively.

further characterized. Downstream of *arcC*, analyses revealed four further ORFs. The first one (1,502 nt) was named *arcD* due to a homology of 70% to an arginine-ornithine antiporter of *S. gordonii* (AAN65258). The second, a 1,358-nt ORF, was named *arcT* because of a homology of 67 to 71% to *arcT* of *S. gordonii* (AF534569). The third ORF, with a size of 4,259 nt, showed a homology of up to 69% to a β-*N*-acetylhexosaminidase of *S. pneumoniae* (L36923). The last ORF, 473 nt, showed a homology of 70% to *arcR* of *S. gordonii* (AF534569). Consistent with what has been described in *S. gordonii* (22), strong similarities were found to regulatory proteins involved in arginine metabolism; e.g., the pI of the first 100 amino acids was basic (9.25), while the pI of the last ones was acidic (3.82). Furthermore, a typical serine-arginine motif essential for DNA binding was found in the N-terminal region of the ArgR sequence, while conserved amino acid residues important for arginine binding were detected at positions 103 (alanine), 125, and 126 (aspartic acid). Thus, this ORF was named *argR*.

Next, we determined which genes of the ADS cluster of *S. suis* were organized in an operon. For this, RNA isolated from *S. suis* cultures grown at 37°C was reverse transcribed with oligonucleotide primers specific for the *flpS* gene, the *arcA* gene, the *arcC* gene, and the *arcD* gene (Fig. 1A). Reverse transcription (RT)-PCR analyses were performed with the respective gene-specific primer pairs. For each cDNA, at least one primer pair consisted of a gene-specific oligonucleotide primer and an additional primer pair specific for the indicated

5′ adjacent genes. As shown in Fig. 1B, all gene-specific RT-PCRs generated the expected PCR products (lanes 2, 4, 7, 8, and 9). However, only the PCR fragments for the intergenic regions between *arcA* and *arcB* and between *arcB* and *arcC* were amplified (lanes 7 and 8), indicating that these genes compose the *arcABC* operon of *S. suis*, whereas genes *flpS* and *arcD* appeared to be located outside the operon.

Analysis of the promoter region and the 5′ untranslated region of the *arcABC* operon. We determined the transcriptional start point of the *arcABC* operon by primer extension analysis with RNA isolated from temperature stress-induced *S. suis* cultures (Fig. 2A). The transcriptional start point was identified 214 nt upstream of the *arcA* start codon, which we identified previously by N-terminal sequencing (53). Sequence analysis of the promoter region (Fig. 2B) revealed a −10 region, **TATAAT**, that matched the typical consensus sequence and a −35 region, **TT**TTAT, 17 nt upstream of the −10 region which, however, shared only two bases (bold letters) with the typical consensus sequence TTGACA.

Based on the homologies of the *flpS* gene to the family of Crp/Fnr transcription regulators and to ArcR homologous proteins, we analyzed the promoter sequence for possible Crp/Fnr DNA-binding sites. A putative binding site for a transcription regulator of the Crp/Fnr family was identified at position −57.5 upstream of the transcriptional start point. This sequence, **TTGA**CAGAAA**C**TC**A**, showed homologies (bold letters) to the Fnr consensus region of *E. coli*, TTGAT-N_1-N_4-A TCAA (21).

In other bacteria, the ADS is inducible by arginine via activation through ArgR. Therefore, we investigated the region upstream of the promoter of *S. suis arcA* with regard to *E. coli* ArgR-binding sites, ANTGAATAATTATTCAN or TNTGA ATTTAAATTCAN, respectively (34). A potential binding site for ArgR (**TTTGAAATTCATATTCAA**) was identified at positions −196 to −214 from the transcriptional start point.

In oral streptococci (20) and *L. sakei* (55), expression of the ADS is under the control of CCR. In AT-rich gram-positive bacteria, CCR is mediated by *trans*-acting catabolite control protein A (CcpA). CcpA binds to *cis*-acting catabolite response elements (*cre*) in the presence of favored carbohydrate sources to repress the expression of catabolic genes and operons. Genes repressed by CcpA generally contain *cre* boxes (TGWAARCGYTWNCW) within and downstream of the promoter (28, 29, 50). We identified two potential CcpA-dependent *cre* sites at +63 (**TGTGAA**GAA**TTTCT**) and +86 (**TGTGAGCGTTTTCA**) with bases matching the consensus (bold letters) within the untranslated region of the *S. suis arcABC* operon (Fig. 2B).

Regulation of the ADS by arginine, reduced oxygen tension, and carbohydrate catabolite repression. The presence of a putative ArgR-binding site, a possible Fnr-binding site, and the two potential *cre*-binding sites suggested that the ADS of *S. suis* is transcriptionally controlled by a multitude of regulator mechanisms. Thus, we examined the regulation of the ADS by variation of growth conditions. First, we determined whether expression of the ADS is induced by arginine (ArgR regulated) and under anaerobic growth conditions (*flpS* regulated). We measured the AD activities of *S. suis* strains I9841/1 and 10 grown in TV minimal medium in the presence and absence of 1% arginine. As shown in Fig. 3A, the AD activities of both strains tested were substantially enhanced when the bacteria were grown in the presence of arginine, indicating that the ADS is inducible by arginine. In addition, we tested the AD activities of *S. suis* grown under aerobic, microaerophilic, and

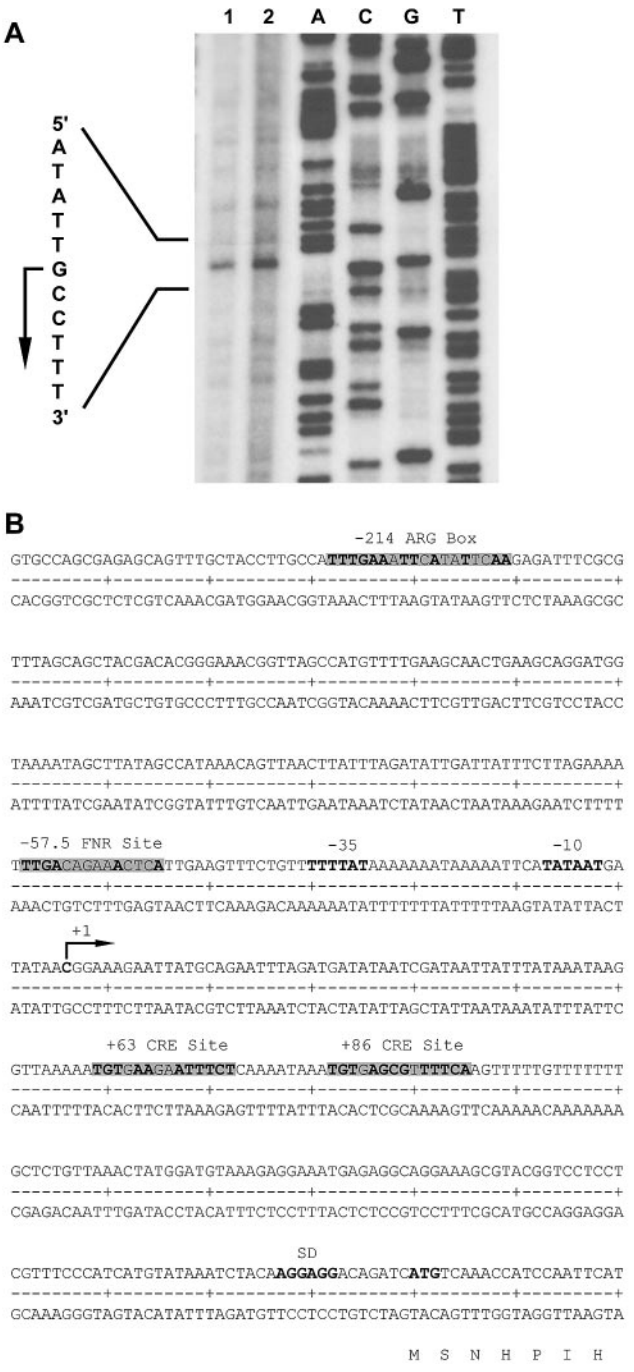

FIG. 2. Analysis of the promoter region and the 5′ untranslated region of the *arcABC* operon. (A) Primer extension experiments with 10 μg (lane 1) and 15 μg (lane 2) of heat-induced (42°C) RNA of *S. suis* strain I9841/1. (B) Sequence of the promoter region of the *S. suis arcABC* operon. The transcriptional start point (arrow), the predicted −10 and −35 boxes, the predicted ARG box, the predicted Fnr-binding site, the predicted *cre*-binding sites, and the predicted Shine-Dalgarno (SD) sequence of *arcA* are indicated.

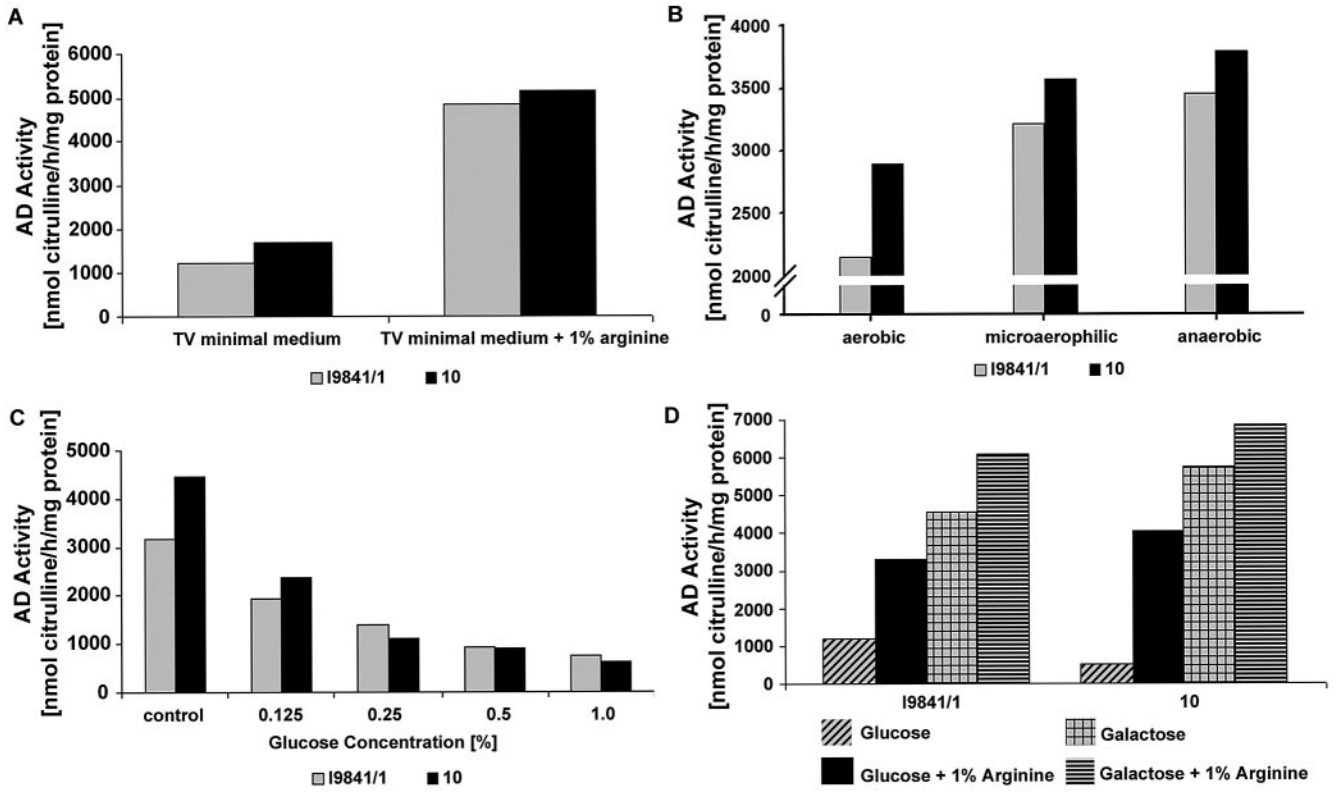

FIG. 3. Regulation of the ADS by arginine, reduced oxygen tension, and carbohydrate catabolite repression. (A) AD activities of *S. suis* serotype 2 strains grown in TV medium with or without 1% arginine (open bars, strain I9841/1; filled bars, strain 10). (B) AD activities of *S. suis* serotype 2 strains grown in THB medium under aerobic, microaerophilic, or anaerobic conditions (open bars, strain I9841/1; filled bars, strain 10). (C) AD activities of *S. suis* serotype 2 strains grown in TSB with increasing amounts of glucose (open bars, strain I9841/1; filled bars, strain 10). (D) *S. suis* serotype 2 strains grown in TV medium with or without 1% arginine and different carbohydrate sources (glucose and galactose). Results of representative experiments are shown. AD activities were determined as described in Materials and Methods. Each experiment was repeated at least twice.

anaerobic culture conditions. Results revealed that the increase in AD activity is correlated with a decrease in O_2 tension (Fig. 3B).

The presence of two potential *cre*-binding sites prompted us to examine whether the ADS of *S. suis* is subject to CCR. Thus, bacteria were grown in glucose-free TSB medium and in medium supplemented with increasing concentrations of glucose. As shown in Fig. 3C, 0.125% glucose reduced AD activity by approximately 45%. AD activities were also determined for *S. suis* grown in TV minimal medium on various other energy sources (Fig. 3D). We expected higher levels of AD activity in the presence of sugars, e.g., galactose, which were not repressive of AD expression in other streptococci (20, 27). Accordingly, higher levels of AD activity were obtained from *S. suis* cultures grown in the presence of 0.2% galactose than from cells grown in the presence of 0.2% glucose. Although the presence of glucose had a repressive effect on AD expression, addition of 1% arginine to the growth medium seemed to partially abolish this effect, which was reflected by an increase in AD activity (Fig. 3D). Supplementation of glucose or galactose to a final concentration of 2% induced CCR in *S. suis* cultures, but addition of 1% arginine seemed to abolish the effect, most notably for cultures grown in the presence of galactose (data not shown).

Role of the ADS in survival of *S. suis* under acidic conditions. Ammonium production by the ADS has been shown to protect bacteria from acidic stress (38). To study the role of the ADS of *S. suis* in an acidic environment, we constructed an *arcA* knockout mutant. The 5′ end of the *arcA* gene and its nontranslated region (positions +35 to +341 bp) were replaced with a spectinomycin (*spc*) resistance gene cassette. The resulting mutant strain was confirmed by PCR (Fig. 4A) and Southern analyses (Fig. 4B) and designated 10Δ*arcA*. Phenotypically, the mutant strain was examined via immunoblot analysis with antisera raised against the recombinant ArcA, ArcB, and ArcC proteins of *S. suis* (Fig. 4B). Whole-cell lysates of *S. suis* strains I9841/1, 10, and 10Δ*arcA* grown at 37°C were prepared and, after SDS-PAGE, blotted onto nitrocellulose membranes. The membranes were probed with anti-ArcA, anti-ArcB, and anti-ArcC antisera, respectively. As shown in Fig. 4C, the mutant strain did not express any of the proteins of the *arcABC* operon, indicating that expression of the complete operon was abolished by insertion of the *spc* resistance gene cassette. Accordingly, there was no detectable AD activity in the mutant strain compared to the wild-type strain (2,762.91 nmol citrulline/h/mg protein).

To analyze the ability of the ArcABC mutant strain to survive under acidic conditions, the mutant and parental strains

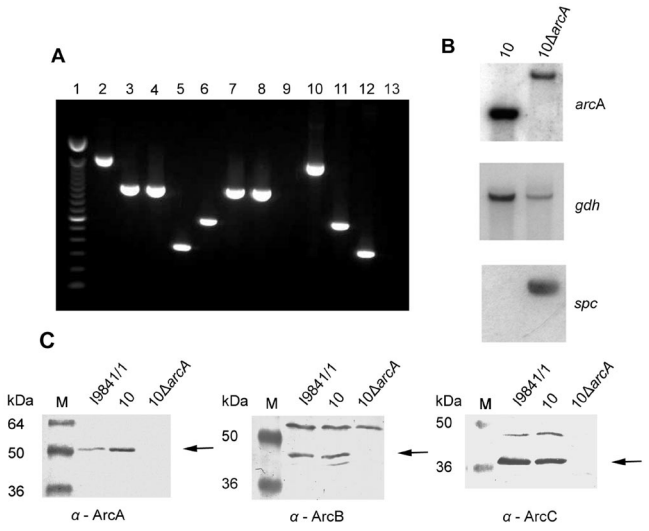

FIG. 4. Construction of an *arcA* knockout mutant. (A) Control of strain 10Δ*arcA* in comparison with wild-type strain 10 by PCR. An ethidium bromide-stained agarose gel with the PCR DNA fragments is shown. Amplification was performed with chromosomal DNAs of strain 10Δ*arcA* (lanes 2 to 5) and wild-type strain 10 (lanes 6 to 9). Δ*arcA*-containing plasmid pICADspc (lane 10), wild-type *arcA*-containing plasmid pBAD (lane 11), and spectinomycin donor plasmid pIC*spc* (lane 12) were used as positive controls, and double-distilled H_2O (lane 13) was used as a negative control. Lane 1, molecular weight marker; lanes 2, 6, and 10, amplification reaction to verify insertion of the *spc* resistance gene cassette (1,609-bp fragment, successful insertion; 614-bp fragment, no insertion); lanes 3 and 7, *arcB*-specific DNA fragment (1,039 bp); lanes 4 and 8, *arcC*-specific DNA fragment (1,027 bp); lanes 5, 9, and 12, *spc* cassette-specific DNA fragment (361 bp). (B) Southern analysis of wild-type strain 10 and mutant strain 10Δ*arcA* with probes specific for the *arcA*, *gdh* (41), and *spc* genes. (C) Immunoblot analyses with whole-cell lysates of *S. suis* strains I9841/1, 10, and 10Δ*arcA* (lane M, molecular size marker). Specific antisera against the ArcA, ArcB, and ArcC (α-ArcA to -C) proteins were used at a 1:200 dilution. The specific protein bands are indicated by arrows.

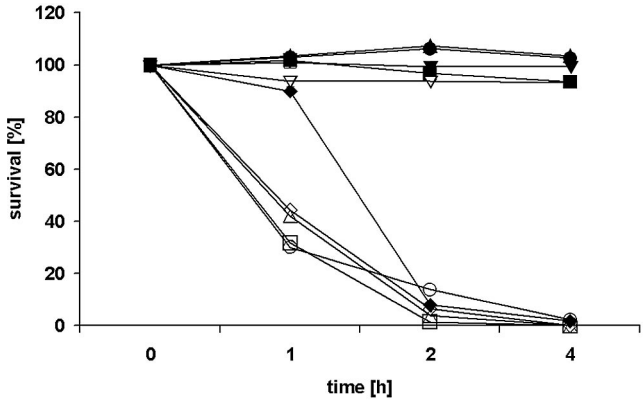

FIG. 5. Survival of *S. suis* in an acidic environment. Wild-type strain 10 (filled symbols) and mutant strain 10Δ*arcA* (open symbols) were grown to stationary phase and harvested, and equal numbers of bacteria were suspended in 20 mM Na_2HPO_4–1 mM $MgCl_2$–25 mM L-arginine–HCl at pH 4 (□), pH 5 (△), pH 6 (○), and pH 7 (▽). Buffer without L-arginine–HCl served as a control (◇). The bacteria were incubated at 37°C, and after 1, 2, and 4 h, the number of viable organisms was determined by plating on THY agar. Results are presented as percent survival (CFU count at the indicated time point compared to that of the initial inoculum). Results of a representative experiment of three are shown.

were exposed to pH values of 4, 5, 6, and 7 in the presence or absence 25 mM L-arginine, respectively. Viable streptococci were quantified by serial platings on THY agar, and results were expressed as percent survival (CFU count at the indicated time point compared to that of the initial inoculum). Both the mutant and wild-type strains were sensitive to acidic pH in the absence of L-arginine (Fig. 5). In the presence of L-arginine, the wild-type strain survived under all pH conditions tested, whereas the ArcABC mutant strain was almost completely killed after a 2-h exposure to acidic pH (pH 4 to 6; Fig. 5). The viability of the mutant strain was not affected at pH 7. The decreased viability of the ArcABC mutant strain indicated that the *arcABC* operon of *S. suis* contributes to survival under acidic conditions.

DISCUSSION

The ADS is a multienzyme pathway that catalyzes the conversion of arginine to ornithine, ammonia, and CO_2, with concomitant production of ATP (56). Due to these properties, ADS-expressing bacteria can overcome oxygen and nutrient starvation and are able to tolerate acidic environments (8, 38). Apart from the genes encoding the catalytic activities, other associated genes were found within the ADS gene cluster (Fig. 6). These genes include regulatory genes belonging to different families of transcription regulators, not-yet-characterized genes, and genes encoding putative transport proteins. Furthermore, the genes encoding the ADS are commonly organized as an operon. However, the gene arrangement, regulation, and physiological role of the ADS differs among species (Fig. 6) (3, 7, 18, 19, 22, 35, 57). The ADS is widely distributed among prokaryotes, and the primary structures of the enzymes involved in the AD pathway have been reasonably conserved throughout evolution. To date, ADS genes have been identified and sequenced in bacteria and archaea; no ADS genes or ADS enzyme activity has been reported for higher eukaryotes (56). In the amitochondriate parasitic protist *Giardia intestinalis*, the first two proteins of the ADS pathway, AD and OCT, were shown to be among 16 immunodominant proteins, underlining the importance of the ADS pathway in this parasite (42). The relevance of the ADS in pathogenic bacteria, however, has been described only for *S. pyogenes*. Here the ADS can be considered as a virulence factor. A protein originally designated streptococcal acid glycoprotein and originally reported to inhibit proliferation of human epidermoid carcinoma cells and human peripheral blood-derived mononuclear cells was ultimately identified as an AD that additionally contributed to acidic survival (15, 16, 54). However, the structure and regulation of the ADS in *S. pyogenes* have not been analyzed in detail.

Recently, we found that genes *adiS*, *octS*, and *ckS* (renamed *arcA*, *arcB*, and *arcC*, respectively) make up the ADS of *S. suis*. Interestingly, we could show that ArcA and ArcB were murein associated and that ArcA was expressed on the cell surface (53). Furthermore, we found that a natural, nonencapsulated, ArcA-deficient *S. suis* strain showed reduced survival after entry into epithelial cells compared to a natural, nonencapsu-

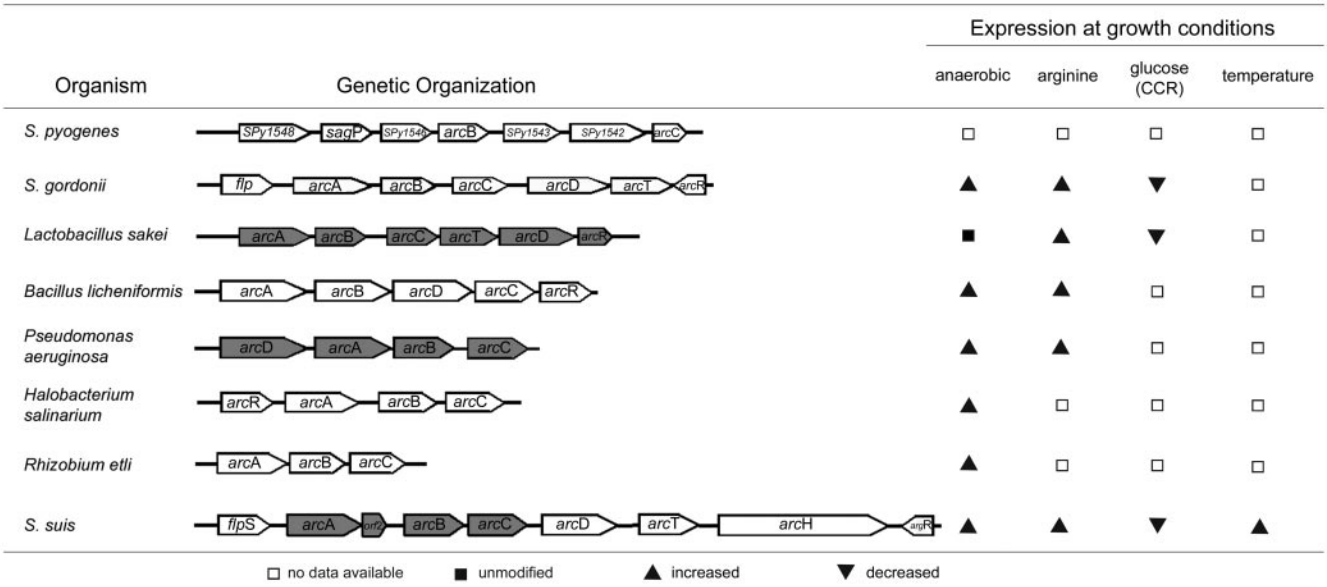

FIG. 6. Comparison of the genetic organization and regulation of known ADSs. The ADSs of *S. pyogenes* (15), *S. gordonii* (19, 20), *L. sakei* (55), *B. licheniformis* (35), *P. aeruginosa* (22), *H. salinarum* (43), *R. etli* (18), and *S. suis* are depicted. Gray gene symbols represent genes with confirmed operon structures.

lated, ArcA-expressing *S. suis* strain (4), emphasizing the putative roles of ArcA and ArcB as virulence factors of *S. suis*.

In the present study, we examined the structure and regulation of the ADS in *S. suis*. We identified an additional ORF upstream of the *arcA* gene named *flpS* with homologies to a Crp/Fnr transcription regulator of *S. pyogenes*, the ArcR protein of *B. licheniformis*, and *flp* of *S. gordonii*, which was required for expression of the ADS under anaerobic induction (19). The two additional ORFs upstream of the *flpS* gene encoded hypothetical proteins that seem not to be functionally associated with the ADS of *S. suis*. Downstream of *arcC*, further ORFs were identified which seem to be associated with the *arcABC* operon. The first one, with high homology to an arginine-ornithine antiporter of *S. gordonii*, was named *arcD* and followed by an Xaa-His dipeptidase (*arcT*) that shared high homologies with *S. gordonii arcT* (20). The putative β-*N*-acetylhexosaminidase (*arcH*), with high homology to an *S. pneumoniae* β-*N*-acetylhexosaminidase, has not been described in ADSs of other bacteria and seems to be a special feature of the ADS of *S. suis*. All these genes appear to be associated with the *arcABC* operon of *S. suis*.

Most of the ADSs are highly regulated (3, 20, 36, 57). Their genetic regulation, however, seems to be only partially conserved among organisms (Fig. 6). In *P. aeruginosa* (33) and *B. licheniformis* (35), the ADS is expressed under anaerobic conditions via regulators of the Crp/Fnr family. In all ADS-expressing bacteria, induction by Crp/Fnr can be further enhanced in the presence of arginine by ArgR, the transcriptional regulator of the arginine operon (33). In some bacteria, such as lactic acid bacteria (55) and oral streptococci (19), expression of the ADS is under the control of CCR and is inducible by arginine. In the present study, we demonstrated that all these regulation mechanisms apply to the ADS of *S. suis*.

The ADS of *S. suis* is induced under microaerophilic and anaerobic growth conditions. Oxygen-dependent regulation of the ADS is probably supported by the presence of the *flpS* gene, which has homologies to the family of Crp/Fnr transcription regulators and to the ArcR homologue protein of *B. licheniformis*, upstream of the *arcABC* operon. Furthermore, the promoter sequence of the *arcABC* operon contains a possible Crp/Fnr DNA-binding site at position −57.5 from the transcriptional start point of the *arcA* gene with homologies to the Fnr consensus region of *E. coli* (21). The members of the Fnr family have been proven to be responsible for anaerobic gene regulation in many gram-negative and some gram-positive bacteria (48). These regulators have been shown to be linked to ADS regulation. For instance, *P. aeruginosa* and *B. licheniformis* utilize the ADS exclusively under anaerobic conditions via regulators of the Crp/Fnr family (33, 35). The position of *flpS* of *S. suis* within the ADS gene cluster is similar to that of *flp* of *S. gordonii*, immediately upstream of *arcA* (19). The predicted amino acid sequence of FlpS of *S. suis* revealed a putative CAP effector domain in the N-terminal region of the protein and a helix-turn-helix motif in the C-terminal region; both structures are characteristic of Crp/Fnr regulator proteins (6, 48). In typical Fnr regulators, four conserved cysteine residues are usually found (48). As in the Fnr-like proteins of *S. gordonii* (19), *Lactobacillus casei* (24), and *Lactococcus lactis* (45), two cysteine residues have been found in the predicted amino acid sequence of FlpS of *S. suis*. Thus, FlpS seems to be more closely related to the ArcR regulators found in other ADS-expressing gram-positive bacteria (3, 35, 57) that have more Crp-like properties and are not typical FeS proteins (32).

We further found that the ADS of *S. suis* is inducible by arginine. In *B. licheniformis* and *S. gordonii*, the AD pathway is activated by the arginine regulators ArgR and ArcR, respectively, in the presence of arginine by binding to conserved ARG boxes. These boxes are located upstream of the promoters apart from the binding sites for Crp/Fnr-type proteins (20, 36). Accordingly, we identified a potential binding site for

ArgR at positions −196 to −214 from the transcriptional start point of the *arcABC* operon, suggesting that the ADS of *S. suis* is regulated similarly to *B. licheniformis* and *S. gordonii*. Furthermore, we identified a putative regulator involved in arginine metabolism named ArgR.

Expression of the ADS in oral streptococci (20) and *L. sakei* (55) is under the control of CCR. In both bacteria, AD expression is induced in the presence of arginine and repressed by glucose. In AT-rich gram-positive bacteria, CCR is mediated by CcpA. CcpA binds to *cis*-acting catabolite response elements (*cre*) in the presence of favored carbohydrate sources to regulate the expression of catabolic genes and operons (50). Sequences resembling Ccp boxes were identified in a number of genes involved in the utilization of secondary carbon sources. In these genes, the target sequence for glucose repression is located within or downstream of the promoter region (28, 50). In the untranslated region of the *S. suis arcA* gene, downstream of the transcriptional start point, two potential CcpA-dependent *cre* sites were identified at positions +63 and +86, suggesting that CcpA contributes to CCR in *S. suis*. CcpA contributes to CCR in *S. gordonii* (19). However, since other pathways, such as CcpB (11), CcpC (31), and the phosphotransferase system, are known to be involved in CCR of some gram-positive bacteria (23, 40, 51), participation of CcpA in CCR of *S. suis* has to be confirmed by further studies.

Nonetheless, the presence of a putative ARG box, a possible Fnr-binding site, and two potential *cre*-binding sites suggests that the ADS of *S. suis* is transcriptionally controlled by a multitude of regulator proteins.

Initially, we detected the ADS of *S. suis* by identification of two cell wall-associated proteins that were induced by a temperature shift from 32°C or 37°C to 42°C (53). Interestingly, temperature-induced ADS expression has not been reported for other bacteria. Even though ArcA and AcrB are extracellularly expressed, the *S. suis* ADS is similar to the ADSs of other, closely related, species. However, there are significant differences in transcriptional organization structure, as emphasized by the presence of ArcH. Elucidation of the temperature regulation and the detailed molecular mechanisms of ADS regulation awaits further studies.

The ADS can be considered a system that protects oral streptococci and *S. pyogenes* against acidic stress (7, 15). Therefore, we examined the role of the *arcABC* operon of *S. suis* regarding survival in an acidic environment by a pH sensitivity test in which we compared an ArcABC mutant strain with its parental strain. The mutant strain was extremely sensitive to low pH, independent of the presence of 25 mM L-arginine, indicating that the ADS facilitates *S. suis* survival in acidified environments. These results were in good agreement with our previous data demonstrating that a natural, nonencapsulated *arcA*-negative *S. suis* strain, in contrast to a nonencapsulated *arcA*-positive *S. suis* strain, was able neither to survive in acidic vacuoles inside human epithelial cells nor to resist exposure to low pH in the presence of arginine (4).

In conclusion, our results show that the ADS enables *S. suis* to overcome oxygen and nutrient starvation and to tolerate acidic environments. Thus, the ADS might facilitate *S. suis* survival within the different niches of the host and thereby probably contributes to *S. suis* pathogenesis. Future investigations have to clarify the importance of the ADS for *S. suis* infections.

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REFERENCES

1. **Allgaier, A., R. Goethe, H. J. Wisselink, H. E. Smith, and P. Valentin-Weigand.** 2001. Relatedness of *Streptococcus suis* isolates of various serotypes and clinical backgrounds as evaluated by macrorestriction analysis and expression of potential virulence traits. J. Clin. Microbiol. **39:**445–453.
2. **Arends, J. P., and H. C. Zanen.** 1988. Meningitis caused by *Streptococcus suis* in humans. Rev. Infect. Dis. **10:**131–137.
3. **Barcelona-Andres, B., A. Marina, and V. Rubio.** 2002. Gene structure, organization, expression, and potential regulatory mechanisms of arginine catabolism in *Enterococcus faecalis*. J. Bacteriol. **184:**6289–6300.
4. **Benga, L., R. Goethe, M. Rohde, and P. Valentin-Weigand.** 2004. Non-encapsulated strains reveal novel insights in invasion and survival of *Streptococcus suis* in epithelial cells. Cell. Microbiol. **6:**867–881.
5. **Burne, R. A., Z. T. Wen, Y. Y. Chen, and J. E. Penders.** 1999. Regulation of expression of the fructan hydrolase gene of *Streptococcus mutans* GS-5 by induction and carbon catabolite repression. J. Bacteriol. **181:**2863–2871.
6. **Busby, S., and R. H. Ebright.** 1999. Transcription activation by catabolite activator protein (CAP). J. Mol. Biol. **293:**199–213.
7. **Casiano-Colon, A., and R. E. Marquis.** 1988. Role of the arginine deiminase system in protecting oral bacteria and an enzymatic basis for acid tolerance. Appl. Environ. Microbiol. **54:**1318–1324.
8. **Champomier Verges, M. C., M. Zuniga, F. Morel-Deville, G. Perez-Martinez, M. Zagorec, and S. D. Ehrlich.** 1999. Relationships between arginine degradation, pH and survival in *Lactobacillus sakei*. FEMS Microbiol. Lett. **180:**297–304.
9. **Chanter, N., P. W. Jones, and T. J. Alexander.** 1993. Meningitis in pigs caused by *Streptococcus suis*—a speculative review. Vet. Microbiol. **36:**39–55.
10. **Charland, N., J. Harel, M. Kobisch, S. Lacasse, and M. Gottschalk.** 1998. *Streptococcus suis* serotype 2 mutants deficient in capsular expression. Microbiology **144**(Pt. 2)**:**325–332.
11. **Chauvaux, S., I. T. Paulsen, and M. H. Saier, Jr.** 1998. CcpB, a novel transcription factor implicated in catabolite repression in *Bacillus subtilis*. J. Bacteriol. **180:**491–497.
12. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
13. **Clifton-Hadley, F. A., and T. J. Alexander.** 1980. The carrier site and carrier rate of *Streptococcus suis* type II in pigs. Vet. Rec. **107:**40–41.
14. **Cunin, R., N. Glansdorff, A. Pierard, and V. Stalon.** 1986. Biosynthesis and metabolism of arginine in bacteria. Microbiol. Rev. **50:**314–352.
15. **Degnan, B. A., M. C. Fontaine, A. H. Doebereiner, J. J. Lee, P. Mastroeni, G. Dougan, J. A. Goodacre, and M. A. Kehoe.** 2000. Characterization of an isogenic mutant of *Streptococcus pyogenes* Manfredo lacking the ability to make streptococcal acid glycoprotein. Infect. Immun. **68:**2441–2448.
16. **Degnan, B. A., J. M. Palmer, T. Robson, C. E. Jones, M. Fischer, M. Glanville, G. D. Mellor, A. G. Diamond, M. A. Kehoe, and J. A. Goodacre.** 1998. Inhibition of human peripheral blood mononuclear cell proliferation by *Streptococcus pyogenes* cell extract is associated with arginine deiminase activity. Infect. Immun. **66:**3050–3058.
17. **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.
18. **D'Hooghe, I., C. Vander Wauven, J. Michiels, C. Tricot, P. de Wilde, J. Vanderleyden, and V. Stalon.** 1997. The arginine deiminase pathway in *Rhizobium etli*: DNA sequence analysis and functional study of the *arcABC* genes. J. Bacteriol. **179:**7403–7409.
19. **Dong, Y., Y. Y. Chen, and R. A. Burne.** 2004. Control of expression of the arginine deiminase operon of *Streptococcus gordonii* by CcpA and Flp. J. Bacteriol. **186:**2511–2514.
20. **Dong, Y., Y. Y. Chen, J. A. Snyder, and R. A. Burne.** 2002. Isolation and molecular analysis of the gene cluster for the arginine deiminase system from *Streptococcus gordonii* DL1. Appl. Environ. Microbiol. **68:**5549–5553.
21. **Eiglmeier, K., N. Honore, S. Iuchi, E. C. Lin, and S. T. Cole.** 1989. Molecular genetic analysis of FNR-dependent promoters. Mol. Microbiol. **3:**869–878.
22. **Gamper, M., A. Zimmermann, and D. Haas.** 1991. Anaerobic regulation of

transcription initiation in the *arcDABC* operon of *Pseudomonas aeruginosa*. J. Bacteriol. **173:**4742–4750.
23. **Gosalbes, M. J., V. Monedero, and G. Perez-Martinez.** 1999. Elements involved in catabolite repression and substrate induction of the lactose operon in *Lactobacillus casei*. J. Bacteriol. **181:**3928–3934.
24. **Gostick, D. O., J. Green, A. S. Irvine, M. J. Gasson, and J. R. Guest.** 1998. A novel regulatory switch mediated by the FNR-like protein of *Lactobacillus casei*. Microbiology **144:**705–717.
25. **Gottschalk, M., and M. Segura.** 2000. The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. Vet. Microbiol. **76:** 259–272.
26. **Gottschalk, M. G., S. Lacouture, and J. D. Dubreuil.** 1995. Characterization of *Streptococcus suis* capsular type 2 haemolysin. Microbiology **141**(Pt. 1)**:** 189–195.
27. **Griswold, A., Y. Y. Chen, J. A. Snyder, and R. A. Burne.** 2004. Characterization of the arginine deiminase operon of *Streptococcus rattus* FA-1. Appl. Environ. Microbiol. **70:**1321–1327.
28. **Henkin, T. M.** 1996. The role of CcpA transcriptional regulator in carbon metabolism in *Bacillus subtilis*. FEMS Microbiol. Lett. **135:**9–15.
29. **Hueck, C. J., and W. Hillen.** 1995. Catabolite repression in *Bacillus subtilis*: a global regulatory mechanism for the gram-positive bacteria? Mol. Microbiol. **15:**395–401.
30. **Jacobs, A. A., P. L. Loeffen, A. J. van den Berg, and P. K. Storm.** 1994. Identification, purification, and characterization of a thiol-activated hemolysin (suilysin) of *Streptococcus suis*. Infect. Immun. **62:**1742–1748.
31. **Jourlin-Castelli, C., N. Mani, M. M. Nakano, and A. L. Sonenshein.** 2000. CcpC, a novel regulator of the LysR family required for glucose repression of the *cit*B gene in *Bacillus subtilis*. J. Mol. Biol. **295:**865–878.
32. **Korner, H., H. J. Sofia, and W. G. Zumft.** 2003. Phylogeny of the bacterial superfamily of Crp-Fnr transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs. FEMS Microbiol. Rev. **27:**559–592.
33. **Lu, C. D., H. Winteler, A. Abdelal, and D. Haas.** 1999. The ArgR regulatory protein, a helper to the anaerobic regulator ANR during transcriptional activation of the *arcD* promoter in *Pseudomonas aeruginosa*. J. Bacteriol. **181:**2459–2464.
34. **Maas, W. K.** 1994. The arginine repressor of *Escherichia coli*. Microbiol. Rev. **58:**631–640.
35. **Maghnouj, A., A. A. Abu-Bakr, S. Baumberg, V. Stalon, and C. Vander Wauven.** 2000. Regulation of anaerobic arginine catabolism in *Bacillus licheniformis* by a protein of the Crp/Fnr family. FEMS Microbiol. Lett. **191:**227–234.
36. **Maghnouj, A., T. F. Sousa Cabral, V. Stalon, and C. Vander Wauven.** 1998. The *arcABDC* gene cluster, encoding the arginine deiminase pathway of *Bacillus licheniformis*, and its activation by the arginine repressor ArgR. J. Bacteriol. **180:**6468–6475.
37. **Marouni, M. J., E. Ziomek, and S. Sela.** 2003. Influence of group A streptococcal acid glycoprotein on expression of major virulence factors and internalization by epithelial cells. Microb. Pathog. **35:**63–72.
38. **Marquis, R. E., G. R. Bender, D. R. Murray, and A. Wong.** 1987. Arginine deiminase system and bacterial adaptation to acid environments. Appl. Environ. Microbiol. **53:**198–200.
39. **Marsh, J. L., M. Erfle, and E. J. Wykes.** 1984. The pIC plasmid and phage vectors with versatile cloning sites for recombinant selection by insertional inactivation. Gene **32:**481–485.
40. **Martin-Verstraete, I., J. Stulke, A. Klier, and G. Rapoport.** 1995. Two different mechanisms mediate catabolite repression of the *Bacillus subtilis* levanase operon. J. Bacteriol. **177:**6919–6927.
41. **Okwumabua, O., J. S. Persaud, and P. G. Reddy.** 2001. Cloning and characterization of the gene encoding the glutamate dehydrogenase of *Streptococcus suis* serotype 2. Clin. Diagn. Lab. Immunol. **8:**251–257.
42. **Palm, J. E., M. E. Weiland, W. J. Griffiths, I. Ljungstrom, and S. G. Svard.** 2003. Identification of immunoreactive proteins during acute human giardiasis. J. Infect. Dis. **187:**1849–1859.
43. **Ruepp, A., and J. Soppa.** 1996. Fermentative arginine degradation in *Halobacterium salinarium* (formerly *Halobacterium halobium*): genes, gene products, and transcripts of the *arcRACB* gene cluster. J. Bacteriol. **178:** 4942–4947.
44. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
45. **Scott, C., J. R. Guest, and J. Green.** 2000. Characterization of the *Lactococcus lactis* transcription factor FlpA and demonstration of an in vitro switch. Mol. Microbiol. **35:**1383–1393.
46. **Smith, H. E., M. Damman, J. van der Velde, F. Wagenaar, H. J. Wisselink, N. Stockhofe-Zurwieden, and M. A. Smits.** 1999. Identification and characterization of the *cps* locus of *Streptococcus suis* serotype 2: the capsule protects against phagocytosis and is an important virulence factor. Infect. Immun. **67:**1750–1756.
47. **Smith, H. E., H. J. Wisselink, U. Vecht, A. L. Gielkens, and M. A. Smits.** 1995. High-efficiency transformation and gene inactivation in *Streptococcus suis* type 2. Microbiology **141**(Pt. 1)**:**181–188.
48. **Spiro, S.** 1994. The FNR family of transcriptional regulators. Antonie Leeuwenhoek **66:**23–36.
49. **Tikkanen, K., S. Haataja, and J. Finne.** 1996. The galactosyl-(α1–4)-galactose-binding adhesin of *Streptococcus suis*: occurrence in strains of different hemagglutination activities and induction of opsonic antibodies. Infect. Immun. **64:**3659–3665.
50. **Titgemeyer, F., and W. Hillen.** 2002. Global control of sugar metabolism: a gram-positive solution. Antonie Van Leeuwenhoek **82:**59–71.
51. **Tobisch, S., J. Stulke, and M. Hecker.** 1999. Regulation of the *lic* operon of *Bacillus subtilis* and characterization of potential phosphorylation sites of the LicR regulator protein by site-directed mutagenesis. J. Bacteriol. **181:**4995–5003.
52. **Vecht, U., H. J. Wisselink, M. L. Jellema, and H. E. Smith.** 1991. Identification of two proteins associated with virulence of *Streptococcus suis* type 2. Infect. Immun. **59:**3156–3162.
53. **Winterhoff, N., R. Goethe, P. Gruening, M. Rohde, H. Kalisz, H. E. Smith, and P. Valentin-Weigand.** 2002. Identification and characterization of two temperature-induced surface-associated proteins of *Streptococcus suis* with high homologies to members of the arginine deiminase system of *Streptococcus pyogenes*. J. Bacteriol. **184:**6768–6776.
54. **Yoshida, J., S. Takamura, and M. Nishio.** 1998. Characterization of a streptococcal antitumor glycoprotein (SAGP). Life Sci. **62:**1043–1053.
55. **Zuniga, M., M. Champomier-Verges, M. Zagorec, and G. Perez-Martinez.** 1998. Structural and functional analysis of the gene cluster encoding the enzymes of the arginine deiminase pathway of *Lactobacillus sake*. J. Bacteriol. **180:**4154–4159.
56. **Zuniga, M., G. Perez, and F. Gonzalez-Candelas.** 2002. Evolution of arginine deiminase (ADI) pathway genes. Mol. Phylogenet. Evol. **25:**429–444.
57. **Zuniga, M., M. del Carmen Miralles, and G. Perez-Martinez.** 2002. The product of *arcR*, the sixth gene of the *arc* operon of *Lactobacillus sakei*, is essential for expression of the arginine deiminase pathway. Appl. Environ. Microbiol. **68:**6051–6058.