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NOTE

Probing the ArcA Regulon in the Rumen Bacterium *Mannheimia succiniciproducens* by Genome-Wide Expression Profiling

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In this study, the putative target genes of the Arc two-component system of the rumen bacterium *Mannheimia succiniciproducens* were determined by analyzing the transcriptome of the ArcA overexpression strain and by the *in silico* scanning of the entire genome sequence with the position weight matrix of the ArcA binding sequence developed for *Escherichia coli*. The majority of 79 repressed genes were involved in energy metabolism and carbohydrate transport and metabolism, while the majority of 82 induced genes were involved in hypothetical or unknown functions. Our results suggest that the Arc system in *M. succiniciproducens* has a specific function that differs from that in *E. coli*.

Keywords: two-component signal transduction system, ArcA response regulator, target genes, *Mannheimia succiniciproducens*

Two-component signal transduction systems, which consist of a membrane-bound histidine sensor kinase and a cytosolic response regulator, play critical roles in cellular processes and adaptation to environmental changes in most bacteria as well as some archaea, fungi, and plants (Stock *et al.*, 1989; Scharf, 2010). The ArcB/A (anoxic redox control) two-component signal transduction system of *Escherichia coli* modulates the expressions of numerous genes depending on the redox conditions of growth (Georgellis *et al.*, 1999, 2001a; Malpica *et al.*, 2006). Under an anoxic condition, the ArcB sensor kinase senses the redox state of the quinone pool and autophosphorylates and transphosphorylates its cognate response regulator ArcA (Georgellis *et al.*, 1997, 1998; Kwon *et al.*, 2003; Malpica *et al.*, 2004; Liu *et al.*, 2009). Phosphorylated ArcA (ArcA-P) can bind to its target promoters and either activate or repress their expressions (Kwon *et al.*, 2000a, 2000b; Rodriguez *et al.*, 2004; Peña-Sandoval *et al.*, 2005). Based on various approaches relying on functional genomics and bioinformatics in combination with genetic manipulation, the functional catalogues of the ArcA target genes in *E. coli* have been expanded to include not only the genes involved in redox metabolism but also numerous genes that are involved in diverse cellular functions (Liu and De Wulf, 2004; Salmon *et al.*, 2005). In addition, Arc systems have species-specific functions according to recent studies of Arc systems in other facultative anaerobic bacteria, including *Haemophilus influenza* and *Shewanella oneidensis* (Georgellis *et al.*, 2001b; Wong *et al.*, 2007; Gao *et al.*, 2008). In this study, we investigated the putative target genes of a recently identified Arc system of the capnophilic rumen bacterium *Mannheimia succiniciproducens*. Functional genomic and bioinformatic approaches were employed due to the limited availability of molecular genetic tools for this organism.

The ArcB/A two-component signal transduction system was identified by a blast search of the complete genome sequence of the rumen bacterium *M. succiniciproducens* and their *in vitro* autophosphorylation and transphosphorylation properties were characterized (Jung *et al.*, 2008). The ArcB of *M. succiniciproducens* shows only 48% of amino acid sequence identity with the *E. coli* ArcB and lacks the PAS domain, which contains the two redox-active cysteine residues critical for redox signaling by the *E. coli* ArcB protein. Moreover, it has been shown that unlike the *E. coli* ArcB, the *in vitro* kinase activity of ArcB of *M. succiniciproducens* is not affected by quinone compounds or by anaerobic metabolites. Thus, it has been suggested that the ArcB sensor kinase of *M. succiniciproducens* employs signaling molecules and regulatory modes that differ from those of the best characterized ArcB protein of *E. coli*. The ArcA protein is a member of the OmpR/PhoB subfamily of response regulators; it has an N-terminus receiver domain with a conserved aspartate residue and a C-terminus helix-turn-helix DNA binding domain. The *M. succiniciproducens* ArcA exhibits a relatively high degree of homology with other bacterial ArcA proteins (Fig. 1). It shows 73% of amino

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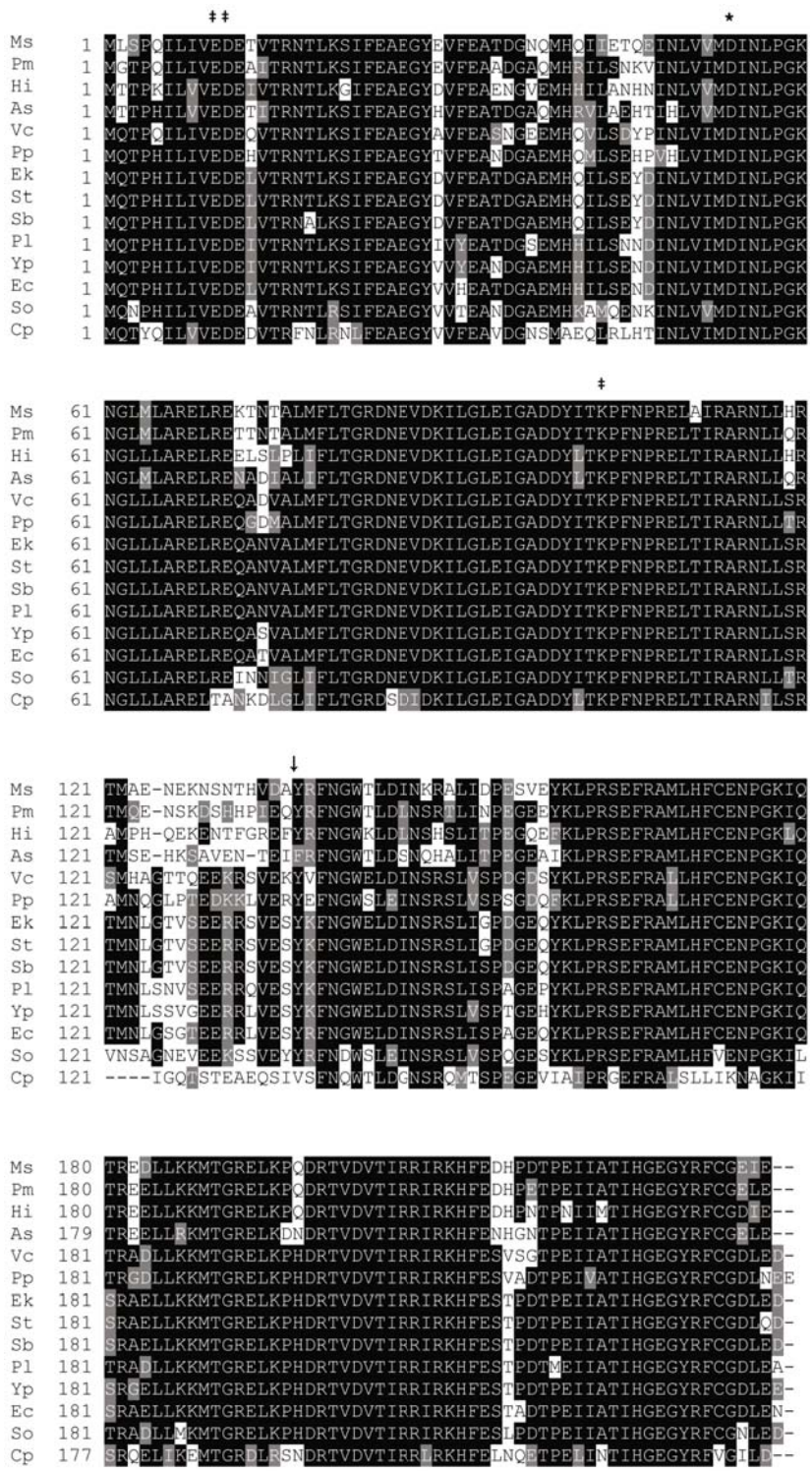

Fig. 1. Alignments of predicted ArcA proteins. ArcA proteins from *Mannheimia succiniciproducens* MBEL55E (Ms, YP_088696), *Pasteurella multocida* multocida Pm70 (Pm, AAK02303), *Haemophilus influenzae* Rd KW20 (Hi, NP_439045), *Actinobacillus succinogenes* 130Z (As, YP_001344184), *Vibrio cholerae* LMA3984-4 (Vc, AEA79276), *Photobacterium profundum* SS9 (Pp, YP_128772), *Escherichia coli* K-12 MG1655 (Ek, NP_418818), *Salmonella enterica* serovar Typhimurium ST4/74 (St, ADX20369), *Shigella boydii* CDC 3083-94 (Sb, ACD10227), *Photorhabdus luminescens* laumondii TTO1 (Pl, NP_927912), *Yersinia pestis* biovar Microtus 91001 (Yp, NP_994996), *Erwinia carotovora* subsp. *atroseptica* SCRI1043 (Ec, YP_051981), *Shewanella oneidensis* MR-1 (So, AE015829_6), and *Colwellia psychrerythraea* 34H (Cp, AAZ24417). * indicates the conserved aspartate residue which receives the phosphoryl group from ArcB sensor kinase. ‡ indicates conserved residues consisting of an acid pocket. ↓ indicates the boundary between receiver domain and helix-turn-helix domain.

acid sequence identity with the *E. coli* ArcA protein, suggesting the possibility to recognize target promoters with a binding motif similar to that of *E. coli* ArcA (Fig. 1).

To identify the putative ArcA target gene in *M. succiniciproducens*, we employed a strategy of examining the changes in the genome-wide expression at the transcriptional level using whole genome DNA microarray in response to the overexpression of the ArcA proteins. In general, a two-component signal transduction system should be activated by signals to modulate the expression of its target genes. However, it has been reported that overexpressed response regulators were able to modulate the expression of their target genes by bind-

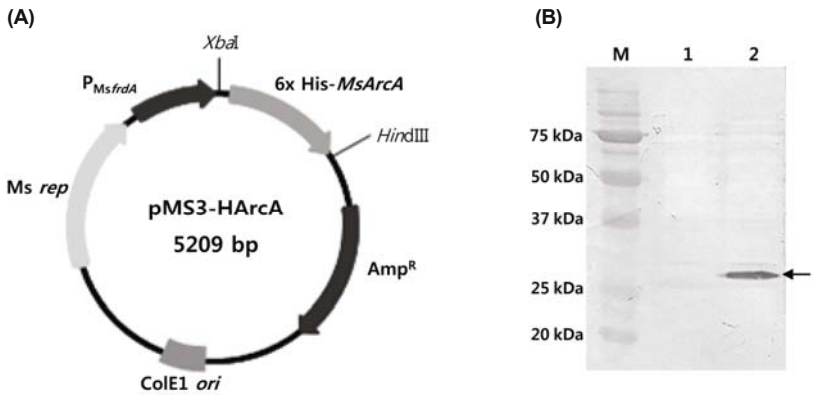

Fig. 2. Overexpression of ArcA in *M. succiniciproducens*. (A) The MsArcA overexpression vector pMS3-HArcA contains the promoter of the fumarate reductase operon of *M. succiniciproducens* to direct the expression of the cloned *MsarcA* gene. (B) The overexpression levels of MsArcA were compared between the transformants harboring the control vector pMS3 (lane 1) and pMS3-HArcA (lane 2) by western blotting. M, protein markers; arrow indicates the 29.5 kDa MsArcA.

ing to promoters even without phosphorylation by cognate sensor kinases in the absence of environmental signals (Masuda and Church, 2002).

To construct the ArcA overexpression vector, plasmid pMS3, an *E. coli-M. succiniciproducens* shuttle vector (Jung *et al.*, 2008) containing the promoter of the fumarate reductase operon of *M. succiniciproducens*, was used. In brief, a DNA fragment containing the sequence of the Ms*arcA* gene and the N-terminus 6xHis-tag of the MsArcA expression vector pMSHarcA (Jung *et al.*, 2008) was subcloned into pMS3, yielding pMS3-HArcA (Fig. 2A). *M. succiniciproducens* MBEL55E (KCTC0769BP) (Lee *et al.*, 2002) containing pMS3 or the pMS3-HArcA plasmid were cultured in a tryptic soy broth (TSB; Difco Laboratories, USA) medium with 25 μg/ml ampicillin until the late log phase (OD_{600}=0.8–1.0) was reached at 39°C in a 10% CO_2 atmosphere. The cell pellets were harvested and mixed with lysis buffer containing 50 mM Tris-Cl, 250 mM NaCl, and a 5× electrophoresis sample buffer. The samples were boiled for 10 min and analyzed by electrophoresis on 12% acrylamide SDS-polyacrylamide gel electrophoresis (PAGE) gels. Proteins were transferred onto a nitrocellulose membrane at 80 V for 30 min and blocked with 50 ml Tris-buffered saline Tween (TBST) and 2.5 g skin milk for 40 min at room temperature. The membrane was incubated with 1/1,000 primary antibody His-tag rabbit polyclonal IgG (Santa Cruz Biotechnology, USA) for 2 h and washed with TBST buffer 3 times. It was then incubated with 1/5,000 secondary antibody horseradish peroxidase (HRP)-labeled anti-rabbit IgG (Santa Cruz Biotechnology, USA) for 40 min and washed 4 times. HRP was detected with the ECL Advance^{TM} western blotting detection kit (GE Healthcare, UK). As shown in Fig. 2B, the MsArcA was overexpressed in the transformant harboring pMS3-HArcA.

For a transcriptome analysis, *M. succiniciproducens* transformants harboring pMS3 or pMS3-HArcA were cultured under the same conditions used for the preparation of the samples for the western blot analysis. Total RNA was isolated by hot phenol method (Sarmientos *et al.*, 1983) and further purified with RNeasy Mini Kits (QIAGEN, USA). The quality and amounts of total RNA were checked by equipment from NanoDrop ND-1000 Spectrophotometer (Nanodrop, USA).

Mixtures of total RNA (20 μg) and random primers (GeneChem, Korea) were heated at 70°C for 10 min and quickly chilled on ice for 1 min. 5× SuperScript III First Strand buffer, 0.1 M DTT, 50× aminoallyl-dNTP mixtures, RNasin, and Superscript III reverse transcriptase (Invitrogen, USA) were sequentially added and incubated at 50°C for 2 h. To the reaction mixture, 1 M NaOH and 0.5 M EDTA were added, after which the mixture was incubated at 65°C for 15 min for the degradation of the RNA before neutralization by adding 1 M HCl. The resulting cDNAs were purified and

Table 1. Primers used in this study

Name	Sequence (5′→ 3′)	Product size (bp)
RT-PCR		
MS0225-F	ATTGTTATGGCACAAATTGCACAA	579 bp
MS0225-R	TGGAATATTATCGGAGCTATCTC	
MS0459-F	GGCAGACGCAGTAAAAGTGA	253 bp
MS0459-R	TACCGCTTTTAAACCTTCG	
MS0271-F	CGTAAAAATGGTAAAGTCGC	325 bp
MS0271-R	GTTCAATCACATCACCGCTG	
MS0831-F	AAAGATCAAGCTCTAACTGC	329 bp
MS0831-R	GAACTGAGACTCAAAAAGAC	
MS1455-F	CCGACACCTTGTTTGGAGCG	269 bp
MS1455-R	AGTAATTTACGGTATGCC	
MS0710-F	GCAGGTCATAGTAAGTGGGC	307 bp
MS0710-R	CGCTTAAACATTCCACCATC	
MS0039-F	TACAGATGTTGTTCTTCGTG	491 bp
MS0039-R	TGTCAATACCTATCTCAAGC	
MS2321-F	TCACCGGCTGGAGAGACG	271 bp
MS2321-R	ACTTGTTCGTCACCGTGTTC	
MS1652-F	GTCTAAGGTCTATCCAATGC	443 bp
MS1652-R	TTTGTACCAGTTTACCTTCC	
EMSA		
MS0458-F	TGCTTATTGTTATTGCTTCC	345 bp
MS0458-R	AGAGTTTCTACTTCTTCACG	
MS0831-F	CTTTTCCTCATAGTTTGGCG	334 bp
MS0831-R	ATTCGTTTAACCAGATTTCC	
MS1455-F	TGCTGTCGAGTGCTTTAGGC	321 bp
MS1455-R	TATCCCAATGCCCTAAACAG	
MS0710-F	TAGGTTATGCATAGTTCAGC	320 bp
MS0710-R	CACTTACTATGACCTGCC	
MS2321-F	TTTGCGTGCGTTTTTGCTCG	328 bp
MS2321-R	TAATTCCATAAAAATCTCCC	

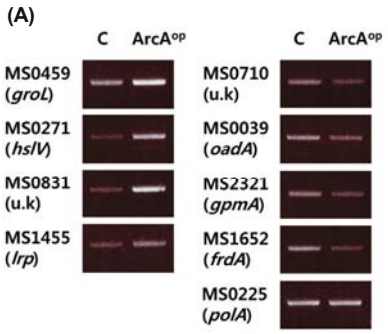

Fig. 3. Semi-quantitative RT-PCR and electrophoretic mobility shift assay. (A) Expressions of four up-regulated genes (left panel), four down-regulated genes (right panel), and *polA* as an internal control in wild-type *M. succiniciproducens* strains transformed with the pMS3 control vector C or pMS3-HArcA (ArcA^{op}) as compared by semi-quantitative RT-PCR. The names of genes are indicated in parentheses. u.k, unknown. (B) The gel shift assay was performed with ^{32}P-labeled DNA probes containing the promoter region of putative ArcA target genes in the presence (+) or absence (-) of the His$_6$-MsArcA protein. 1, MS0458; 2, MS0831; 3, MS1455; 4, MS0710; 5, MS2321.

Table 2. Differentially expressed genes of *M. succiniciproducens* in response to overexpression of ArcA protein

FunCat[a]	Up-regulated genes (Gene name, Fold change[b])	Down-regulated genes (Gene name, Fold change[b])
C	MS1352 (*sucC*, 2.32), MS2371 (*gltA*, 2.25), MS0587 (*torC*, 2.24)	MS2221 (*ppa*, -2.08), MS2347 (*atpG*, -2.15), MS0999 (*ackA*, -2.38), MS2369 (*acnB*, -2.74), MS0308 (*nqrB*, -2.9), MS1655 (*frdD*, -3.06), MS1653 (*frdB*, -3.08), MS0401 (*pflD*, -3.16), MS1652 (*frdA*, -3.44), MS1336 (*aceE*, -3.8), MS1654 (*frdC*, -3.88), MS0305 (*nqrE*, -3.92), MS0307 (*nqrC*, -4.41), MS0309 (*nqrA*, -5.12), MS0040 (*oadG*, -5.21), MS0038 (*oadB*, -6.68), MS0306 (*nqrD*, -7.1), MS0039 (*oadA*, -7.81), MS0292 (*hmp*, -8.13)
D	MS1662 (*ftsA*, 4.35), MS1454 (*ftsK*, 2.79)	
E	MS1627 (*metC*, 5.51), MS1199 (*dcp*, 2.88), MS0265 (*dapA*, 2.21)	MS0001 (*ubiE*, -2.09), MS2223 (*ilvB*, -2.15), MS1177 (*dapD*, -2.38), MS0812 (*malK*, -2.51), MS1319 (*ilvB*, -2.51), MS1613 (*lysC*, -3.15), MS0809 (*potD*, -3.49), MS0196 (*gdhA*, -5.03)
F	MS1738 (*gmk*, 2.29)	MS1477 (*cmk*, -2.09), MS2318 (*udp*, -2.1), MS1582 (*udk*, -2.55)
G	MS0187 (*glpR*, 4.3), MS1606 (*dAK1*, 3.22), MS1744 (*rpiA*, 2.58), MS1612 (*rbsB*, 2.5), MS0199 (*araH*, 2.38)	MS2254 (*glpX*, -2.04), MS0998 (*pta*, -2.18), MS2180 (*ptsN*, -2.31), MS0256 (*eno*, -2.32), MS1509 (*ptsA*, -2.35), MS1739 (*gapA*, -2.55), MS1508 (*nagE*, -2.56), MS1233 (*rbsK*, -2.99), MS0245 (*pgk*, -3.15), MS1122 (*glgX*, -3.42), MS2321 (*gpmA*, -6), MS0617 (*manY*, -12.01)
H	MS1254 (*cysG*, 2.5), MS1752 (*ribF*, 2.11)	
I		MS1174 (*accD*, -2.02), MS1535 (*ispE*, -2.24)
J	MS0963 (*ftsJ*, 5.34), MS0240 (*trmA*, 3.9), MS0375 (u.k, 2.82), MS0132 (*sUA5*, 2.69), MS0493 (*pnp*, 2.16), MS0440 (*rpsP*, 2.07), MS2025 (*rpsK*, 2.07), MS0556 (*pth*, 2.06)	MS2036 (*rplE*, -2.06), MS1756 (*rpsT*, -2.09), MS1558 (*queA*, -2.12), MS1933 (*rpsB*, -2.2), MS2049 (*rpsJ*, -2.3), MS2367 (*trmA*, -2.46), MS0209 (*rplJ*, -2.47), MS1943 (*rpmG*, -2.54), MS2048 (*rplC*, -2.77), MS1762 (*rpsU*, -2.84), MS2043 (*rplV*, -4.86), MS1090 (*pheT*, -7.33)
K	MS2228 (*rpoE*, 3.61), MS0473 (*vacB*, 3.56), MS1455 (*lrp*, 3.04)	MS1322 (*hns*, -2.39),
L	MS1135 (*dinP*, 5.61), MS1368 (*uvrD*, 3.03), MS0712 (*ruvA*, 2.93), MS0803 (*hlpB*, 2.91), MS0131 (*topA*, 2.13)	
M	MS0188 (*glmS*, 3.24), MS1830 (*rlpA*, 3.17), MS0865 (*kefA*, 3.07), MS0189 (*glmS*, 2.95), MS0267 (*nlpB*, 2.9), MS0417 (*wbbJ*, 2.65), MS1664 (*ddlA*, 2.6), MS1750 (*lspA*, 2.5), MS0489 (u.k, 2.3), MS1568 (*dltE*, 2.18)	MS1337 (*ompC*, -2.07), MS1220 (*ompA*, -2.46), MS1515 (*amiC*, -2.54), MS1534 (*lolB*, -2.54), MS1670 (*rfe*, -2.59), MS1734 (*murI*, -2.78), MS1218 (*ompA*, -3.27), MS1219 (*ompA*, -10.14)
O	MS0458 (*groS*, 10.67), MS0459 (*groL*, 7.67), MS0271 (*hslV*, 5.06), MS0993 (*degQ*, 5.06), MS0964 (*hflB*, 2.42), MS1561 (*sirA*, 2.14)	MS2199 (*slpA*, -3.35)
P	MS0320 (*corA*, 3.82), MS0376 (*mMT1*, 3.31), MS0160 (*dsrF*, 3.17)	MS0430 (*nhaB*, -2.83)
Q	MS1711 (u.k, 2.42), MS1712 (u.k, 2.09)	
S	MS1256 (7.07), MS0825 (5.29), MS0831 (4.9), MS1644 (4.25), MS2210 (3.75), MS1645 (3.68), MS1328 (3.6), MS2141 (3.55), MS0177 (3.32), MS0841 (3.23), MS0012 (3.18), MS1688 (3.18), MS2209 (3.12), MS0861 (2.68), MS0568 (2.67), MS1369 (2.62), MS0158 (2.54), MS0802 (2.5), MS0318 (2.43), MS0134 (2.31), MS0475 (2.24), MS0936 (2.18), MS0355 (2.15), MS2304 (2.1)	MS1787 (-2.02), MS2308 (-2.31), MS0482 (-2.34), MS0372 (-2.78), MS2317 (-2.81), MS1139 (-4.63), MS0289 (-9.71), MS0710 (-10.27), MS1733 (-30.69)
T	MS1504 (*arcA*, 4.57), MS2229 (*rseA*, 3.72)	
U	MS0494 (*nrfG*, 4.1), MS0514 (*exbD*, 2.66), MS0515 (*tolQ*, 2.42)	MS1563 (*secD*, -2.75), MS1782 (*flp1*, -3.99), MS0723 (*tolB*, -4.72)

[a] Letters indicate the functional categories of the proteins encoded by DEG. C, energy production and conversion; D, cell cycle control, mitosis, and meiosis; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; G, carbohydrate transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; J, translation; K, transcription; L, replication, recombination, and repair; M, cell wall/membrane biogenesis; O, posttranslational modification, protein turnover, and chaperones; P, inorganic ion transport and metabolism; Q, secondary metabolites biosynthesis, transport, and catabolism; S, function unknown; T, signal transduction mechanisms; U, intracellular trafficking and secretion.
[b] Positive and negative numbers indicate fold changes of induction and repression upon ArcA overexpression, respectively.
u.k : unknown genes

concentrated using a Microcon YM-30 device (Millipore, Ireland). These cDNAs of *M. succiniciproducens* with pMS3-HArcA or pMS3 were mixed with 1 M Na_2CO_3 and labeled with Cy5- and Cy3-, respectively, using an Aminoallyl Post DNA Labeling Kit (GeneChem, Korea) according to manufacturer's instruction.

The labeled cDNAs were purified and incubated with a hybridization buffer at 95°C for 5 min, applied to a prehybridized *M. succiniciproducens* oligo chip (Genotech, Korea) slide, and incubated in a dark humidified chamber at 42°C for 16 h. 2,400 of 55-mer probes covering the ORF in the whole genome of *M. succiniciproducens* MBEL55E were spotted on the oligo chip in duplicates. The chip slides were washed with 2× SSC and 0.1% SDS, 2× SSC, 0.2× SSC, and HPLC water in that order and were analyzed using a GenePix 4000B microarray scanner and GenePix Pro 4.0 software (Axon, USA).

To validate the DNA microarray data, we carried out semi-quantitative reverse transcription PCR (RT-PCR) as described previously (Lee *et al.*, 2012). Expressions of nine genes, including four up-regulated, four down-regulated, and *polA* as a control, were analyzed by PCR using specific primer pairs (Table 1) using the cDNAs synthesized from the total RNAs of *M. succiniciproducens* transformants harboring pMS3 or pMS3-HArcA. As shown in Fig. 3A, there was a close correlation between the microarray data and the RT-PCR analysis, validating the results of the DNA microarray. Furthermore, to test whether ArcA indeed interacts with the putative target genes identified by the microarray and the *in silico* prediction, we performed an electrophoretic mobility shift assay (EMSA). Recombinant His_6-tagged MsArcA protein was prepared as described previously (Jung *et al.*, 2008). The probes used for EMSA were prepared by PCR using *M. succiniciproducens* genomic DNA as a template and primer pairs (Table 1) and were end-labeled using [γ-^{32}P] ATP and T4 polynucleotide kinase (Fermentas, USA). The binding reaction was performed with 2 fmol (0.2 nM) labeled probes and 50 pmol (5 μM) protein in 10 μl of binding buffer containing 10 mM Tris (pH 7.5), 10% glycerol, 1 mM EDTA, 50 mM KCl, 10 mM $MgCl_2$, 2 mM DTT, and 0.2 mg/ml polydeoxyinosinic-deoxycytidylic acid sodium salt (poly d(I-C), Sigma, USA) for 20 min at room temperature and resolved on pre-run 4% polyacrylamide native gel as described previously (Lynch and Lin, 1996; Hellman and Fried, 2007). The gel was dried and analyzed with a BAS-1500 phosphoimage analyzer (Fujifilm, Japan). As shown in Fig. 3B, the His_6-MsArcA protein was able to bind DNA fragments containing the upstream region of the initiation codon of the putative ArcA target genes. However, further studies, such as EMSA and DNaseI protection assays using phosphorylated or mutated ArcA proteins are required to determine the detailed features pertaining to the interaction between ArcA and its target promoters.

When the transcript profile of the *M. succiniciproducens* strain harboring the ArcA overexpression vector pMS3-HArcA was compared to that of the *M. succiniciproducens* strain harboring the pMS3 control vector, the expressions of 161 genes were changed by more than two-fold (Table 2). When these differentially expressed genes (DEGs) were grouped according to their functional categories based on COG (Hong *et al.*, 2004), the majority of the down-regulated genes were determined to belong to functional categories related to energy production and conversion and carbohydrate transport and metabolism. The majority of the up-regulated genes were grouped into functional categories related to cell wall and membrane biogenesis and translation. However, it is important to note that more than 20% of the total DEGs belonged to the group of hypothetical genes encoding proteins with unknown functions.

Transcription of the 79 genes was down-regulated in the ArcA overexpression strain compared to a control strain. 12 and 19 of these genes encode proteins in carbohydrate transport and metabolism and in energy production and conversion, respectively. The overexpression of ArcA resulted in the repression of many genes involved in glycolysis (Fig. 4). The expressions of several genes involved in pyruvate metabolism, such as *oadBAG* (oxaloacetate decarboxylase), *pflD* (pyruvate formate lyase), *aceE* (pyruvate dehydrogenase E1 subunit), and *ackA* (acetate kinase), were also repressed. In addition, the expressions of genes involved in the tricarboxylic acid (TCA) cycle, such as the *frdABCD* (fumarate reductase) and *acnB* (bifunctional aconitate hydratase 2/2-methylisocitrate dehydratase), were down-regulated by ArcA overexpression. It was reported earlier that in *E. coli*, ArcA functions as a repressor for some genes involved in carbon and energy metabolism, such as TCA-cycle components under anaerobic growth conditions (Salmon *et al.*, 2005). However, it appears that ArcA can repress the expression of genes involved in a broader range of metabolism in *M. succiniciproducens* compared to that in *E. coli*. Another interesting feature associated with the ArcA-dependent gene repression in *M. succiniciproducens* is the fact that many genes involved in translation, such as ribosomal proteins, were down-regulated upon ArcA overexpression.

Transcription of the 82 genes in *M. succiniciproducens* was up-regulated more than two-fold in the ArcA overexpression strain compared to the control strain. Ten genes involved in cell wall/membrane biogenesis, such as *glmS* (glucosamine 6-phosphate synthetase) and *ddlA* (D-alanyl-D-alanine ligase), were up-regulated by ArcA overexpression. In addition, several genes involved in translation and post-translational modification, protein turnover, and chaperones were up-regulated upon ArcA overexpression. This category includes the most strongly induced genes by ArcA overexpression, *groS* and *groEL*, which encode the GroS and GroEL proteins of GroESL chaperonin, respectively, as well as several protease genes, such as *degQ*, *hslV*, and *hflB*. The remainder of the up-regulated genes includes two genes involved in the TCA cycle, *gltA* encoding citrate synthase and the *sucC* encoding succinyl-CoA synthetase beta subunit. The expressions of *rpoE* encoding sigma factor E, *rseA* encoding negative regulator of sigma E activity, and *arcA* were also induced upon ArcA overexpression. Surprisingly, the observed level of *arcA* induction was lower than expected when considered the expression from the multi-copy pMS3-HArcA vector. This may have been due to the repression of the promoter of the *frdABCD* operon, as described above.

As an *in silico* search for the putative ArcA target genes, we inspected the genome sequence of *M. succiniciproducens* with the ArcA recognition sequence developed for *E. coli*

K12 (Liu and De Wulf, 2004) using the web-based program Virtual Footprint, version 3.0 (http://prodoric.tu-bs.de/vfp/). Virtual Footprint is a program for DNA pattern prediction in whole prokaryotic genomes using position weight matrices (PMWs) provided by a database of prokaryotic gene expression and transcription factor binding sites (Munch *et al.*, 2003, 2005). When the genome sequence of *M. succiniciproducens* was scanned with this program using a 15 bp *E. coli* ArcA recognition weight matrix provided by the DPInteract database (http://arep.med.harvard.edu/ecoli_matrices), 37 genes in total with an ArcA recognition sequence in their intergenic regions were identified. When this list of genes was compared with the list of DEGs obtained using a microarray upon ArcA overexpression, it was found that

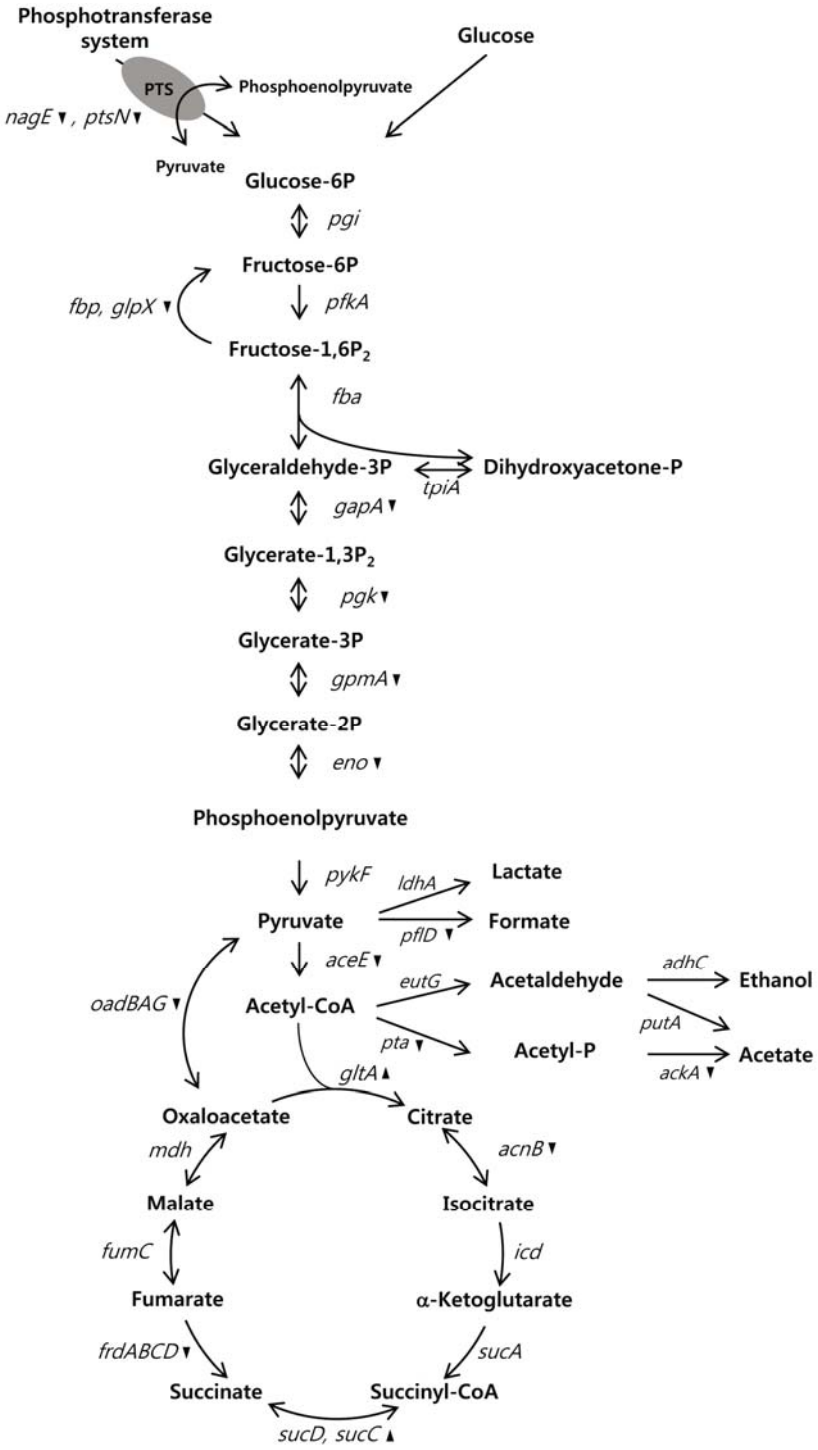

Fig. 4. Graphical representation of the changes in the levels of the expression genes involved in the central metabolism of *M. succiniciproducens* upon ArcA overexpression. Triangles and inverted triangles next to the gene names indicate the induced genes and the reduced genes, respectively.

Table 3. Putative ArcA regulons in *M. succiniciproducens* MBEL55E

ORF No.	Gene or operon	Strand	Binding sequences[a]	Function of genes[b]	Fold change
MS0001		+	GTTAAATAATATTTT…(N_{177})…ATG	Hypothetical protein	-2.09
MS0040	*oadG*	-	TTTAATATTAATAAA …(N_{93})…ATG	Oxaloacetate decarboxylase, γ subunit	-5.21
MS0196	*gdhA*	+	GTTGAATGAAATATA…(N_{120})…TTG	Glutamate dehydrogenase	-5.03
MS0202		-	TTTAATTAAAAGTAT…(N_{56})…ATG	Hypothetical protein	N/A
MS0239	*recO*	+	GTTAAAGAATCCGGA…(N_{299})…GTG	DNA repair protein	N/A
MS0245	*pgk*	-	GTTAAAAATAATAAA…(N_{16})…ATG	Phosphoglycerate kinase	-3.15
MS0289		-	GTAAATATTAACTCA…(N_{12})…ATG	Predicted permease	-9.71
MS0355		-	GTTAATTCAGCTGAT…(N_{248})…TTG	Hypothetical protein	2.15
MS0372		-	GTTAACAAAATTTAA…(N_{173})…ATG	Autonomous glycyl radical cofactor GrcA	-2.79
MS0373	*ung*	+	TTAAATTTTCAACTA…(N_{310})…ATG	Uracil-DNA glycosylase	N/A
MS0430	*nhaB*	-	GTTAATTAAGTATAT…(N_{21})…ATG	Na^+/H^+ antiporter	-2.83
MS0458	*groS*	+	GTTATCAATAGCGGA…(N_{331})…TTG	GroS protein Co-chaperonin GroES (HSP10)	10.67
MS0484	*rpmH*	-	GTTAATTGAAAAATA…(N_{346})…ATG	50S ribosomal protein L34	N/A
MS0556	*pth*	-	GTTAAGTAGAATTAA…(N_{51})…ATG	Peptidyl-tRNA hydrolase	2.06
MS0618		-	GTTAAAGACCTGAAA…(N_{403})…ATG	Hypothetical protein	N/A
MS0710		+	GTTAAGCAAATTAAA…(N_{104})…ATG	Uncharacterized ACR	-10.27
MS0825		+	TTTAATGGCAAGTCT…(N_{58})…ATG	Hypothetical protein	5.29
MS0831		-	GTTAATCGTTTGCTT…(N_{172})…ATG	Hypothetical protein	4.90
MS0863	*seqA*	+	GTTAATTGCAGGGTT…(N_{325})…ATG	Replication initiation regulator	N/A
MS0964	*hflB*	+	GTTAATAAATTGTTA…(N_{21})…TTG	ATP-dependent Zn protease	2.42
MS1218	*ompA*	-	GTTAAAGAAATGAAT…(N_{158})…ATG	Outer membrane protein and related peptidoglycan-associated (lipo)protein	-3.27
MS1319	*ilvB*	-	TTTAATTAAATTAGA…(N_{130})…GTG	Acetolactate synthase 3 catalytic subunit	-2.51
MS1322	*hns*	+	GTTAAAAAACAGGTT…(N_{169})…ATG	DNA-binding protein H-NS	-2.39
MS1336	*aceE*	-	GTTAAAAAAATGTAA…(N_{195})…ATG	Pyruvate dehydrogenase	-3.80
MS1455	*lrp*	-	GTTAATAAGTTGTTA…(N_{177})…ATG	Leucine-responsive transcriptional regulator	3.05
MS1534	*lolB*	+	GTTAATTATTAAGTT…(N_{112})…ATG	Outer membrane lipoprotein involved in outer membrane biogenesis	-2.54
MS1582	*udk*	-	GTTAAATAACATGAT…(N_{161})…ATG	Uridine kinase	-2.55
MS1612	*rbsB*	+	GTTAAATCACAAAAT…(N_{127})…ATG	Periplasmic sugar-binding protein	2.50
MS1688		-	GTTAATTGTGAATTA…(N_{111})…ATG	Hypothetical protein	3.18
MS1713		-	GTTAATAAATAAAGC…(N_{211})…TTG	Hypothetical protein	N/A
MS1752	*ribF*	-	GTTAAATAAATAGAA…(N_{11})…ATG	Bifunctional riboflavin kinase / FMN adenylyltransferase	2.11
MS1756	*rpsT*	+	GTTAATTTCATCGCT…(N_{164})…ATG	30S ribosomal protein S20	-2.09
MS2199	*slpA*	+	GTTAAAAAAATTTTT…(N_{18})…ATG	FKBP-type peptidyl-prolyl cis-trans isomerase 2	-3.35
MS2223	*ilvB*	-	GTTAAATAAGAATAA…(N_{38})…ATG	Acetolactate synthase 2 catalytic subunit	-2.15
MS2254	*glpX*	+	TTTAACTCTTCCACT…(N_{273})…ATG	Fructose 1,6-bisphosphatase II	-2.04
MS2321	*gpmA*	+	GTTAAATAATATCAT…(N_{109})…ATG	Phosphoglyceromutase	-6.00
MS2370	*icd*	-	TTTAAATAATCCTAT…(N_{86})…ATG	Isocitrate dehydrogenase	N/A

[a] Positions of the inverted repeats of the potential ArcA-binding sites are indicated for the given DNA strand with respect to the translation initiation codon.
[b] Known and putative functions for each regulon member based on genome annotations are indicated.
N/A, less than two-fold change.

29 genes were overlapped (Table 3).

In this study, we attempted for the first time to elucidate the putative functions of the Arc system in the rumen bacterium *M. succiniciproducens*. It may not be possible to conclude whether each putative target is regulated by the Arc system directly or indirectly, as they were selected solely based on their differential expressions in response to ArcA overexpression. However, it is noteworthy that 70% of the putative ArcA target genes identified by the *in silico* prediction method were differentially expressed under this condition. When considering the high degree of amino acid sequence homology between the ArcA proteins of *E. coli* and *M. succiniciproducens*, it can be assumed that MsArcA binds to a target sequence similar to that of the ArcA of *E. coli*, as demonstrated previously with ArcA of *S. oneidensis* (Gao *et al.*, 2008). Thus, our results taken together indicate that the Arc system of *M. succiniciproducens* may be involved in adaptive redox dependent gene regulation in response to signals other than quinone compounds, as suggested based on an *in vitro* study of recombinant Arc proteins (Jung *et al.*, 2008). This system may be responsible for a stress response which requires a central metabolism reduction and the induction of mechanisms of recovery from damage. However, further molecular genetic and functional genomic characterizations of the predicted ArcA target genes in wild-type and *arc* mutant strains under various growth conditions

are necessary to understand the physiological role of the Arc system in *M. succiniciproducens*.

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References

Gao, H., Wang, X., Yang, Z.K., Palzkill, T., and Zhou, J. 2008. Probing regulon of ArcA in *Shewanella oneidensis* MR-1 by integrated genomic analyses. *BMC Genomics* **9**, 42.

Georgellis, D., Kwon, O., De Wulf, P., and Lin, E.C. 1998. Signal decay through a reverse phosphorelay in the arc two-component signal transduction system. *J. Biol. Chem.* **273**, 32864–32869.

Georgellis, D., Kwon, O., and Lin, E.C. 1999. Amplification of signaling activity of the arc two-component system of *Escherichia coli* by anaerobic metabolites - An *in vitro* study with different protein modules. *J. Biol. Chem.* **274**, 35950–35954.

Georgellis, D., Kwon, O., and Lin, E.C. 2001a. Quinones as the redox signal for the Arc two-component system of bacteria. *Science* **292**, 2314–2316.

Georgellis, D., Kwon, O., Lin, E.C., Wong, S.M., and Akerley, B.J. 2001b. Redox signal transduction by the ArcB sensor kinase of *Haemophilus influenzae* lacking the PAS domain. *J. Bacteriol.* **183**, 7206–7212.

Georgellis, D., Lynch, A.S., and Lin, E.C. 1997. *In vitro* phosphorylation study of the Arc two-component signal transduction system of *Escherichia coli*. *J. Bacteriol.* **179**, 5429–5435.

Hellman, L.M. and Fried, M.G. 2007. Electrophoretic mobility shift assay (EMSA) for detecting protein-nucleic acid interactions. *Nat. Protoc.* **2**, 1849–1861.

Hong, S.H., Kim, J.S., Lee, S.Y., In, Y.H., Choi, S.S., Rih, J.K., Kim, C.H., Jeong, H., Hur, C.G., and Kim, J.J. 2004. The genome sequence of the capnophilic rumen bacterium *Mannheimia succiniciproducens*. *Nat. Biotechnol.* **22**, 1275–1281.

Jung, W.S., Jung, Y.R., Oh, D.B., Kang, H.A., Lee, S.Y., Chavez-Canales, M., Georgellis, D., and Kwon, O. 2008. Characterization of the Arc two-component signal transduction system of the capnophilic rumen bacterium *Mannheimia succiniciproducens*. *FEMS Microbiol. Lett.* **284**, 109–119.

Kwon, O., Georgellis, D., Lynch, A.S., Boyd, D., and Lin, E.C. 2000a. The ArcB sensor kinase of *Escherichia coli*: genetic exploration of the transmembrane region. *J. Bacteriol.* **182**, 2960–2966.

Kwon, O., Georgellis, D., and Lin, E.C. 2000b. Phosphorelay as the sole physiological route of signal transmission by the arc two-component system of *Escherichia coli*. *J. Bacteriol.* **182**, 3858–3862.

Kwon, O., Georgellis, D., and Lin, E.C. 2003. Rotational on-off switching of a hybrid membrane sensor kinase Tar-ArcB in *Escherichia coli*. *J. Biol. Chem.* **278**, 13192–13195.

Lee, E.G., Kim, S., Oh, D.B., Lee, S.Y., and Kwon, O. 2012. Distinct roles of β-galactosidase paralogues of the rumen bacterium *Mannheimia succiniciproducens*. *J. Bacteriol.* **194**, 426–436.

Lee, P.C., Lee, S.Y., Hong, S.H., and Chang, H.N. 2002. Isolation and characterization of a new succinic acid-producing bacterium, *Mannheimia succiniciproducens* MBEL55E, from bovine rumen. *Appl. Microbiol. Biotechnol.* **58**, 663–668.

Liu, X. and De Wulf, P. 2004. Probing the ArcA-P modulon of *Escherichia coli* by whole genome transcriptional analysis and sequence recognition profiling. *J. Biol. Chem.* **279**, 12588–12597.

Liu, X., Peña Sandoval, G.R., Wanner, B.L., Jung, W.S., Georgellis, D., and Kwon, O. 2009. Evidence against the physiological role of acetyl phosphate in the phosphorylation of the ArcA response regulator in *Escherichia coli*. *J. Microbiol.* **47**, 657–662.

Lynch, A.S. and Lin, E.C. 1996. Transcriptional control mediated by the ArcA two-component response regulator protein of *Escherichia coli*: characterization of DNA binding at target promoters. *J. Bacteriol.* **178**, 6238–6249.

Malpica, R., Franco, B., Rodriguez, C., Kwon, O., and Georgellis, D. 2004. Identification of a quinone-sensitive redox switch in the ArcB sensor kinase. *Proc. Natl. Acad. Sci. USA* **101**, 13318–13323.

Malpica, R., Sandoval, G.R., Rodriguez, C., Franco, B., and Georgellis, D. 2006. Signaling by the Arc two-component system provides a link between the redox state of the quinone pool and gene expression. *Antioxid. Redox. Signal.* **8**, 781–795.

Masuda, N. and Church, G.M. 2002. *Escherichia coli* gene expression responsive to levels of the response regulator EvgA. *J. Bacteriol.* **184**, 6225–6234.

Munch, R., Hiller, K., Barg, H., Heldt, D., Linz, S., Wingender, E., and Jahn, D. 2003. Prodoric: prokaryotic database of gene regulation. *Nucleic Acids Res.* **31**, 266–269.

Munch, R., Hiller, K., Grote, A., Scheer, M., Klein, J., Schobert, M., and Jahn, D. 2005. Virtual footprint and prodoric: an integrative framework for regulon prediction in prokaryotes. *Bioinformatics* **21**, 4187–4189.

Pena-Sandoval, G.R., Kwon, O., and Georgellis, D. 2005. Requirement of the receiver and phosphotransfer domains of ArcB for efficient dephosphorylation of phosphorylated ArcA *in vivo*. *J. Bacteriol.* **187**, 3267–3272.

Rodriguez, C., Kwon, O., and Georgellis, D. 2004. Effect of *D*-lactate on the physiological activity of the ArcB sensor kinase in *Escherichia coli*. *J. Bacteriol.* **186**, 2085–2090.

Salmon, K.A., Hung, S.P., Steffen, N.R., Krupp, R., Baldi, P., Hatfield, G.W., and Gunsalus, R.P. 2005. Global gene expression profiling in *Escherichia coli* K12: effects of oxygen availability and ArcA. *J. Biol. Chem.* **280**, 15084–15096.

Sarmientos, P., Sylvester, J.E., Contente, S., and Cashel, M. 1983. Differential stringent control of the tandem *E. coli* ribosomal RNA promoters from the *rrnA* operon expressed *in vivo* in multicopy plasmids. *Cell* **32**, 1337–1346.

Scharf, B.E. 2010. Summary of useful methods for two-component system research. *Curr. Opin. Microbiol.* **13**, 246–252.

Stock, J.B., Ninfa, A.J., and Stock, A.M. 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. *Microbiol. Rev.* **53**, 450–490.

Wong, S.M., Alugupalli, K.R., Ram, S., and Akerley, B.J. 2007. The ArcA regulon and oxidative stress resistance in *Haemophilus influenzae*. *Mol. Microbiol.* **64**, 1375–1390.