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Characterization of the Arginine Deiminase Operon of *Streptococcus rattus* FA-1

Ann Griswold, Yi-Ywan M. Chen, Jennifer A. Snyder,† and Robert A. Burne*

Department of Oral Biology, University of Florida, Gainesville, Florida

The arginine deiminase system (ADS) is of critical importance in oral biofilm pH homeostasis and microbial ecology. The ADS consists of three enzymes. Arginine is hydrolyzed by AD (ArcA) to generate citrulline and ammonia. Citrulline is then converted to ornithine and carbamoylphosphate via ornithine carbamoyltransferase (ArcB). Finally, carbamate kinase (ArcC) transfers a phosphate from carbamoylphosphate to ADP, yielding ATP. Ammonia production from this pathway protects bacteria from lethal acidification, and ATP production provides a source of energy for the cells. The purpose of this study was to initiate a characterization of the *arc* operon of *Streptococcus rattus*, the least cariogenic and sole ADS-positive member of the mutans streptococci. Using an *arcB* gene fragment obtained by degenerate PCRs, the FA-1 *arc* operon was identified in subgenomic DNA libraries and sequence analysis was performed. Results showed that the genes encoding the AD pathway in *S. rattus* FA-1 are organized as an *arcABCDT-adiR* operon gene cluster, including the enzymes of the pathway, an arginine-ornithine antiporter (ArcD) and a putative regulatory protein (AdiR). The *arcA* transcriptional start site was identified by primer extension, and a σ⁷⁰-like promoter was mapped 5′ to *arcA*. Reverse transcriptase PCR was used to establish that *arcABCDT* could be cotranscribed. Reporter gene fusions and AD assays demonstrated that the operon is regulated by substrate induction and catabolite repression, the latter apparently through a CcpA-dependent pathway.

The oral cavity is a diverse ecosystem in which resident bacteria must withstand constant changes in environmental pH and energy source in order to survive. Following a carbohydrate challenge, the pH of the mouth can drop to values below 4, mainly due to glycolysis by *Streptococcus mutans* and other acid-tolerant bacteria (34, 38). Repeated acidification of dental plaque can contribute to demineralization of tooth enamel and development of dental caries (28). Oral bacteria have developed a variety of strategies to cope with environmental acidification, including mounting an adaptive acid tolerance response and production of alkali (9). A relatively small, yet abundant, subset of oral bacteria uses one of two primary ammonia-generating mechanisms: the hydrolysis of urea by urease, or catabolism of arginine via the arginine deiminase system (ADS) (9). The ADS is thought to be a critical factor in oral biofilm pH homeostasis that may inhibit the emergence of a cariogenic flora (9).

Genes encoding the ADS are commonly arranged as an operon, although the gene order varies among bacteria (4, 19, 30, 40, 42). Typically, the operons contain *arcA*, encoding arginine deiminase (AD), which hydrolyzes arginine to generate citrulline and ammonia. A second gene, *arcB*, encodes a catabolic ornithine carbamoyltransferase (cOTC), which converts citrulline to ornithine and carbamoylphosphate. Finally, *arcC* encodes a catabolic carbamate kinase, which transfers a phosphate group from carbamoylphosphate to ADP, generating ATP, CO_2, and ammonia. Many ADS operons also include a

fourth gene encoding an arginine-ornithine antiporter (*arcD*). In some organisms, a gene encoding a putative aminopeptidase (*arcT*) and one encoding a transcriptional regulator of the Crp/Fnr family (*arcR*) or ArgR/AhrC family are also associated with the ADS gene cluster (19, 42). The generation of ATP in accordance with equimolar hydrolysis of substrate allows the use of arginine as a sole energy source. Ammonia generated from this pathway also helps neutralize the cytoplasm (12) and alkalinize the surroundings. Thus, arginine metabolism via the ADS has been shown to protect bacteria from acid killing (12).

The ADS has been identified in a variety of prokaryotes (1), although the physiological role of the system, as well as the mode of regulation, can be different between species. In the oral cavity, the ADS is believed to be used by plaque bacteria associated with dental health, such as *Streptococcus gordonii* and *Streptococcus sanguis*, to counteract acid production by cariogenic mutans streptococci and lactobacilli (9). Regulation of the ADS in these bacteria is highly dependent on growth conditions. AD activity in *S. gordonii* and *S. sanguis* is induced by arginine and is subject to carbon catabolite repression (CCR), with lower activity observed in the presence of the repressing sugar glucose (19, 21). In *S. gordonii*, the *trans*-acting ArcR protein activates AD gene expression and CcpA represses expression in the presence of glucose (19).

Streptococcus rattus is similar to other ADS-positive oral bacteria in that it is relatively acid sensitive in the absence of arginine and has not been linked to caries development in humans. However, *S. rattus* is a member of the mutans group of streptococci and is most closely related to *S. mutans*, which is noted for exceptional cariogenicity and acid tolerance. In fact, a major factor distinguishing *S. rattus* from *S. mutans* is the ability to catabolize arginine via the ADS (16). By initiating a molecular characterization of the *arc* operon of *S. rattus*

FA-1, a clearer understanding of the evolution and diversity of the mutans streptococci in relation to pH adaptation will be obtained.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and reagents. *S. rattus* FA-1 was grown in brain heart infusion (Difco Laboratories, Detroit, Mich.) broth at 37°C, in 5% CO_2 and 95% air. To monitor ADS expression, wild-type *S. rattus* FA-1 was grown in a low-carbohydrate tryptone-vitamin (TV)-based broth (11) containing 0.2 or 2% glucose or galactose, with or without 10 mM arginine. Recombinant *Escherichia coli* strains were maintained on L agar supplemented, when indicated, with 25 μg of chloramphenicol (Cm) ml^{-1} or 50 μg of kanamycin (Km) ml^{-1}. All chemical reagents were obtained from Sigma (St. Louis, Mo.).

DNA manipulations. Genomic DNA from *S. rattus* FA-1 was isolated as previously described (13). Plasmid DNA was isolated from *E. coli* by the method of Birnboim and Doly (6). Plasmid DNA used in sequencing reactions was prepared from *E. coli* DH10B using the QIAprep Spin plasmid kit (Qiagen Inc., Valencia, Calif.). Cloning and electrophoretic analysis of DNA fragments were carried out according to established protocols (3). Southern hybridization and high-stringency washes were performed as previously described (27). Restriction and DNA-modifying enzymes were purchased from Life Technologies Inc. (Rockville, Md.) or New England Biolabs (Beverley, Mass.).

To prepare subgenomic DNA libraries of *S. rattus* FA-1, chromosomal DNA was digested to completion using *Xba*I or *Eco*RV. The digested DNA fragments were separated on agarose gels, and the *Xba*I fragments of 6 to 8 kbp or *Eco*RV fragments of 4 to 5 kbp were enriched by gel purification using the Elu-Quik DNA purification kit (Schleicher & Schuell, Keene, N.H.). The isolated DNA fragments were ligated onto *Xba*I- or *Hinc*II-digested, phosphatase-treated pSU20 (5), respectively. The ligation mixtures were used to transform *E. coli* DH10B, and Cm-resistant transformants were selected.

Development of an *arcB*-specific probe. An internal fragment of the *S. rattus* FA-1 *arcB* gene, encoding cOTC, was generated by PCRs using degenerate primers based on alignments of known anabolic and catabolic OTCs (19). Using this fragment as a probe, a lambda clone containing the *arcA* gene and a partial *arcB* gene was identified (data not shown). Specific primers internal to *arcB* were designed, and the subsequent PCR product was used to screen a subgenomic library. Primer arcBS, 5′-CAAGTATTTCAGGGACGC-3′, encoded amino acid (aa) residues 3 through 8 of cOTC. Primer arcBAS, 5′-CATCTGTCAAGCCA TTCC-3′, encoded the antisense sequence, corresponding to aa residues 127 to 132 of cOTC. Each reaction consisted of 25 cycles at a stringent annealing temperature (55°C). The product with the correct predicted size was gel purified prior to cloning into pCRII to generate pJZ22. Southern blot analysis confirmed hybridization of the PCR product to the *S. rattus* FA-1 chromosome at high stringency. Sequence analysis and BLAST searches were performed to confirm that the product shared high degrees of homology with other cOTCs.

RNA isolation, primer extension, and reverse transcriptase PCR (RT-PCR) analysis. The protocol used to isolate total RNA from *S. rattus* FA-1 was the same as that originally described for *Streptococcus salivarius* RNA isolation (15). *S. rattus* FA-1 was grown to mid-exponential phase in TV medium containing 2% galactose and 1% arginine. Primer extension analysis was used to map the *arcA* transcription initiation site. Primer ArcAS (5′-GACGATGTAACATTACCTT CTT-3′) encoded the antisense sequence of *arcA* located 50 bases downstream from the translational start site. Incubation of radiolabeled primers with 50 μg of total RNA at 42°C for 90 min was followed by reverse transcription, and the products were separated by electrophoresis and disclosed by autoradiography. A DNA sequencing reaction using the same primer was included on the gel to allow identification of the start site.

To determine if the *arc* genes of *S. rattus* FA-1 could be cotranscribed, RT-PCR was performed using the SuperScript first-strand synthesis system (Invitrogen, Carlsbad, Calif.). RNA was isolated from *S. rattus* FA-1 grown in TV plus 2% galactose and 1% arginine. PCR amplification of the cDNA was performed using various primer pairs: arcABS (5′-CTGTGTATGTCTATGCCATTTG-3′) and arcABAS (5′-AGCTAGGAAACTGCGTCCCT-3′), arcDTS (5′-TTTAGA CTCTTTACAGGACAGATT-3′) and arcDTAS (5′-TGAATATTCATCTGTT TACCCCTT-3′), and arcTadiRS (5′-AGTGAGTTGTCTGAGTTTCTA-3′) and arcTadiRAS (5′-TTTATCTTACTTTGGCGCAATA-3′), flanking the intergenic region of *arcAB*, *arcDT*, and *arcTadiR*, respectively.

Construction of promoter fusions and CAT assays. The *arcA* promoter and deletion derivatives were amplified via recombinant PCR (23) using sense primers (arcASacI-S400, TTGCTCTA**GAGCTC**TCAAATGACACGAA; arcASacI-S150, TTATAAATTC**GAGCTC**CAAAAAACGTGAA; and arcASacI-S100, TA

AATAACAATTC**GAGCTC**GAAAAAAATCTTA) in conjunction with the antisense primer arcABamHI-AS (TTTTGAGTCAT**GGATCC**TACTCCTTTC GAT). These primers allowed insertion of *Sac*I and *Bam*HI restriction sites (bold) to facilitate cloning. The PCR products were ligated to the 5′ end of a promoterless chloramphenicol acetyltransferase (CAT) gene (*cat*) from *Staphylococcus aureus* (20) and cloned onto the integration vector pMJB8A (14). The constructs were then electroporated into *E. coli* DH10B cells, screened for the correct configurations, and then introduced into *S. gordonii* DL-1 by natural transformation. *S. rattus* FA-1 cultures were grown in TV medium containing 2% glucose or galactose, with or without 1% arginine, to an optical density at 600 nm of 0.6.

Biochemical assays. *S. rattus* FA-1 was grown in TV medium supplemented with 0.2 or 2% glucose or galactose, with or without 1% arginine, at 37°C in 5% CO_2 and 95% air. AD activity was measured by monitoring production of citrulline from arginine as previously described (2). CAT assays were performed using the spectrophotometric method of Shaw (36). Enzyme activities were normalized to protein concentration, which was determined by the method of Bradford (7) using a kit (Bio-Rad) with bovine serum albumin as the standard.

Nucleotide sequence accession number. The complete sequences of the *arc* operon and *adiR* reported in this article have been deposited in the GenBank database, and the accession number is AY396288.

RESULTS AND DISCUSSION

Isolation of the ADS genes of *S. rattus* FA-1. To prepare a subgenomic DNA library, chromosomal DNA isolated from *S. rattus* FA-1 was digested to completion using various restriction enzymes. The digested DNA fragments were separated on a 0.8% agarose gel and screened for the presence of *arcB* by stringent hybridization with a 0.35-kbp PCR product internal to the *S. rattus arcB* gene. Results indicated that the *arcB* gene was contained on an *Xba*I fragment of approximately 7 kbp. To clone this fragment, a subgenomic DNA library of *Xba*I fragments was constructed in the intermediate-copy-number plasmid, pSU20 (5). The library was screened by colony hybridization under stringent conditions with an *arcB*-specific probe. A positive clone, containing a 7.0-kbp DNA insert (pJZ29), was identified. Southern blot analysis confirmed that the 7.0-kbp *Xba*I DNA fragment originated from *S. rattus* and demonstrated that the fragment was continuous on the chromosome (data not shown).

Results of DNA sequence analysis performed on the 7.0-kbp *Xba*I fragment indicated that this fragment contained the 3′ portion of a partial open reading frame (ORF) that shared homology with other known *arcA* genes, followed by five complete ORFs. To obtain genomic DNA fragments containing the complete ORF and to potentially identify other genes tightly linked to the arginine catabolism cluster, a subgenomic DNA library of *Eco*RV fragments was constructed and screened with a 1-kbp DNA fragment containing the 3′ portion of *arcA*. A 4-kbp *Eco*RV fragment was subsequently isolated, and a Southern blot analysis under stringent conditions confirmed that the fragment originated from *S. rattus*.

Nucleotide sequence analysis of the ADS genes. Using a series of nested deletions generated by exonuclease III, the complete sense and antisense nucleotide sequences of the 7-kbp *Xba*I fragment and 4-kbp *Eco*RV fragment were determined. The nucleotide sequences were translated and found to encode six ORFs arranged in an apparent operon (Fig. 1). Each ORF began with an ATG start codon and was preceded by a putative Shine-Dalgarno sequence. Between the fifth and sixth ORF, a stable stem-loop structure that could possibly act as a terminator was identified. Based on similarity to known *arc*
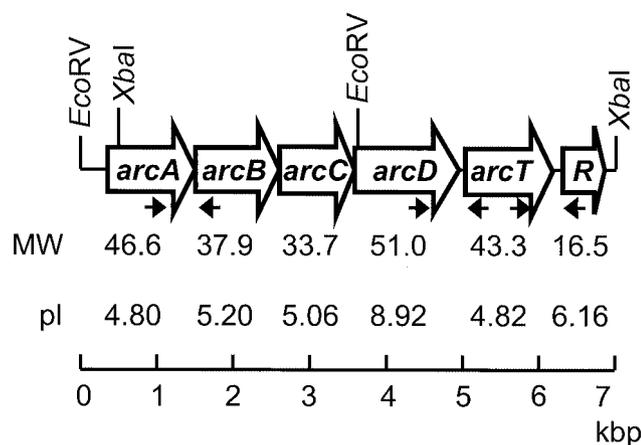
FIG. 1. The *S. rattus* FA-1 *arc* operon. Restriction sites used to isolate the *arc* gene fragment from a subgenomic library are noted. The arrows indicate positions of primers used in RT-PCR.

genes, the ORFs were designated *arcA*, *-B*, *-C*, *-D*, and *-T* and *adiR*.

The predicted amino acid sequences of the six ORFs were determined, and BLAST searches were used to identify sequence similarity to other known proteins. *S. rattus* AD, encoded by *arcA*, was 85, 84, 80, and 79% identical to the AD enzymes of *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *S. gordonii*, respectively. *S. rattus* AD also shared homology with the AD proteins of *Enterococcus faecalis*, *Clostridium perfringens*, *Bacillus licheniformis*, *Lactobacillus sakei*, and *S. aureus*. Several conserved regions were identified in *S. rattus* ArcA, including the signature arginine deiminase motifs SEIGKLKKVML (aa 11 to 21), FTRD (aa 164 to 167), EGGD (aa 220 to 223), and MHLDTVF (aa 274 to 280) (26).

S. rattus cOTC, encoded by *arcB*, shared 88, 86, 85, and 85% identity with the cOTCases of *S. pyogenes*, *S. agalactiae*, *S. gordonii*, and *S. pneumoniae*, respectively. Additional homologies were observed with cOTC enzymes from *E. faecalis*, *L. sakei*, *B. licheniformis*, and *S. aureus*. Conserved carbamoylphosphate binding and catalysis motifs, STRTR and HPTQ, were identified at aa residues 57 to 61 and 135 to 138, respectively (24). The conserved ornithine binding site (LHCLP) was identified at positions 270 to 274.

The carbamate kinase encoded by *S. rattus* arcC was 73 and 72% identical to that of *S. gordonii* and *S. pneumoniae*, respectively. Homologies were also observed with the carbamate kinases of *Listeria monocytogenes*, *S. pyogenes*, *S. agalactiae*, *S. mutans*, *L. sakei*, and *B. licheniformis*. Aside from the highly conserved arginine residues at aa residues 157 and 160, no other conserved motifs were identified in the *S. rattus* arcC, as was the case for *S. gordonii* and *E. faecalis* (19, 31).

The arginine-ornithine antiporter encoded by *S. rattus* arcD shared 49 and 42% identity with those of *L. sakei* and *S. aureus*, respectively. Conservation was also observed with the arginine-ornithine antiporters of *S. agalactiae*, *C. perfringens*, and *Pseudomonas putida*. Twelve predicted transmembrane helices were identified in ArcD of *S. rattus* using the dense alignment surface method from the DAS-Transmembrane Prediction server (http://www.sbc.su.se/~miklos/DAS) (17).

S. rattus arcT, encoding a putative peptidase, shared 58% identity with the ArcT of *L. lactis* and 59% identity with SMU.816, a putative aminotransferase of *S. mutans* UA159. Additional homologies were observed with the ArcT proteins of *Lactobacillus lactis* and *L. sakei*, as well as a *Lactobacillus plantarum* aminotransferase.

The amino acid sequence of an apparent regulatory protein linked to the *arc* operon was compared to those of regulatory proteins controlling arginine metabolism in other bacteria. The most significant level of similarity was observed with the putative *S. mutans* UA159 ArgR (80% identity), which apparently regulates arginine biosynthesis. The second highest identity was with ArgR of *S. agalactiae* (58%), followed by putative arginine repressors in *S. pyogenes* and *S. pneumoniae* and AhrC of *L. lactis*. The AhrC protein was originally identified in *Bacillus subtilis* as a repressor of the arginine biosynthetic genes (32). An AhrC homologue, ArgR, has been identified in *B. licheniformis*, where it both represses the anabolic ornithine carbamoyltransferase and activates *arc* operon expression in the presence of arginine (30). In contrast to the *B. licheniformis* ArgR, it is not known whether the regulatory protein associated with the *arc* operon in *S. rattus* also regulates arginine biosynthetic genes. Additionally, only a very low level of similarity was shared between *S. rattus* AdiR and known Crp/Fnr-like proteins, such as *S. gordonii* ArcR (30%) and *B. licheniformis* ArcR (no similarity). To avoid generating confusion of this apparent *S. rattus* ADS regulatory protein with the global regulator ArcR or regulators repressing arginine biosynthesis (ArgR/AhrC), we have designated this protein AdiR to reflect its putative role as a regulator of the AD gene cluster.

We analyzed the predicted amino acid sequence of *S. rattus* AdiR to determine if any conserved DNA or arginine binding residues were present. The conserved SR-RE motif thought to be involved in DNA contact by the *E. coli* ArgR (39) was found at aa residues 44 to 49 of the *S. rattus* AdiR, consistent with the theory that the N terminus of ArgR is involved in DNA binding. Conserved amino acid residues thought to be important for arginine binding in the *E. coli* ArgR (8, 41) were identified near the C terminus at positions 101 (alanine) and 124 (aspartic acid). In addition, a conserved glycine residue involved in oligomerization was identified at position 122 (41).

Most ADS genes appear to be highly regulated (4, 19, 30, 43). Three transcriptional activators of the *E. faecalis* arc operon have been identified, including ArcR and two ArgR/AhrC-type regulators (4). In *B. lichenformis*, ArgR activates *arc* operon expression in the presence of arginine by binding to a conserved ARG box located upstream of the *arcA* promoter (30). Transcriptional regulation of ADS by multiple proteins has also been proposed for *B. licheniformis*, as putative binding sites for both ArgR/AhrC-type and Crp/Fnr-type proteins have been identified 5' to the transcriptional initiation site (30). ArcR, a Crp/Fnr-type transcriptional regulator, activates the *arc* genes by binding to a Crp-like consensus sequence upstream of the *arcA* promoter. We analyzed the sequence upstream of *S. rattus* arcA for possible ArgR or ArcR DNA binding sites. Potential binding sites for both ArgR and ArcR (22, 39) were identified 200 and 52 bases upstream of the *arcA* transcriptional start site, respectively (Fig. 2B). The presence of a putative Arg box (**AATGAATTTATA**AGTTAA) and an imperfect palindrome possibly constituting a Crp binding site
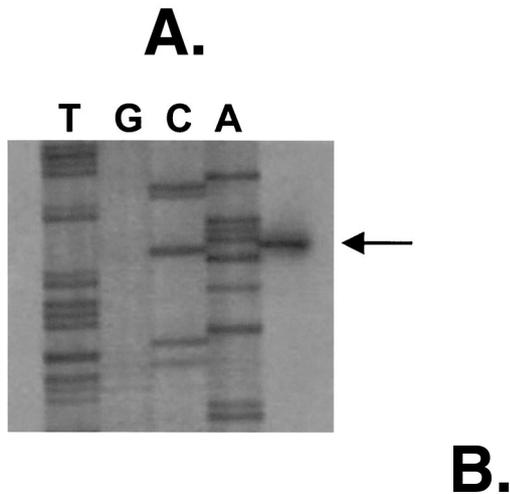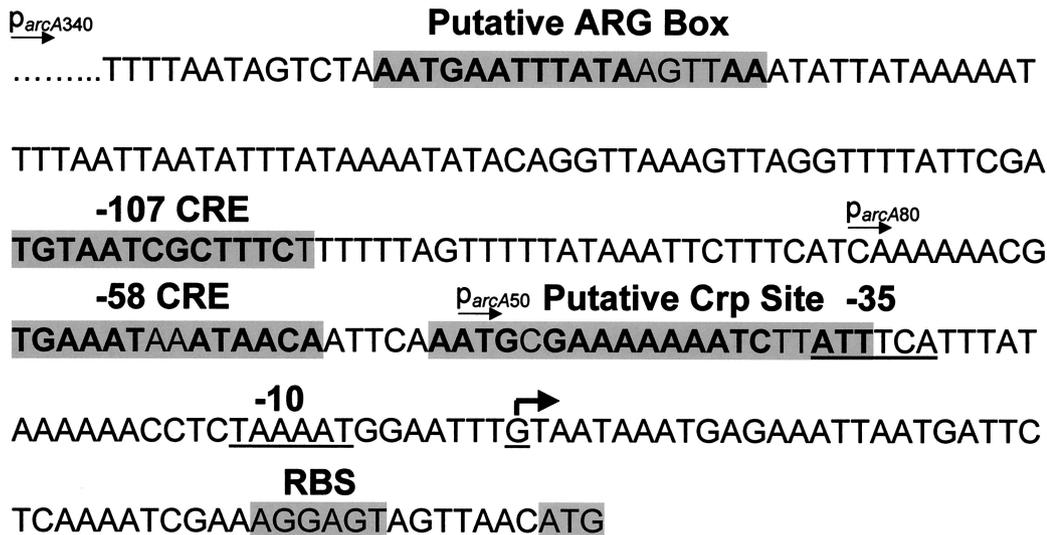
A.

T G C A

FIG. 2. (A) Primer extension analysis of *S. rattus arcA*. The arrowhead indicates initiation of transcription at a G residue, 49 bases 5′ to the ATG translational start codon. (B) Upstream sequence of *S. rattus* FA-1 *arcA*, with putative regulatory elements highlighted. The −10 and −35 promoter elements are underlined, and the arrowhead indicates the transcription start site.

B.

p*arcA*340

………TTTTAATAGTCTA**AATGAATTTATA**AGTT**AA**ATATTATAAAAT

Putative ARG Box

TTTAATTAATATTTATAAAATATACAGGTTAAAGTTAGGTTTTATTCGA

-107 CRE

p*arcA*80

TGTAATCGCTTTCTTTTTTAGTTTTTATAAATTCTTTCATCAAAAAACG

-58 CRE

p*arcA*50 Putative Crp Site -35

TGAAATAA**ATAACA**ATTCA**AATGC**G**AAAAAAAATC**TT<u>ATT</u>TCATTTAT

-10

AAAAAACCTC<u>TAAAAT</u>GGAATTT<u>G</u>TAATAAATGAGAAATTAATGATTC

RBS

TCAAAATCGAAAGGAGTAGTTAACATG

(**AATGCGA**-N$_6$-**TCTTATT**) (bases in bold match the *E. coli* consensus sequences) (29) suggest that the ADS in *S. rattus* may be under the control of a Crp/Fnr family member, in addition to an ArgR/AhrC-type protein (30), most likely the *S. rattus* AdiR protein. The role of these putative binding sites in regulation of the *S. rattus* ADS is currently under investigation.

Localization of p$_{arcA}$ and reporter gene fusions. Primer extension analysis was used to map the *arcA* promoter region. A single band was observed corresponding to a G residue 49 bases upstream of the *arcA* start codon (Fig. 2A). Examination of the upstream sequence revealed a putative promoter that was most similar to σ^{70}-type promoters. The −10 region (**TA AAAT**) shared 5 out of 6 bases with the consensus sequence (bold), whereas the −35 region (**ATTTCA**) identified 17 bases upstream of the −10 region shared only 3 bases with the consensus (bold). To determine which *arc* genes could be transcribed from the *arcA* promoter, RT-PCR analysis was performed on RNA isolated from *S. rattus* FA-1 grown in TV plus 2% galactose plus 1% arginine (Fig. 3). RT-PCR results sug-
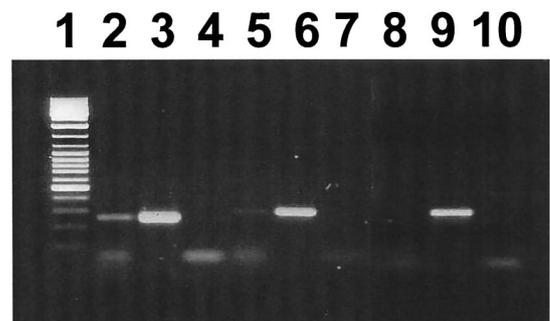

1 2 3 4 5 6 7 8 9 10

FIG. 3. RT-PCR analysis of mRNA from *S. rattus* grown in TV broth containing 2% galactose and 1% arginine. Primers specific to the *arc* intergenic regions were used to amplify cDNA. Lane 1, molecular weight markers; lanes 2 to 4, *arcAB* intergenic region; lanes 5 to 7, *arcDT* intergenic region; lanes 8 to 10, *arcTR* intergenic region. The order within each triplicate (2 to 4, 5 to 7, 8 to 10) is cDNA, chromosomal DNA, and a control in which a reaction containing mRNA with no RT was used in the amplification reaction.
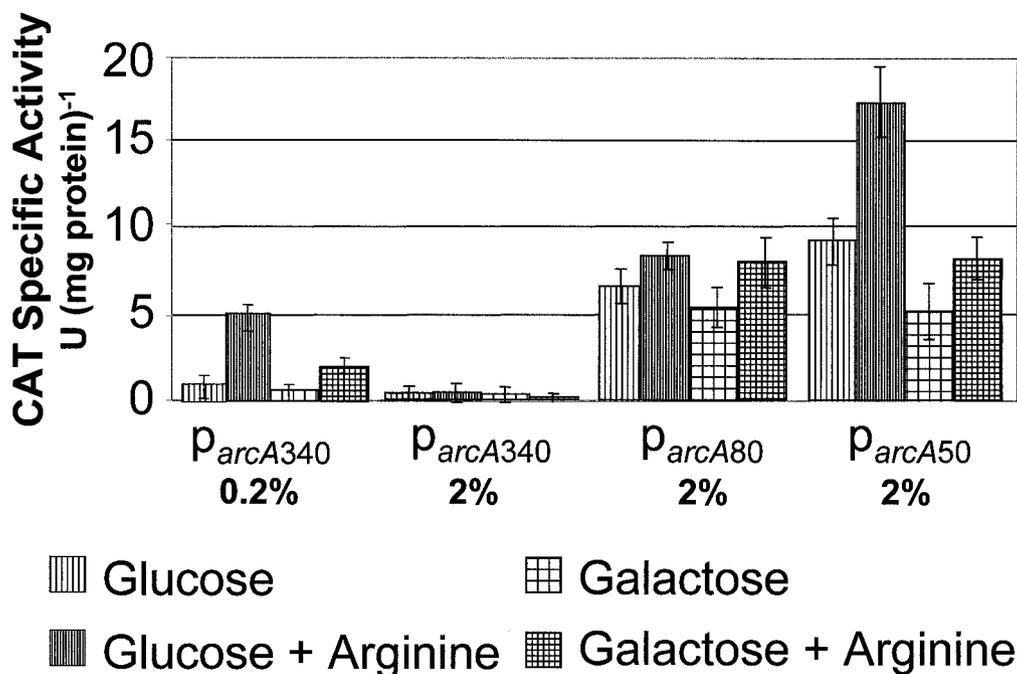
FIG. 4. CAT specific activity, in units per milligram of protein of the wild-type *S. rattus* FA-1 *arcA* promoter (p$_{arcA340}$) and derivatives in which one *cre* has been deleted (p$_{arcA80}$) or both *cre* have been deleted (p$_{arcA50}$). Bacterial cultures used in the CAT assays were grown in TV containing 0.2 or 2% carbohydrate, with or without 1% arginine.

gested the presence of a polycistronic *arcABCDT* transcript. No transcript could be detected between the intergenic region of *arcT* and *adiR*, suggesting that *adiR* is transcribed from a separate promoter.

Expression of the *arc* operon in *L. sakei* (42), as well as some oral streptococci (19), is under the control of CCR. ADS expression in these bacteria appears to be up-regulated in the presence of arginine (33) and repressed by glucose (18, 37). In AT-rich gram-positive bacteria, CCR is mediated by the *trans*-acting catabolite control protein A (CcpA), which binds to *cis*-acting catabolite response elements (*cre*) in the presence of preferred carbohydrate sources to regulate the expression of catabolic genes and operons (35). Two potential CcpA-dependent *cre* were identified at −58 (**TGAAATAAATAACA**) and −107 (**TGTAATCGCTTTC**T) relative to the *arcA* transcriptional start site, with bases matching the consensus shown in bold (25). The presence of these elements is consistent with the observation that the ADS in *S. rattus* FA-1 is regulated by CCR (10).

To assess the functionality of p$_{arcA}$ and the putative *cre* sites found upstream of the promoter region, p$_{arcA}$ and deletion derivatives were fused to a *cat* gene from *S. aureus*. Three *arcA* promoter fusions were constructed: (i) 340 bases upstream of the *arcA* transcriptional start site, including both putative *cre* sites; (ii) 80 bases upstream, including one *cre* site; and (iii) 50 bases upstream, lacking both *cre* sites. All of the promoter fusions were constructed such that expression of *cat* was driven by the cognate *arcA* ribosome binding sequence. Since an efficient system of genetic transformation has not yet been established for *S. rattus* FA-1, the promoter fusions were integrated into the *gtfG* gene on the chromosome of the naturally competent, ADS-positive organism *S. gordonii* DL-1 using a

previously described integration vector (14). Results showed that the intact p$_{arcA}$ was functional in *S. gordonii*, and deletion of the putative *cre* sites resulted in increased promoter activity relative to the wild type, providing evidence that expression from p$_{arcA}$ is regulated via CcpA-dependent catabolite repression (Fig. 4).

Expression of AD in *S. rattus*. The identification of putative *cre* sites upstream of p$_{arcA}$, as well as the ADS regulation patterns observed in *S. gordonii*, prompted us to investigate the role of catabolite repression in *S. rattus* FA-1 *arc* regulation. Wild-type *S. rattus* FA-1 was grown in TV broth containing glucose or galactose, with or without supplemental arginine, to mid-exponential phase and AD activity was measured. Galactose was included in the analysis because it is not repressive for AD expression in *S. gordonii* (19). In wild-type *S. rattus* grown in 2% carbohydrate, peak AD activity was found in cells grown in galactose and arginine, while the expression level decreased by 75% in cells grown in glucose, regardless of the presence of arginine (Fig. 5). In agreement with previous studies (10, 18), catabolite repression of ADS in *S. rattus* FA-1 was not evident when the amount of glucose or galactose present in the growth medium was reduced to 0.2%. This differs from CCR of the ADS in other closely related oral streptococci, including *S. gordonii*, where inclusion of as little as 0.2% glucose in the growth medium strongly represses ADS expression. The different susceptibilities of ADS expression to CCR may reflect different physiological roles of the system in these bacteria. Specifically, susceptibility to CCR at lower carbohydrate concentrations would indicate that the ADS in *S. gordonii* may be primarily involved in energy generation and not acid tolerance, since the system may be repressed when conditions exist that favor low pH, i.e., higher carbohydrate availability. In contrast,
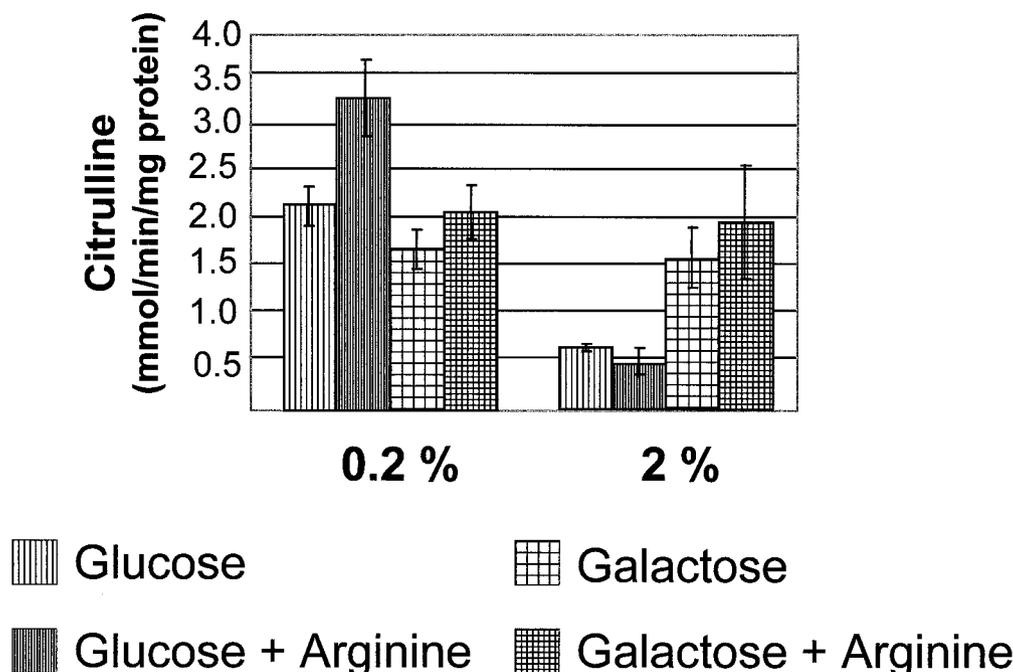
FIG. 5. AD enzyme activity of wild-type (WT) *S. rattus* FA-1 grown in TV broth containing 0.2 or 2% carbohydrate, with or without 1% arginine.

a lack of repression of the ADS in the presence of comparatively high levels of sugar may allow *S. rattus* to derive ATP from arginine and carbohydrate concurrently, and the ammonia generated could enhance the growth in, or protect the organisms from, a low-pH environment. As shown in Fig. 4 and 5, there is some induction of the *S. rattus* ADS by arginine, especially at low carbohydrate concentrations, but the induction is not of the magnitude that is seen in other organisms. This observation lends credence to the idea that there are fundamental differences in regulation of ADS in *S. rattus* compared to that in other bacteria. Further physiological characterization of the *S. rattus arc* operon is ongoing to understand the contribution of the ADS to the acid tolerance of mutans streptococci.

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