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Identification of the *cadBA* operon from *Vibrio vulnificus* and its influence on survival to acid stress

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Abstract

By a transposon-tagging method, *cadBA* genes encoding a lysine/cadaverin antiporter and a lysine decarboxylase were identified and cloned from *Vibrio vulnificus*. The deduced amino acid sequences of *cadBA* were 64–97% similar to those reported from other Enterobacteriaceae. Functions of *cadBA* genes on acid tolerance were assessed by comparing acid tolerances of *V. vulnificus* and its isogenic mutants, whose *cadBA* genes were separately inactivated by allelic exchanges. The results demonstrated that gene products of *cadBA* contribute to acid tolerance of *V. vulnificus*, and that their contribution is dependent on prior exposure of cells to moderately acidic pH.

Keywords: Acid tolerance; *Vibrio vulnificus cadBA*

1. Introduction

Vibrio vulnificus is an opportunistic Gram-negative pathogen that commonly contaminates raw oysters, and is the causative agent of food-borne diseases such as gastroenteritis and life-threatening septicemia. Predisposed individuals, with underlying immunocompromised conditions, liver damage, or excess levels of iron, who consume raw oysters, can die within days from sepsis. Even otherwise healthy people are susceptible to serious wound infections after contact with shellfish or water contaminated with *V. vulnificus* [1]. Mortality from septicemia is very high ($>50\%$), and death may occur within 1 to 2 days after the first signs of illness.

Bacteria have evolved with elaborate protection systems to allow survival and/or growth during exposure to environmental change. Change in the external acidity is one of the most common environmental stresses that bacteria routinely encounter (see [2,3]). Like many other food-borne pathogenic bacteria, *V. vulnificus* has to cope with ever changing acidity in their growth environments to ensure developing illness. The pathogen has to survive acidic stresses imposed not only by natural ecosystems and present control practices, such as adding acid to suppress growth in raw seafood, but also by the human gastric barrier. However, until now a definitive analysis of the acid tolerance of *V. vulnificus* has not been made, and the molecular mechanisms by which the bacterium can survive in low pH environments have not yet been characterized.

It is obvious that characterization of the acid protection system of *V. vulnificus* at the molecular level is useful in delineating novel strategies to control this pathogen present in foods. Accordingly, in an effort to identify the genes involved in acid tolerance, a library of *V. vulnificus* mutants was constructed, and a mutant that was more sensitive to low pH was screened. By a transposon-tagging method, a *cadBA* operon encoding a lysine/cadaverin antiporter and a lysine decarboxylase was identified and cloned from *V. vulnificus*. The nucleotide and deduced amino acid sequences of each gene of the *cadBA* operon were analyzed. *V. vulnificus* null mutants whose *cadBA* genes were separately disrupted were also constructed, and the acid tolerance of the mutants was compared to that of the parental wild-type.

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2. Materials and methods

2.1. Strains, plasmids, and culture media

The strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strains used for plasmid DNA replication or conjugational transfer of plasmids were grown in Luria–Bertani (LB) broth or on LB broth containing 1.5% (w/v) agar. Unless noted otherwise, *V. vulnificus* strains were grown in LB medium supplemented with 2.0% (w/v) NaCl (LBS). When required, appropriate antibiotics were added to the media as follows: ampicillin 100 μg ml^{-1}, kanamycin 50 μg ml^{-1}, and tetracycline 10 μg ml^{-1}.

2.2. Cloning of the V. vulnificus cadBA operon

A mutant, strain JR991, that was more sensitive to low pH was screened from a library of *V. vulnificus* mutants generated by a random transposon mutagenesis using a mini-Tn5 *lacZ*1 [4]. A DNA segment flanking the transposon insertion was amplified by PCR as described previously [5]. Since the deduced amino acid sequence of the resulting PCR product, a 150-bp DNA fragment, revealed 62% identity with that of *E. coli cadB*, the DNA was labeled with [α-^{32}P]dCTP and named CadBP. To clone the full genes of the *cadBA* operon, a cosmid library of *V. vulnificus* ATCC 29307 constructed using pLAFR3 (J.H. Rhee, unpublished data) was screened using CadBP as a probe. A colony showing a positive signal was isolated, and the cosmid DNA was purified and named pJR990 (Fig. 1). A 9.3-kb band and a 4.3-kb band from the cosmid DNA digested with *Xho*I and *Eco*RI, respectively, were purified and ligated into pGEM7zf(+) (Promega, Madison, WI, USA) to result in pPR991 and pJR991, as shown in Fig. 1.

2.3. Generation of the cad::nptI mutants

To inactivate *cadB*, a 1.2-kb *nptI* DNA conferring resistance to kanamycin was isolated from pUC4K (Pharmacia, Piscataway, NJ, USA) and inserted into a unique *Acc*I site present within the open reading frame (ORF) of *cadB*. The 3.7-kb *cadB::nptI* cartridge from the resulting construct (pJR002) was liberated and ligated with *Sma*I-digested pCVD442 [6], forming pJR0021. To inactivate the *cadA*, a 2.2-kb DNA fragment carrying whole *cadA* was amplified by PCR using primers CAD0005 (5′-GCTCTA-

Table 1
Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics[a]	Reference or source
Bacterial strains		
V. vulnificus		
ATCC 29307	Clinical isolate; virulent	Laboratory collection
MO6-24/O	Clinical isolate; virulent	Laboratory collection
JR991	MO6-24/O with *cadB*::mini-Tn5 *lacZ*1	This study
JR202	ATCC 29307 with *cadB::nptI*; Km^r	This study
JR203	ATCC 29307 with *cadA::nptI*; Km^r	This study
E. coli		
DH5α	*supE44 Δ lacU169* (*ϕ80 lacZ* ΔM15) *hsdR17 recA1 endA1 gyrA96 relA1 relA1 thi-I rel AI*; plasmid replication	Laboratory collection
SM10(λ*pir*)	*thi thr leu tonA lacY supE recA*::RP4-2-Tc::Mu λ*pir*; Km^r; host for π-requiring plasmids; conjugal donor	[6]
Plasmids		
pUT::mini-Tn5 *lacZ*1	R6K γ *ori*; suicide vector; *oriT* of RP4; Ap^r	[4]
pGEM7zf(+)	pMB1 *ori*; Ap^r	Promega
pUC4K	pUC4 with *nptI*; Ap^r, Km^r	Pharmacia
pCVD442	R6K γ *ori*; *sacB*; suicide vector; *oriT* of RP4; Ap^r	[6]
pRK415	IncP *ori*; broad host-range vector; *oriT* of RP4; Tc^r	[13]
pPR991	9.3-kb *Xho*I fragment containing *cadBA* in pGEM7zf(+); Ap^r	This study
pJR990	Cosmid containing *cadBA*	This study
pJR991	4.3-kb *Eco*RI fragment containing *cadB*, part of *cadA* in pGEM7zf(+); Ap^r	This study
pJR992	2.5-kb *Hin*dIII fragment of pJR991 in pGEM7zf(+); Ap^r	This study
pJR9914	2.2-kb *cadA* fragment in pGEM7zf(+); Ap^r	This study
pJR002	pJR992 with *cadB::nptI*; Ap^r, Km^r	This study
pJR003	pJR9914 with *cadA::nptI*; Ap^r, Km^r	This study
pJR0021	pCVD442 with *cadB::nptI*; Ap^r, Km^r	This study
pJR0031	pCVD442 with *cadA::nptI*; Ap^r, Km^r	This study
pJR0022	pRK415 with *cadB*; Tc^r	This study
pJR0032	pRK415 with *cadA*; Tc^r	This study

[a] Ap^r, ampicillin resistant; Km^r, kanamycin resistant; Tc^r, tetracycline resistant.

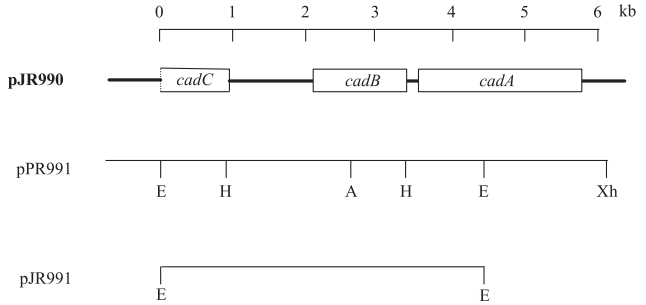

Fig. 1. Physical map of *V. vulnificus cadBA* genes. Plasmids pJR991 and pPR991 were used to determine the nucleotide sequence of *cadBA*. The open boxes and thick lines represent the coding regions of *cadBA* genes and chromosomal DNA, respectively. Part of *cadC*, a box with dashed line, appears at the left end of *cadBA*. The nucleotide sequence of *cadBA* genes of *V. vulnificus* ATCC 29307 was deposited into GenBank under accession number AF324470. Abbreviations: A, *Acc*I; E, *Eco*RI; H, *Hin*dIII; Xh, *Xho*I.

GACCAAATGAATATTTTCGCTATCTT-3′) and CAD-0006 (5′-CGGGATCCTGCGTTAAATCATTGACCTA-ATTT-3′) and ligated with pGEM7zf(+) to yield pJR9914. The *cadA* in pJR9914 was inactivated by inserting the *nptI* fragment into the *Eco*RI site of *cadA* ORF, to result in pJR003. The *cadA::nptI* cartridge was isolated after digestion of pJR003 with *Xba*I and *Bam*HI, and ligated with pCVD442 to construct pJR0031, following the procedures for pJR0021.

E. coli SM10 (λ*pir*), *tra* (containing pJR0021 or pJR0031) was used as a conjugal donor to generate the *cad::nptI* mutants of *V. vulnificus* ATCC 29307 by homologous recombination (Fig. 2A). The conjugation and isolation of the transconjugants were conducted using the methods previously described [7], and a double crossover, in which each wild-type *cad* gene was replaced with the *cad::nptI* allele, was confirmed by PCR as shown in Fig. 2B. The *V. vulnificus* mutants chosen for further analysis were named JR202 for the *cadB* mutant and JR203 for the *cadA* mutant, respectively.

2.4. Measurement of cadaverin excretion and lysine decarboxylase activities

Cultures of *V. vulnificus* strains, buffered at pH 5.8 with 10 mM 2-(*N*-morpholino)ethanesulfonic acid (Sigma, St. Louis, MO, USA) or pH 7.6 with 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Sigma), were incubated at 30°C under aeration and harvested at the indicated times to measure cell density, cadaverin excretion and lysine decarboxylase activities. Bacterial growth was monitored by measuring the OD_{600} of cultures. The lysine decarboxylase activity in the cells and the cadaverin excretion in the supernatants were determined according to procedures described previously [8,9]. A unit of enzyme activity was defined as an increase in absorbance ($1000 \times A_{340}$) of the reaction solution per minute per cell density (OD_{600}) of cultures. For statistical analysis, Student's *t*-test was used to evaluate the differences between the enzyme activities and the survival rates for the various strains (SAS software, SAS Institute Inc., Cary, NC, USA). Averages and standard errors of the mean (S.E.M.) were calculated from at least three independent trials.

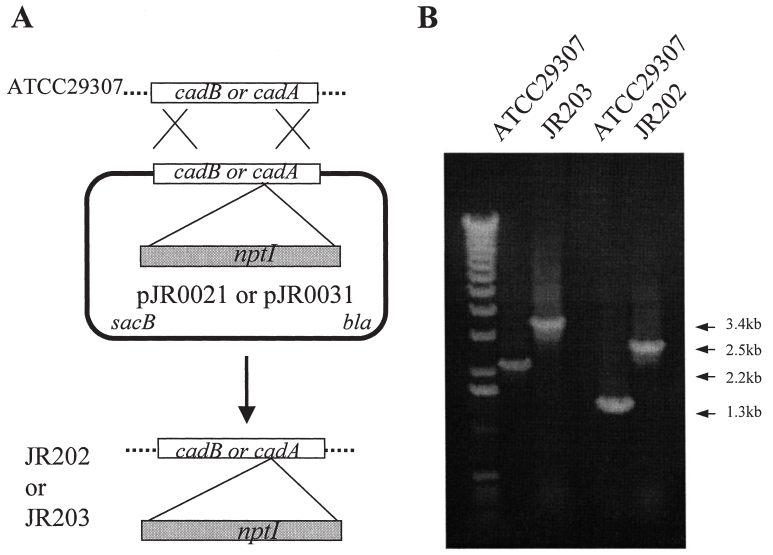

Fig. 2. Allelic exchange procedure and construction of *cad::nptI* isogenic mutants. A: Double homologous recombinations between strain ATCC 29307 and plasmids pJR0021 or pJR0031 led to interruption of respective *cad* genes and resulted in construction of mutants JR202 or JR203, respectively. Dashed lines represent the bacterial chromosome; a full line, the plasmid DNA; open boxes, the target *cad* genes; shaded boxes, the *nptI* gene; large X's represent genetic crossing over. Abbreviations: *sacB*, levansucrase gene; *bla*, β-lactamase gene. B: PCR analysis of genomic DNA from ATCC 29307 with primers CAD0003 and CAD0004 produced a 1.3-kb fragment, whereas genomic DNA from JR202 resulted in an amplified DNA fragment approximately 2.5 kb in length. The 2.5-kb fragment is in agreement with the projected size of the DNA fragment containing wild-type *cadB* (1.3 kb) and the *nptI* gene (1.2 kb). Similarly, the construction of JR203 was confirmed by PCR using primers CAD0005 and CAD0006. Molecular size markers (1-kb ladder, Gibco-BRL, Gaithersburg, MD, USA) appear in the end lane of the gel.

2.5. Acid challenges

Acid tolerance was assessed in a 10 mM sodium citrate buffer (pH 4.4) supplemented with 2.0% NaCl (SCBN) and filter-sterilized. Cultures prepared essentially as described above, and grown for 4 h were used to inoculate flasks containing 50 ml of SCBN to achieve a final concentration of ca. 10^5 colony forming units (CFU) ml^{-1}. Following inoculation, the flasks were incubated at 30°C with shaking (150 rpm) for 90 min. The samples then removed were plated in duplicate on LBS and incubated at 30°C. The percentage of survivors was calculated by using the CFU ml^{-1} as determined immediately after inoculation as 100%.

3. Results and discussion

3.1. Sequence analysis of the V. vulnificus cadBA operon

The nucleotide sequences of the DNA fragments in pJR991 and pPR991 were determined by primer walking (Korea Basic Science Research Center, Kwang-Ju, South Korea). The nucleotide sequence revealed two coding regions consisting of 1332 nucleotides and 2136 nucleotides, respectively, 131 bp apart from each other (Fig. 1). A database search for nucleotide sequences similar to those of the coding regions revealed three other *cadBA* genes cloned from *E. coli*, *Vibrio cholerae* and *Salmonella typhimurium* strains with high levels of identity. The *cadBA* from these bacteria was 64–85% identical in nucleotide sequence with the coding regions in pPR991 and pJR991 (data not shown). This information proposed that the coding regions are homologs of *cadBA* genes reported from other Enterobacteriaceae, and led us to name the coding regions *cadBA* of *V. vulnificus*.

The amino acid sequence deduced from the *cadB* nucleotide sequence revealed a protein, a lysine/cadaverin antiporter composed of 443 amino acids with a theoretical molecular mass of 46 753 Da and a p*I* of 6.51. The amino acid sequence of the *V. vulnificus* lysine/cadaverin antiporter, CadB, was 64% and 93% identical to those of the CadB of *E. coli* and *V. cholerae*, respectively (data not shown, http//www.ebi.ac.uk/clustalw). The predicted profile of the hydrophobicity (http//www.expasy.ch) is significantly similar to that of the CadB of *E. coli* [10], and is consistent with the fact that the CadB protein is a membrane spanning protein (data not shown).

The amino acid sequence deduced from the *cadA* coding sequence revealed a protein, a lysine decarboxylase composed of 711 amino acids with a theoretical molecular mass of 80 432 Da and a p*I* of 5.53. The amino acid composition and molecular mass of this CadA are quite similar to those of the CadA from other Enterobacteriaceae. The amino acid sequence of the *V. vulnificus* CadA was 73–97% identical to those of the CadA from *E. coli*, *S. typhimurium* and *V. cholerae*, and their identity appears evenly throughout the whole proteins (data not shown, http//www.ebi.ac.uk/clustalw). Furthermore, the hydrophobicity analysis revealed that the CadA is a cytosolic soluble protein as observed previously from lysine decarboxylases of *E. coli* [10]. All of this information confirmed that the *cadBA* operon encodes the lysine/cadaverin antiporter and lysine decarboxylase of *V. vulnificus*.

3.2. Lysine decarboxylase activity of V. vulnificus ATCC 29307

It has previously been observed that the *cad* genes of *E. coli* are induced by low pH [11]. Consistent with this, the lysine decarboxylase activity of strain ATCC 29307 appeared at the beginning of growth and reached a maximum during the mid-exponential phase in a culture adjusted to pH 5.8 (Fig. 3). The lysine decarboxylase activity began to decrease when the pH of the culture broth increased. However, when the culture adjusted to pH 7.6 was analyzed, it appeared to produce much less lysine decarboxylase, although the level of the enzyme activity was still evident. It is apparent from this result that the lysine decarboxylase of *V. vulnificus* is also subject to induction by low pH. Therefore, cultures of ATCC 29307 and the *cad* mutants, buffered at pH 5.8 and grown for 4 h, were used to compare lysine decarboxylase activities.

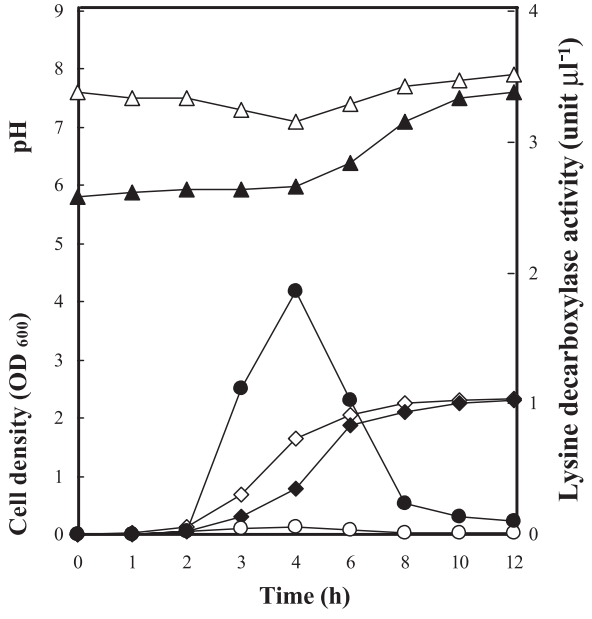

Fig. 3. Growth kinetics and activities of lysine decarboxylase in *V. vulnificus* ATCC 29307. Cultures of *V. vulnificus* ATCC 29307 were grown in LBS, buffered at pH 5.8 (filled symbols) or at pH 7.6 (open symbols) as described in the text. Samples removed at the indicated times from cultures were analyzed for cell density (♦, ◇), lysine decarboxylase activity (●, ○), and pH (▲, △).

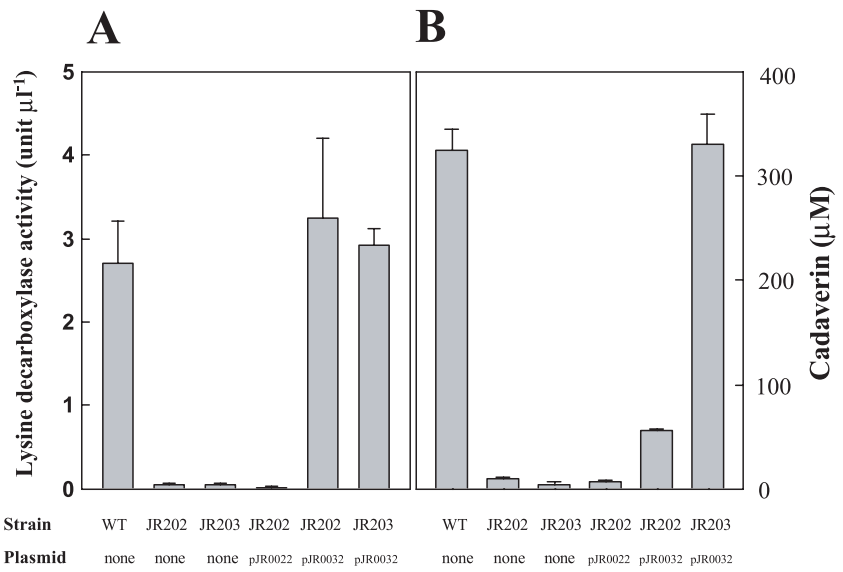

Fig. 4. Effects of mutations in *cad* genes on lysine decarboxylase activity and cadaverin excretion. For both panels, cultures of ATCC 29307 and each isogenic mutant were grown in LBS adjusted to a pH of 5.8. After 4 h incubation, samples were removed and analyzed for lysine decarboxylase activity (A) and for cadaverin excretion (B). Complementations of the mutants with a functional *cadB* (pJR0022) or *cadA* (pJR0032) are also presented as indicated. Relative activities of lysine decarboxylase, and levels of cadaverin excretion were calculated as described in the text. Error bars represent the S.E.M.

3.3. Effects of mutations in cad genes on lysine decarboxylase activity and cadaverin excretion

For ATCC 29307, lysine decarboxylase was produced and reached a maximum 3.0 units (Fig. 4A). The disruption of *cadA* in the mutant JR203 resulted in reduced production of lysine decarboxylase activity ($P < 0.05$). The residual level of lysine decarboxylase activity in JR203 corresponded to less than one-tenth of that of the wild-type. These data demonstrated that the *cadA* gene encoded the lysine decarboxylase of *V. vulnificus*. However, the lysine decarboxylase activity in JR203 was still evident and its level was significantly higher than the background levels observed when control assays were carried out in the absence of lysine. This observation that the mutant still exhibited lysine decarboxylase activity indicated the existence of at least one more lysine decarboxylase (or its homolog) being produced by *V. vulnificus* ATCC 29307. This assumption that *V. vulnificus* has multiple lysine decarboxylases is supported by recent reports that *E. coli* has a second lysine decarboxylase. The second lysine decarboxylase of *E. coli*, encoded by the *Idc* gene located apart from *cadA*, is expressed at a low level under various conditions [8,12].

To rule out the possibility that the decrease of lysine decarboxylase activity by more than 90% resulted from polar effects of the *cadA* insertional mutation on downstream genes, we examined if reintroduction of pJR0032 carrying recombinant *cadA* could complement the decrease of lysine decarboxylase activity of JR203 cells. For this purpose, pJR0032 was constructed by subcloning the *cadA* amplified by PCR using primers CAD0005 and CAD0006 and then digested with *Xba*I and *Bam*HI into the broad host-range vector pRK415 [13] linearized with the same enzymes. The resulting plasmid was mobilized into *V. vulnificus* by conjugation. The lysine decarboxylase activity of the JR203(pJR0032) was restored to a level comparable to the wild-type level of ATCC 29307 (Fig. 4A). Therefore, the decreased lysine decarboxylase activity of JR203 resulted from inactivation of functional *cadA* rather than any polar effects on any genes downstream of *cadA*.

When JR202, a *cadB* mutant, was compared with its parental wild-type, it appeared to produce a significantly lower level of lysine decarboxylase ($P < 0.05$), and the level of the lysine decarboxylase was comparable to that of the *cadA* mutant (Fig. 4A). One possible explanation for this decrease in lysine decarboxylase in JR202 is that the *cadBA* is one transcriptional unit and insertional mutation of *cadB* gives the effect of polarity on *cadA*. To test this possibility further, recombinant *cadB* was reintroduced into JR202 and the lysine decarboxylase activity was determined. The ORF and upstream region of the *cadB* was amplified by PCR using the pairs of primers CAD0003 (5′-CGGGATCCGAGATGTTATGTCATCTGATACT-A-3′) and CAD0004 (5′-CGGAATTCGCCAGCGGAA-TCAATCAGTGTTG-3′). The amplified *cadB* was digested with *Eco*RI and *Bam*HI and then ligated with pRK415 digested with the same enzymes, to result in pJR0022. The level of lysine decarboxylase of JR202(pJR0022) was not restored and remained close to that of JR202. However, the reintroduction of pJR0032 carrying *cadA* into JR202 restored the decreased activity of lysine decarboxylase to the level of the wild-type (Fig.

4A). These results are in agreement with our assumption that *cadBA* is one transcriptional unit and *cadA* is not functional in JR202.

The excretion of cadaverin results from the combined activity of lysine decarboxylase and cadaverin/lysine antiporter [10]. When the cadaverin excretion was determined in the *cadB* mutant (JR202) and *cadA* mutant (JR203), the cadaverin excretion of both strains decreased significantly, and the trend of that decrease was similar to that for lysine decarboxylase (Fig. 4B). Introduction of the recombinant *cadA* (pJR0032) into JR203 restored cadaverin excretion to the level found in the wild-type. However, in contrast to the lysine decarboxylase, neither pJR0022 nor pJR0032 was able to complement *cadB* mutation to restore the decrease of cadaverin excretion in JR202 (Fig. 4B). This observation again supports our assumption that neither *cadA* nor *cadB* is functional in JR202.

3.4. Acid tolerance of the cad mutants

The survival of the parent strain (ATCC 29307) was significantly greater ($P < 0.05$) than that of the *cadA* mutant (JR203) when cells grown at pH 5.8 were used for acid challenge in SCBN (Fig. 5A). The parent strain decreased ca. 1.0 $\log_{10}$ CFU ml^{-1} (90%), while the JR203 strain decreased ca. 3.0 $\log_{10}$ CFU ml^{-1} (99.9%) after a 90-min acid challenge. This indicated that the *cadA* mutant is more sensitive to acid than the wild-type, and that lysine decarboxylase plays an important role in the acid tolerance of *V. vulnificus*. Similar to results with JR203 cells, the survival of JR202 was significantly lower ($P < 0.05$) than that of the parental wild-type (Fig. 5A). Complementation of *cadA::nptI* in JR203 with a functional *cadA* (pJR0032) restored acid tolerance to a level equivalent to that of the parent strain (Fig. 5A). In agreement with the observation that *cadA* is not functional in JR202, reintroduction of recombinant *cadB* could not complement the decrease of survival of JR202 cells. Introduction of a control plasmid (pRK415) into JR203 did not significantly affect survival during an acid challenge (data not shown).

The survival of cells grown at pH 7.6 in acid challenges is shown in Fig. 5B. The survival of the parent strain and mutants is significantly lower (ca. 3–4 log CFU ml^{-1} reduction) than the survival of cells grown at pH 5.8. Additionally, compared to the results with cells grown at pH 5.8, smaller differences in the survival of the parent strain and the *cad* mutants were observed (Fig. 5B). It is apparent that the contribution of the *cadBA* genes for the acid tolerance of *V. vulnificus* is substantial, but dependent on prior exposure to acidic pH.

These results are consistent with the previous observation that the lysine decarboxylase of *V. vulnificus* is induced by the exposure of cells to low pH (Fig. 3). Recent studies noted that many other degradative decarboxylases, such as lysine decarboxylase of *E. coli*, are also induced at acidic pH as well as by high concentrations of the respective amino acids [10,14]. The expression of the *E. coli cadBA* operon is regulated by CadC, an activator, and by LysP, a repressor, as a function of the pH and concentration of lysine [15,16]. Likewise, CadC positively regulates the expression of *cadBA* genes in *V. cholerae*, a species closely related to *V. vulnificus* [17]. In the course of our sequencing analysis, the part of *cadC* homology located upstream of *cadB* was found (Fig. 1). It is not clear whether the same elements observed in the regulation of *cadBA* of other Enterobacteriaceae are also involved in the regulation of the *cadBA* of *V. vulnificus* or not. Neverthe-

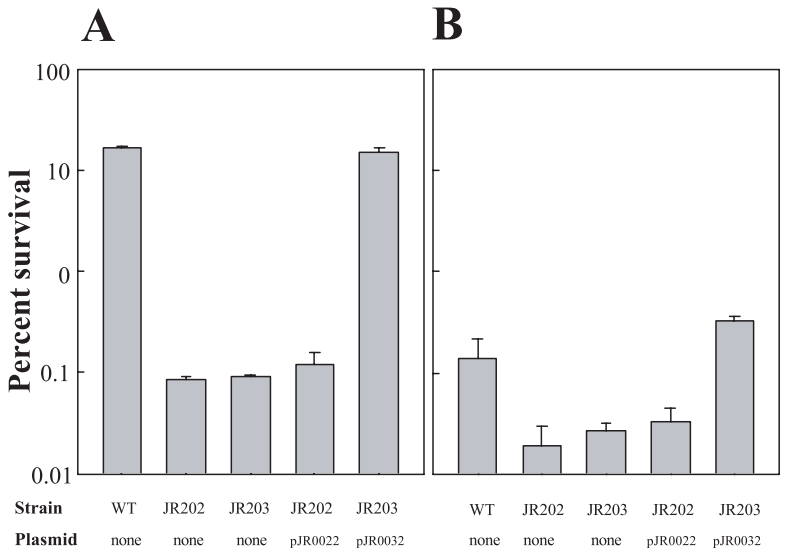

Fig. 5. Acid tolerance of ATCC 29307 and isogenic *cad* mutants. Cultures, grown in LBS adjusted to a pH of 5.8 (A) or pH of 7.6 (B), were used for acid challenge. The percent of survivors was calculated using the CFU ml^{-1} of ATCC 29307 that survived immediately after the challenge as 100%. Survivors of the mutants complemented with a functional *cadB* (pJR0022) or *cadA* (pJR0032) are also presented as indicated. Error bars represent the S.E.M.

less, from our observation that the lysine decarboxylase is induced by exposing cells to low pH as observed in *E. coli*, and that the *cadC* homology is present, it is most likely that the *cadBA* of *V. vulnificus* would be regulated in a manner analogous to the regulation of *E. coli cadBA*.

Acknowledgements

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References

[1] Klontz, K.C., Lieb, S., Schreiber, M., Janowski, H.T., Baldy, L.M. and Gunn, R.A. (1988) Syndromes of *Vibrio vulnificus* infections. Clinical and epidemiologic features in Florida cases, 1981–1987. Ann. Intern. Med. 109, 318–323.

[2] Bearson, S., Bearson, B. and Foster, J.W. (1997) Acid stress responses in enterobacteria. FEMS Microbiol. Lett. 147, 173–180.

[3] Foster, J.W. (2000) Microbial responses to acid stress. In: Bacterial Stress Responses (Storz, G. and Hengge-Aronis, R., Eds.), pp. 99–115. American Society for Microbiology, Washington, DC.

[4] DeLorenzo, V., Herrero, M., Jakubzik, U. and Timmis, K. (1990) Mini-Tn5 transposon derivatives for insertion mutagenesis, promoter probing, and chromosomal insertion of cloned DNA in Gram-negative eubacteria. J. Bacteriol. 172, 6568–6572.

[5] Surette, M.G., Miller, M.B. and Bassler, B.L. (1999) Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. Proc. Natl. Acad. Sci. USA 96, 1639–1644.

[6] Donnenberg, M.S. and Kaper, J.B. (1991) Construction of an *eae* deletion mutant of enteropathogenic *Escherichia coli* by using a positive-selection suicide vector. Infect. Immun. 59, 4310–4317.

[7] Jeong, H.S., Jeong, K.C., Choi, H.K., Park, K.J., Lee, K.H., Rhee, J.H. and Choi, S.H. (2001) Differential expression of *Vibrio vulnificus* elastase gene in a growth phase-dependent manner by two different types of promoters. J. Biol. Chem. 276, 13875–13880.

[8] Lemonnier, M. and David, L. (1998) Expression of the second lysine decarboxylase gene of *Escherichia coli*. Microbiology 144, 751–760.

[9] Phan, A.P.H., Ngo, T.T. and Lenhoff, H.M. (1982) Spectrophotometric assay for lysine decarboxylase. Anal. Biochem. 120, 193–197.

[10] Meng, S.Y. and Bennett, G.N. (1992) Nucleotide sequence of the *Escherichia coli cad* operon: a system for neutralization of low extracellular pH. J. Bacteriol. 174, 2659–2669.

[11] Neely, M.N. and Olson, E.R. (1996) Kinetics of expression of the *Escherichia coli cad* operon as a function of pH and lysine. J. Bacteriol. 178, 5522–5528.

[12] Kikuchi, Y., Kojima, H., Tanaka, T., Takatsuka, Y. and Kamio, Y. (1997) Characterization of a second lysine decarboxylase isolated from *Escherichia coli*. J. Bacteriol. 179, 4486–4492.

[13] Keen, N.T., Tamaki, S., Kobayashi, D. and Trollinger, D. (1988) Improved broad-host-range plasmids for DNA cloning in Gram-negative bacteria. Gene 70, 191–197.

[14] Sabo, D.L., Boeker, E.A., Byers, B., Waron, H. and Fischer, E.H. (1974) Purification and physical properties of inducible *Escherichia coli* lysine decarboxylase. Biochemistry 13, 662–670.

[15] Neely, M.N., Dell, C.L. and Olson, E.R. (1994) Roles of LysP and CadC in mediating the lysine requirement for acid induction of the *Escherichia coli cad* operon. J. Bacteriol. 176, 3278–3285.

[16] Watson, N., Dunyak, D.S., Rosey, E.L., Slonczewski, J.L. and Olson, E.R. (1992) Identification of elements involved in transcriptional regulation of the *Escherichia coli cad* operon by external pH. J. Bacteriol. 174, 530–540.

[17] Merrell, D.S. and Camilli, A. (2000) Regulation of *Vibrio cholerae* genes required for acid tolerance by a member of the 'ToxR-like' family of transcriptional regulators. J. Bacteriol. 182, 5342–5350.