Vibrio vulnificus Secretes an Insulin-degrading Enzyme That Promotes Bacterial Proliferation in Vivo*

In Hwang Kim†, Ik-Jung Kim‡, Yancheng Wen†, Na-Young Park‡, Jinyoung Park‡, Keun-Woo Lee‡, Ara Koh‡, Ji-Hyun Lee†, Seung-Hoi Koo‡, and Kun-Soo Kim§

From the †Department of Life Science, Sogang University, Seoul 121-742, Korea, and the §Division of Life Science, Korea University, Seoul 136-701, Korea

Background: Vibrio vulnificus produces SidC, an extracellular insulin-degrading enzyme.

Results: SidC causes degradation of insulin, leading to proliferation of the pathogen, and sidC was expressed in low-glucose conditions.

Conclusion: Degradation of insulin by SidC correlated with the proliferation of the pathogen.

Significance: V. vulnificus manipulates host endocrine signals through SidC, making the host environment more favorable for its own proliferation.

We describe a novel insulin-degrading enzyme, SidC, that contributes to the proliferation of the human bacterial pathogen Vibrio vulnificus in a mouse model. SidC is phylogenetically distinct from other known insulin-degrading enzymes and is expressed and secreted specifically during host infection. Purified SidC causes a significant decrease in serum insulin levels and an increase in blood glucose levels in mice. A comparison of mice infected with wild type V. vulnificus or an isogenic sidC-deletion strain showed that wild type bacteria proliferated to higher levels. Additionally, hyperglycemia leads to increased proliferation of V. vulnificus in diabetic mice. Consistent with these observations, the sid operon was up-regulated in response to low glucose levels through binding of the cAMP-receptor protein (CRP) complex to a region upstream of the operon. We conclude that glucose levels are important for the survival of V. vulnificus in the host, and that this pathogen uses SidC to actively manipulate host endocrine signals, making the host environment more favorable for bacterial survival and growth.

Pathogens have evolved elaborate ways to survive and thrive within their hosts to improve their own chance for survival. Elucidation of the mechanisms behind such strategies can be an important step in defining targets for the development of novel antibacterial drugs. Little is known about the ways in which pathogens are able to utilize host catabolic substrates, and although there is a plethora of information about how pathogens modify host proteins, to our knowledge, little is known about bacterial modification of endocrine signals, including insulin.

Vibrio vulnificus causes infection when contaminated seafood is consumed or when it invades an open wound, and this infection can often lead to fatal septicemia in people who are immunocompromised or have an underlying condition such as liver disease, alcoholism, or diabetes mellitus (1, 2). Several virulence factors, including hemolysin, elastolytic protease, Rtx toxin, and siderophores, have been identified in V. vulnificus (1–3). However, it is clear that the disease elicited by this pathogen requires the complex interaction of numerous factors, including many yet to be identified.

The existence of an insulin-degrading enzyme (IDE)2 in human that plays a role in insulin turnover in tissues was suggested as early as the 1940s (4). However, attempts to identify and characterize IDEs were not successful because of its low concentration and poor stability (5). In addition to insulin, glucagon, β-endorphin, insulin-like growth factors I and II, and amyloid-β have also been reported as substrates for IDEs (5, 6). Recently, IDE has attracted attention for its role in type 2 diabetes and Alzheimer disease, but additional studies are needed (6). A homologous bacterial protein was discovered in Escherichia coli, and the enzyme, called pitrilysin (or protease III), has a specificity for small substrates and is able to degrade both insulin and peptides smaller than 7 kDa (7). Pitrilysin is localized within the periplasmic space (8). This enzyme, like other IDEs, has an unusual metal-binding motif (HXXEH) characteristic of the inverzincin family of zinc metalloproteases (9, 10). This motif is an inversion of the more common zinc-binding motif (HEXXH) of other metalloproteases (11). Biochemical properties such as substrate specificity, enzyme kinetics, and metal stoichiometry have been studied (12); however, no obvious phenotypic deficiencies were observed in pitrilysin-deficient E. coli mutants (13), and not much is known about the physiological functions of this enzyme in bacteria. IDE homologs are highly conserved between bacteria and mammals and, in bacteria, they are localized to the cytosol, the plasma membrane, or the periplasm (5). A previous study described a cytoplasmic insulin-degrading enzyme (vIDE) in V. vulnificus (14) and the gene encoding this enzyme was shown to be regulated by CRP-cAMP and by the sugar phosphotransferase system.

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† To whom correspondence should be addressed. Tel.: 822-705-8460; Fax: 822-704-3601; E-mail: kskim@sogang.ac.kr.

2 The abbreviations used are: IDE, insulin-degrading enzyme; CRP, cAMP-receptor protein; IPGTT, intraperitoneal glucose tolerance test; OGGT, oral glucose tolerance test; TPEN, N,N′,N′-tetrakis(2-pyridylmethyl) ethylenediamine.
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TABLE 1
Strains and plasmids

| Strains and plasmids | Relevant characteristics | Sources/Ref. |
|----------------------|-------------------------|-------------|
| E. coli | | |
| BL21(DE3) | F’ ompT hsdSB (rB−,mB−) gal dcm (DE3) | Novagen |
| V. vulnificus | | |
| MO6–24/OΔsidC | Pathogenic clinical isolate | Our collection |
| Δcrp | Derivative of MO6–24/O with a deletion in sidC | This study |
| Δcrp | Derivative of MO6–24/O with a deletion in crp | 19 |
| Δcrr | Derivative of MO6–24/O with a deletion in crr | 32 |
| Plasmids | | |
| pASK-IBA3 | Expression vector for recombinant proteins with the C-terminal Strep-tag, Ap* | IBA |
| pASK-SidC | pASK-IBA3 containing the V. vulnificus sidC operon | This study |
| pASK-CRP | pASK-IBA3 containing the V. vulnificus crp operon | This study |
| pRK415 | lncP ori, broad-host-range vector; oriT of RP4, Tc* | 49 |
| pRK-sidC | pRK415 with V. vulnificus sidC | This study |
| pGEM-T Easy | TA-cloning vector, lacZ, Fl origin, Ap* | Promega |
| pHK0011 | pRK415 with a romoterless luxAB, Tc* | 19 |
| pPsid-lux | pHK0011 with the sidA promoter region fused to luxAB (−257 to +435)* | This study |
| pDM4 | Suicide vector for allelic exchange, sacB, Cm* | 50 |
| pDM4-sidKO | pDM4 with the sidC containing an in-frame deletion | This study |
| pBRI-MCS2 | Broad range cloning vector, K* | 51 |
| pBRR1-crp | pBRR1-MCS2 with V. vulnificus crp | This study |
| pGEM-sidA-crpmt | pGEM-T Easy vector with the mutated CRP binding site of the sidA promoter region (−344 to +246)* | This study |
| pPsidcmt-lux | pHK0011 with the mutated CRP binding site of the sidA promoter region fused to luxAB (−257 to +435)* | This study |

* Numbers indicate nucleotide positions relative to the translational start site.

(subcloned into the pASK-IBA3 vector (IBA BioTAGnology, Göttingen, Germany). The resulting vector pASK-SidC was transformed into E. coli BL21(DE3). Expression of SidC with a Strep-tag at the carboxyl terminus was induced with 0.2 μg/ml of anhydrotriacycline. After centrifugation, the bacterial pellets were suspended in buffer W (100 mM Tris-Cl, 150 mM NaCl), sonicated, and centrifuged at 7,000 rpm for 10 min. The resulting supernatant was passed through Strep-Tactin® affinity resin (IBA BioTAGnology, Göttingen, Germany) and bound protein was eluted using buffer E (100 mM Tris-Cl, 150 mM NaCl, 2.5 mM desthiobiotin) according to the manufacturer’s protocol. CRP was purified using a similar strategy. A DNA fragment encoding 310 amino acids of CRP was amplified by PCR using the primers crp-OEF and crp-OEB (Table 2). The amplified PCR product was cloned into pASK-IBA3. The resulting plasmid pASK-CRP, which encodes CRP fused to a Strep-tag at the C terminus, was expressed in E. coli BL21(DE3) as described above. The purified SidC and CRP proteins were dialyzed and concentrated with buffer S (50 mM Tris-Cl (pH 8.0), 100 mM NaCl, 2 mM diithiothreitol, 10% glycerol) and with buffer WG (buffer W with 30% glycerol) using an Ultracell®-30K centricron (Millipore, MA), respectively. To construct a V. vulnificus strain overexpressing SidC, a 3,076-bp DNA fragment of sidC was amplified by PCR using primers sid-comp-F1 and sid-comp-R1 (Table 2) and the resulting product was cloned behind the lac promoter in the vector pRK415 using HindIII and XbaI sites to generated pRK-sidC. The resulting construct was introduced into V. vulnificus strains.

Fractionation of Cellular Compartments and Subcellular Localization of SidC and Western Blot Analysis—Overnight cultures of the V. vulnificus strains were subcultured into fresh brain heart infusion broth (Difco, BD Biosciences, NJ) and cells and supernatants were separated by centrifugation when the optical density at 600 nm of the culture reached ~2.2. The supernatant was filtered by passing the culture through a 0.22-μm syringe filter (Millipore, MA), and was concentrated using Ultracell®-30K centricron (Millipore, MA). Fractionation of different cell compartments of V. vulnificus was carried out as described previously (16). After obtaining each fraction, the same volume of each subcellular fraction was separated by SDS-PAGE and transferred to a Hybond-P membrane (GE Healthcare). The membrane was incubated with polyclonal rat antisera against purified SidC (1:1,000 dilution in blocking solution), and subsequently incubated with goat anti-rat immunoglobulin-G-AP (1:2000) (Promega, WI). Alkaline phosphatase activity was measured quantitatively as described previously (16).

Assessing the Degradation of Insulin and Other Substrates and Determination of Cleavage Sites of Insulin Digested by SidC—Purified SidC (0.05–1 μM) was incubated with 20 μg of recombinant human insulin or other substrates (glucagon, TGF-α, IGF1, and IGF2, Sigma) in reaction buffer (50 mM Tris-Cl, pH 7.5) for 2 h at 37 °C and then separated by 20% SDS-PAGE. To test the effectiveness of protease inhibitors, either EDTA or TPEN at a final concentration of 5 mM was added. The reaction was terminated by boiling the mixture in 1 × SDS-

(15). However, a functional role for this enzyme remains to be elucidated.

Here we describe a novel secreted IDE from V. vulnificus named SidC that contributes to proliferation and survivability of the pathogen in a host through proteolytic degradation of the host endocrine signal insulin.

Experimental Procedures

Strains, Plasmids, and Culture Conditions—The bacterial strains and plasmids used in this study are listed in Table 1. E. coli strains were cultured in Luria-Bertani (LB) broth supplemented with appropriate antibiotics at 37 °C. V. vulnificus strains were cultured in LB broth or thiosulfate citrate bile salt sucrose (TCBS) agar supplemented with appropriate antibiotics at 30 or 37 °C.

Expression and Purification of SidC and CRP—A DNA fragment encoding 938 amino acids of SidC without an aminoterminal signal peptide region was amplified by PCR using the primers sid-OEF and sid-OEB (Table 2) and the product was
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TABLE 2

| Purpose/name | Nucleotide sequence (5′ to 3′)* |
|--------------|--------------------------------|
| Cloning of sidC and crp | ACCATGTGCCCTCCTCCTCCATACCCCTGTCAGCCTCGGAGATCC | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |
| sid-OEF | ACTCCCTCAGAGTGCAGGAGATCTGCTCTGAGCTGTTTACCTACCCCTGTCAGCCTCGGAGATCC |
| sid-OEB | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |
| crp-OEF | CCCCCCTGAGGCTGAGGAGATCGGAGATCC |
| crp-OEB | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |
| sid-comp-F1 | AAGGCTGCAGGCTGAGGAGATCGGAGATCC |
| sid-comp-R1 | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |
| crp-F | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |
| crp-B | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |

Construction of a luxAB fusion

| Purpose/name | Nucleotide sequence (5′ to 3′)* |
|--------------|--------------------------------|
| sid-pro-F1 | GGTACGCGCCGGCTGATGCTTTAG |
| sid-pro-B1 | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |

Construction of a sidC in-frame deletion mutant

| Purpose/name | Nucleotide sequence (5′ to 3′)* |
|--------------|--------------------------------|
| sid-KO-F1 | GGTACGCGCCGGCTGATGCTTTAG |
| sid-KO-B1 | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |
| sid-KO-F2 | GGTACGCGCCGGCTGATGCTTTAG |
| sid-KO-B2 | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |

Site-directed mutagenesis

| Purpose/name | Nucleotide sequence (5′ to 3′)* |
|--------------|--------------------------------|
| sid-mtF1 | GGTACGCGCCGGCTGATGCTTTAG |
| sid-mtB1 | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |
| sid-mtF2 | GGTACGCGCCGGCTGATGCTTTAG |
| sid-mtB2 | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |

Electrophoresis mobility shift assay

| Purpose/name | Nucleotide sequence (5′ to 3′)* |
|--------------|--------------------------------|
| sid-F | TGGTGAATTTAAAGGCTGATGCTTTAG |
| sid-B | GACCAGCGCATCCCTCATCCAGGCTGTCGAGGATCGGAGATCC |

* Nucleotides modified for the generation of restriction sites or for site-directed mutagenesis are underlined.

PAGE loading buffer containing 5% β-mercaptoethanol. The reaction mixtures were loaded onto a 20% SDS-PAGE gel and stained with Coomassie Brilliant Blue. To identify cleavage sites of insulin digested by SidC, 30 μg of recombinant human insulin was incubated with 30 μM SidC in reaction buffer for 6 h at 37 °C and then analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an UltiMate 3000 HPLC system (Dionex) and a MicroQ-TOF III mass spectrometer (Bruker Daltonics, 255748, Germany) at PROTEINWORKS Inc. (Daejeon, Korea). The chromatography was performed with solvent A (0.2% fluorooacetic acid in water) and solvent B (0.2% fluorooacetic acid in acetonitrile) on an Acclaim RSLC 120 C18 column (2.1 × 100 mm, 2.2 μm, Dionex) at a flow rate 200 μl/min. The capillary temperature of mass spectrometer was 180 °C.

Construction of a sid-lux Fusion and an In-Frame Deletion of sidC—The upstream region (−257 to +43, with respect to the translational start site) of the sid operon was amplified by PCR using primers sid-pro-F1 and sid-pro-B1 (Table 2), and cloned into the pGEM-T Easy vector (Promega). The resulting plasmid was digested with KpnI and BamHI and cloned into pHK0011 (Table 1) to generate pPsid-lux, in which the promoter region of the sid operon is fused to luxAB. To construct an in-frame sidC deletion mutant, a 771-bp DNA fragment comprising the upstream region, and a 773-bp DNA fragment comprising the downstream region of the sidC were amplified using primers sid-KO-F1 and sid-KO-B1 and primers sid-KO-F2 and sid-KO-B2 (Table 2), respectively. Each fragment was digested with the restriction enzyme KpnI, and ligated with pGEM-T Easy vector. The resulting plasmids were digested by Apal and SacI, and cloned into the pDM4 vector (Table 1) to generate pDM4-sidKO, which was then introduced into V. vulnificus MO6–24/O by conjugation. Double crossover selection to construct a deletion of sidC in the chromosome was performed as described previously (17). The resulting strain was named ΔsidC.

Evaluation of the Effect of Purified SidC on the Concentration of Insulin Secreted by INS-1 Cells—The amount of insulin secreted by INS-1 cells was measured. Cells were preincubated in KRBB buffer (25 mM HEPES, pH 7.4, 115 mM NaCl, 5 mM KCl, 2.5 mM CaCl2, 1 mM MgCl2, 25 mM NaHCO3) containing 0.5% BSA for 3 h and stimulated with either 3 (LG) or 15 mM glucose (HG), with or without SidC, for an additional hour as described (18).

Cytotoxicity Assays—Cytotoxicity assays were performed using the CytoTox96® Non-radioactive Cytotoxicity assay kit (Promega). Briefly, HepG2 was seeded at 2 × 10^5 cells per well to 24-well culture plates and grown overnight in DMEM at 37 °C in the presence 5% CO2. Zero, 1, and 2 μM SidC were added to HepG2, incubated for 1–2 h, then cytotoxicity was determined by measuring the level of released lactate dehydrogenase according to the manufacturer’s protocol.

Assessment of Insulin Activity, Glucose Level, and Proliferation of V. vulnificus in the Blood of Infected Mice—C57BLKS/db/db mice were purchased from DBL (Korea), and ICR (CD-1) mice were purchased from Orient Bio Inc. (Korea). All mice used to assess the effects of SidC were fasted for 16 h before the experiment and only water was provided. For the intraperitoneal glucose tolerance test (IPGTT), db/db mice were injected intraperitoneally with glucose (1 g/kg of body weight) along with 100 μl of either the concentration buffer alone or 1.6 μl SidC per mouse. Blood was drawn from the tail vein at the designated times and was used to detect glucose and insulin levels as described previously (18). To determine the effect on insulin and glucose levels in the host after infection of vibrio strains, wild type V. vulnificus MO6–24/O, ΔsidC, MO6–24/O (pRK-sidC), and Δcrr (pRK-sidC) cells were cultured overnight in LB medium and subcultured into fresh LB
brought. When the culture reached a cell density \( (A_{600}) \) of \( \sim 0.7 \) to the same cell density, using phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM NaHPO\(_4\), and 2 mM KH\(_2\)PO\(_4\), pH 7.4). V. vulnificus cells (about \( \sim 10^7 \) colony forming units) were subcultured into a 

2-h time points after injection of V. vulnificus strains, the mice were administered filter-sterilized glucose (1.5 g/kg body weight) orally. Blood samples were collected from the heart at time points after the V. vulnificus injection. The blood glucose level was measured immediately using Accu-check\textsuperscript{TM} Performa (Roche Diagnostics). To determine the level of proliferation of the strains within the host, the number of viable cells present in the blood was measured as follows. A blood sample was diluted in PBS and the diluted samples were then spread onto LBS agar (LB agar with 2% NaCl) in duplicate. The rest of the blood sample was incubated at 4 \( ^\circ \)C for coagulation and centrifuged for serum separation. Insulin levels were determined in serum using an Ultrasensitive EIA kit (Alpco Diagnostics, NH) as per the manufacturer’s instructions. The results were analyzed using GraphPad Prism 5.

Cloning of crp—The 886-bp DNA fragment comprising the promoter region and the coding region of the crp was amplified by PCR using the primers crp-F and crp-B (Table 2). The resulting product was cloned into pBRR1-MCS2 (Table 1) to construct pBRR1-crp. Plasmids pBRR1-MCS2 and pBRR1-crp were conjugated into wild type V. vulnificus MO6–24/O and the \( \Delta \text{crp} \) strain (19).

Luciferase Assay—Overnight cultures of V. vulnificus strains were inoculated into appropriate medium. Samples were diluted 125-fold with PBS. After adding 0.006% (v/v) n-decylaldehyde, luminescence was measured using a luminometer (Lumat LB 9507, Berthold Technologies, Bad Wildbad, Germany). Specific transcriptional levels were expressed as light units normalized to cell density (relative light units), as described previously (20).

Electrophoresis Mobility Shift Assay—A 395-bp DNA fragment of the upstream region of the sid operon (−247 to +148, with respect to the translation start site) was amplified by PCR using primers sid-F and \( ^{32} \text{P}-\text{labeled sid-B} \) (Table 2). For the gel shift assay, 5 ng of the labeled probe was incubated with increasing amounts of purified CRP protein (0 to 400 nM) in a 20-μl reaction in the binding buffer containing 10 mM Tris-Cl (pH 8.0), 75 mM NaCl, 1 mM dithiothreitol (DTT), and 1 mM cyclic AMP (cAMP) for 30 min at 37 \( ^\circ \)C. The binding mixtures were resolved on a 5% neutral polyacrylamide gel after addition of 5% glycerol. Gels were exposed to a BAS-MP 2040s IP plate (Fujifilm, Tokyo, Japan) and scanned by BAS-1500 (Fujifilm, Tokyo, Japan).

Site-directed Mutagenesis of the CRP Binding Site in the Upstream Region of the sid Operon—For site-directed mutagenesis of the CRP binding site, the 266-bp DNA fragment (−344 to −78, with respect to the translation start site) and 343-bp DNA fragment (−97 to +246, with respect to the translation start site) of the sid upstream region were amplified by PCR using primers sid-mtF1 and sid-mtB1 and primers sid-mtF2 and sid-mtB2 (Table 2). The resulting products were cloned into the pGEM-T easy vector (Promega) to generate pGEM-sid-crpm using the In-fusion\textsuperscript{TM} HD cloning kit (Clontech Laboratories, Takara Bio Inc., Shiga, Japan). The region upstream to sidA (−257 to +43, with respect to the translation start site) containing the mutated CRP binding site was amplified by PCR from pGEM-sidA-crpm using primers sid-pro-F1 and sid-pro-B1, and then cloned into pKH0011 as described above. The resulting plasmid was named pSid\textsuperscript{mut}-lux, in which the region upstream to the sid operon with a mutation at the CRP-binding site is transcriptionally fused to luxAB, and was conjugated into V. vulnificus strains.

Semi-quantitative Measurement of Levels of Cytokines in Blood of Mice Infected by V. vulnificus—Serum was obtained from ICR mice infected with wild type V. vulnificus MO6–24/O and the \( \Delta \text{sidC} \) strain. Levels of cytokines were semi-quantitatively determined using a mouse cytokine antibody array C3 kit (RayBiotech, Inc.) following the manufacturer’s instructions. The results were analyzed using a GelQuant Pro v13 (DNR/Bio-Imaging Systems Ltd., Israel).

Results

SidC Is a Virulence Factor Regulating V. vulnificus Survival within the Host—To adapt and survive within the host, V. vulnificus generates factors to manipulate the physiological status of the host, making the host environment favorable for its own survival. We took two approaches to identify novel virulence factors in V. vulnificus: transcriptomic comparisons of V. vulnificus isolates with different pathogenic potencies (21) and in vivo expression technology (22, 23) to identify genes that were expressed at higher levels in a mouse model than in vitro (24). From these two independent methods, a putative insulin-degrading enzyme with the conventional N-terminal secretory signal peptide was identified (annotated as: VVMO6_00510 for strain MO6–24/O (accession number YP_004187735)). Analysis of the deduced amino acid sequence of this gene using the Blastx database in NCBI showed that there is a conventional signal leader sequence at the amino terminus. This protein also contains the inverted zinc-binding motif that is typical to IDEs (data not shown). Because both of these methods identified a specific protease with putative insulin-degrading activity, we hypothesized that this enzyme would be expressed preferentially within the host and therefore, may be important for survival and pathogenicity of V. vulnificus. VVMO6_00510 is encoded by the third gene in an operon that also includes genes encoding VVMO6_00508 (a putative NADPH:quinone reductase) and VVMO6_00509 (a hypothetical protein). We named the three genes in this operon sidABC for secreted-insulin degrading enzyme. The sidA and sidB genes have no detectable effect on the expression or function of SidC (data not shown).

To gain insight into the role of SidC in the host and its role in the survival of V. vulnificus, we compared the survival of wild type V. vulnificus (MO6–24/O), a sidC knock-out strain (\( \Delta \text{sidC} \)), and wild type harboring the sidC gene cloned in a multicopy vector (Fig. 1A). Compared with wild type, \( \Delta \text{sidC} \) can barely survive within the host, whereas the strain complemented with sidC in high copy can survive at least 2-fold more than wild type cells. We also compared the survival of wild type and \( \Delta \text{sidC} \) inside wild type mice and mice suffering type 2 diabetes (db/db mice, C57BLKS-m Lepr\textsuperscript{db}db) (25) (Fig. 1B).

Conclusion

Hypothesis

We have shown that the putative NADPH:quinone reductase encoded by the third gene in an operon that also includes genes encoding VVMO6_00508 and VVMO6_00509 (hypothetical protein) is the putative insulin-degrading enzyme that generates factors to manipulate the physiological status of the host, making the host environment favorable for its own survival.
db/db mice are characterized by extreme physiological changes, hyperinsulinemia and hyperglycemia, compared with wild type mice. Infection with wild type V. vulnificus resulted in significantly lower insulin levels in both mouse strains. Glucose levels in db/db mice are, as expected, significantly higher than in wild type mice. However, there were no significant differences in glucose levels between mice infected by wild type V. vulnificus or /H9004 sidC. There were at least 4-fold more viable wild type bacterial cells in the db/db mice compared with those in non-diabetic mice (Fig. 1B), suggesting that there were favorable factors for V. vulnificus growth and survival in the diabetic mice. Growth of /H9004 mutants in the diabetic mice, however, was greatly impaired (Fig. 1B). These results imply that there may be factors that V. vulnificus must overcome via SidC despite the existence of favorable factors in a diabetic host. In addition to decreased survival within the host, /H9004 bacteria were also less pathogenic than wild type (Fig. 2). Wild type V. vulnificus killed 50% of infected mice in less than 6 h, whereas a /H9004 took 10 h to reach this level of killing (Fig. 2). These results suggest that SidC may play an important role not only in survival within the host, but also in pathogenicity, and that high levels of blood glucose could provide a condition favorable for V. vulnificus proliferation in vivo.

SidC Is a Secreted Insulin-degrading Enzyme—SidC is a member of the inverzincin family of zinc metalloproteases (9, 11), but is only distantly related to other IDEs (Fig. 3, A and B). SidC is distinct from other IDEs in that it has a conventional secretory sequence (26) at the N terminus that is absent in the IDE of E. coli (7) or in the cytosolic IDE of V. vulnificus (14) (Fig. 3C). Mammalian enzymes such as human IDE also can be secreted extracellularly (6, 27). However, those IDEs are secreted in a non-conventional way mediated by the SlyX motif (EKPPHY) located at the C terminus (28). We found that SidC of V. vulnificus was detected in supernatant, periplasmic cell fractions, and cytoplasmic fractions from bacterial culture suggesting that SidC may have an extracellular role (Fig. 3D).

These results led us to test whether SidC has insulin-degrading activity, as does human IDE. V. vulnificus SidC protein that was overexpressed and purified from E. coli showed insulin-degrading activity in a concentration-dependent manner (Fig.
Like other zinc metalloproteases, zinc is required for SidC activity because TPEN, a zinc-specific chelator, and EDTA abolished the insulin-degrading activity of SidC (Fig. 4A).

Cleavage sites on insulin digested by SidC were determined by analyzing the enzymatic products using LC-MS/MS (Fig. 4B). SidC cleaves more sites than human IDE (hIDE) or rat IDE (rIDE) (29, 30). Among 14 cleavage sites, four of them (Leu13-Tyr14 of chain A, and His10-Leu11, Ala14-Leu15, and Phe25-Tyr26 of chain B) were identical with those of both hIDE and rIDE. One cleavage site (Tyr16-Leu17 of chain B) and four cleavage sites (Ser12-Leu13 of chain A, Arg22-Gly23, Gly23-Phe24, and Phe24-Phe25 of chain B) were identical with those of hIDE or rIDE, respectively (29, 30). Five cleavage sites were unique to SidC.

**FIGURE 3.** SidC is novel insulin-degrading enzyme secreted from *V. vulnificus*. A, alignment of the deduced amino acid sequences of the zinc-binding motif in SidC with those of other insulin-degrading enzymes in the inverzincin family. The zinc-binding motifs are marked with asterisks and common amino acids are in boldface; hIDE, human insulin-degrading enzyme (GenBank™ accession number, AAA52712); rIDE, rat insulin-degrading enzyme (NP_037291); dIDE, *Drosophila* insulin-degrading enzyme (AAA28439); yIDE, yeast processing-enhancing protein (NP_013493); PTR, *E. coli* protease III (pitrilysin, YP_491026); vIDE, *V. vulnificus* cytoplasmic insulysin (VVMO6_00860). B, the neighbor-joining phylogenetic tree of insulin-degrading enzymes. Phylogenetic analysis was conducted using MEGA version 4 (48). The distance scale is shown under the phylogenetic tree. C, sequence of the amino terminus of SidC. Charged amino acid residues and hydrophobic residues characteristic of a conventional leader sequence are indicated by asterisks and in boldface, respectively. D, the protein encoded by SidC is exported outside of the cell. Subcellular localization of SidC was determined by Western blot hybridization using polyclonal rat antiserum against purified SidC. 1, Wild type *V. vulnificus* MO6/24-O; 2, ΔsidC; 3, MO6 (pRK-sidC). Alkaline phosphatase activity was measured quantitatively as described previously (16). Alkaline phosphatase and β-galactosidase activity values are the average from three independent experiments, and standard deviations are indicated.
Next, to show insulin-degrading activity within a cellular system, we employed INS-1 pancreatic beta cells, which can secrete insulin in response to high glucose. Insulin levels were reduced in the presence of SidC in a concentration-dependent manner (Fig. 4C). To determine whether the lower insulin levels were due to a cytotoxic effect of the protein, we determined the effects of SidC on the human liver carcinoma cell line HepG2 (31). As shown in Fig. 5A, SidC does not have a significant cytotoxic effect on the HepG2 cell line. We also examined the cytotoxic effect of SidC in INS-1 cells and determined that it is not cytotoxic up to a concentration of 2 μM of SidC (Fig. 5B).

**SidC Reduces Insulin Levels but Increases Glucose Levels in Vivo**—SidC is a unique IDE because it can be secreted via the N-terminal signal peptide (Fig. 3, C and D). Therefore, we hypothesized that after infecting a host, *V. vulnificus* secretes SidC to degrade insulin in the bloodstream, thereby increasing blood glucose levels, improving survivability and enhancing pathogenicity. To test this hypothesis, we injected mice with purified SidC and measured both insulin and glucose levels in the bloodstream using the IPGTT. Interestingly, glucose clearance was significantly reduced in SidC-injected mice compared with the control throughout the experiment, showing that SidC promoted glucose intolerance in mice (Fig. 6A). Because the treatment of SidC reduced glucose-elevated insulin levels in cultured beta cells, we suspected that impaired glucose tolerance might be attributed to the reduction in plasma insulin levels by SidC. Indeed, whereas the intraperitoneal injection of glucose promoted higher insulin concentration in the plasma of control mice, SidC-injected mice showed reduced plasma insulin levels even in the presence of a supra-physiological concentration of glucose (Fig. 6B). This result led us to speculate that after infecting a host, *V. vulnificus* may actively degrade insulin by secreting SidC to affect the host environment.

**SidC Facilitates Survival of *V. vulnificus* in Mice by Modulating Host Insulin Levels**—The effects of injection of purified SidC into mice should likely be different from an active infection with *V. vulnificus*, which will secrete SidC during proliferation and consume host nutrients, such as glucose, for survival. To determine the effects of SidC produced by *V. vulnificus* within the host, we infected mice with both wild type and sidC bacteria and monitored both insulin and glucose levels as well as viable bacterial cell counts over time (Fig. 7). Four hours after
infection, insulin levels in the blood of mice infected with wild type *V. vulnificus* were significantly lower than in mice infected with the ΔsidC mutant (Fig. 7A) confirming that secreted SidC degrades insulin in vivo. We also examined glucose levels and observed that 4 h after infection, glucose levels in the blood of mice infected with wild type *V. vulnificus* were significantly lower than in mice infected with the ΔsidC mutant (Fig. 7B). Importantly, viable cell counts of wild type *V. vulnificus* were significantly higher than those of the ΔsidC mutant at 4 h post-infection and thereafter (Fig. 7C). Taken together, these results demonstrate that SidC is important for *V. vulnificus* survival and proliferation within the host by degrading insulin.

We expected that, due to a lower level of insulin, the level of glucose in blood from mice infected by wild type *V. vulnificus* would be higher than that in mice infected by ΔsidC. But our results did not match this prediction (Fig. 7B). One possible explanation for the low level of glucose in the blood from mice infected by wild type *V. vulnificus* is that SidC produced by infecting *V. vulnificus* also degrades glucagon, resulting in a decreased blood glucose level. We actually observed that glucagon can serve as a substrate for SidC (data not shown). Another possibility is that glucose was being consumed rapidly by the pathogen. To test these possibilities, we employed the *V. vulnificus* strain Δcrr that has a null mutation in *crr* (Table 1), which encodes a crucial factor for glucose uptake; thus Δcrr cannot utilize glucose as a carbon source (32). We compared insulin levels, glucose levels, and viable cell counts in blood samples of mice infected by wild type MO6–24/O, ΔsidC, Δcrr(pRK-sidC), or MO6–24/O(pRK-sidC). We assessed the effects of SidC produced by the pathogen employing the oral glucose tolerance test (OGTT) to minimize the influence of SidC on glucagon (33).

As expected, levels of insulin in blood samples from mice infected with wild type MO6–24/O, Δcrr(pRK-sidC), or MO6–24/O(pRK-sidC) were significantly lower than levels from mice infected with ΔsidC (Fig. 8A). The viable cell counts of wild type MO6–24/O and MO6–24/O(pRK-sidC) are lower (Fig. 8C). These data suggest that the elevated levels of glucose caused by SidC degradation of insulin are subsequently lowered by infecting the mice with *V. vulnificus*, which consumes the glucose to support growth. Taken together, these results show that SidC facilitates growth of *V. vulnificus* in the host by reducing insulin levels, and that degradation of insulin was correlated with increased proliferation of the infecting bacteria.

**Expression of SidC Is Repressed by Glucose via CRP-cAMP—** To determine how sidC is regulated, we sought to determine the growth conditions where sidC is best transcribed. Among various bacterial growth media, sidC was expressed more quickly and at higher levels during growth in brain heart infusion medium, which is an undefined medium made from animal tissue, compared with LB-rich medium or AB minimal medium (3). Also, sidC was expressed more abundantly at 37 °C compared with 28 °C (Fig. 9A), which is consistent with our previous *in vivo* expression technology screening results, which showed that sidC is expressed at higher levels *in vivo* compared with *ex vivo* (24). Numerous genes associated with sugar metabolism are regulated in response to glucose levels through cAMP and the activator protein CRP (34), and it has been shown that expression of the *V. vulnificus* cytoplasmic IDE, insulysin, is regulated in this way (15). We predicted that *sidC* expression may be regulated in a similar manner. In fact, expression of the *sid* operon generally increased as cells entered stationary phase, and subsequent addition of glucose (but not sucrose) led to a dramatic reduction in expression levels (Fig. 9B). In a *crp*-null mutant, expression levels were significantly lower (Fig. 9C), suggesting a role for CRP-mediated regulation. We located a consensus CRP binding site (35) in the region upstream of the *sid* operon (Fig. 9D) and gel mobility shift assays showed that the CRP-cAMP complex bound to this region of the DNA (Fig. 9E). Furthermore, site-directed mutagenesis of the putative CRP-binding site (Fig. 9D) abolished this binding (Fig. 9E). Using *luxAB* reporter fusions, we then showed that both the presence of CRP and the presence of an intact CRP-binding site are required for expression of the *sid* genes (Fig. 9F). We conclude that *sidC* is up-regulated under glucose-limiting conditions, and that this regulation is mediated by the CRP-cAMP complex.

**Discussion**

Many pathogenic bacteria express enzymes that modify host proteins during infection. Some of these enzymes catalyze the addition of chemical groups onto host factors and others modify amino acids (36). Proteolysis of host factors is also known to...
occur, as exemplified by the cleavage of adhesin by a Bacteroides fragilis enterotoxin (37) and the cleavage of host factors by botulinum toxin (38). Through these post-translational modifications, an infecting pathogen can disrupt host signal transduction pathways, important cellular defense mechanisms, or normal cellular processes to facilitate its own survival and propagation. However, specific hydrolysis of human endocrine signals has not yet been documented. In this study, we identified and characterized SidC, a novel bacterial insulin-degrading enzyme that enhances the pathogenicity of *V. vulnificus*. SidC is the first example of an extracellular enzyme secreted by a pathogenic bacterial species that affects the physiology of the host through the proteolytic degradation of a host endocrine signal, insulin.

It is challenging for pathogens to acquire nutrients and energy during infection of a host. Some viruses stimulate an increase in host intracellular glucose levels to increase the energy available for replication by affecting signal transduction pathways associated with sugar metabolism (39, 40). Intracellular Salmonellae have been shown to relay on host intracellular glucose (41). Intracellular *Brucella abortus* also needs glucose for chronic infection (42). However, little is known about how extracellular bacterial pathogens access host metabolites (43). We observed that diabetic mice supported the growth of infecting *V. vulnificus* to higher levels than wild type mice (Fig. 1B), suggesting that higher blood glucose levels are advantageous to *V. vulnificus*. The opportunistic pathogen *Saccharomyces cerevisiae* uses glucose as a primary carbon source in vivo, and the ability to sense and respond to glucose is important for its survival (44). These results suggest that host blood glucose could be an important source of carbon and energy for other extracellular pathogens, including *V. vulnificus*.

We believe that SidC is directly secreted into the bloodstream of mice because we detected *V. vulnificus* in the blood of infected mice. However, the mechanism by which *V. vulnificus* gains access to the bloodstream has yet to be studied. SidC is expressed at higher levels when *V. vulnificus* infects a host, and was also detected at higher levels when cells were grown in an undefined, animal-tissue containing medium. This, together with the fact that low sugar levels stimulated *sidC* expression, led us to speculate that the role of this enzyme is to manipulate glucose levels in the host. Injection of purified SidC led to a reduction in insulin levels and an elevation of glucose levels in the blood of mice (Fig. 6). Blood insulin levels and glucose levels

**FIGURE 7. Effect of SidC on insulin levels, glucose levels, and viability of *V. vulnificus*.** A, 6-week-old female ICR mice (n = 45) were infected with either wild type *V. vulnificus* MO6–24/O (solid square) or ΔsidC (open square). Blood samples were collected every hour for 5 h (n = 5) and tested for insulin levels (A), glucose levels (B), and viable bacterial cell counts (C) as described under “Experimental Procedures.” Error bars denote the standard deviations. The area under the curve (AUC) was calculated by the trapezoid method (33) between the 2- and 5-h time point.
An Insulin-degrading Enzyme Facilitates Host Infection

![Graphs showing effects of Δcrr on insulin levels, glucose levels, and the viability of V. vulnificus.](image)

**FIGURE 8.** Effect of Δcrr on insulin levels, glucose levels, and the viability of *V. vulnificus*. Six-week-old female ICR mice were infected with wild type *V. vulnificus* MO6–24/O (solid square), ΔsidC (empty square), Δcrr (prK-sidC) (solid triangle), and MO6–24/O (prK-sidC) (empty triangle), and orally administered with glucose as described under “Experimental Procedures” (*n* = 8). Blood samples were collected 4 h after infection, and tested for insulin levels (A), viable bacterial cell counts (B), and glucose levels (C) as described under “Experimental Procedures.” Each symbol represents an individual mouse. Long bars indicate the mean, and upper and lower bars represent the mean ± S.E. for each group. Statistical significance was determined using the Mann-Whitney test.

In mice infected with wild type *V. vulnificus* were both significantly lower than those in mice infected with a sidC-deletion strain (Fig. 7). It is possible that blood glucose levels increased as a result of SidC activity, and then decreased as the sugar was consumed by the pathogen. Alternatively, it is possible that the reduced blood glucose level may be due to decreased levels of glucagon caused by SidC-mediated degradation, because we observed that SidC can also use glucagon as a substrate (data not shown). A recent study indicated that the route of glucose administration determines the effect of IDE on glucose tolerance (33). The insulin level in IPGTT is significantly lower than that in OGTT. Therefore, during IPGTT, IDE degrades proportionally more glucagon and thereby results in lower glucose levels during IPGTT (33). Meanwhile, OGTT does not decrease the blood insulin level, providing a better condition to monitor the effect of IDE on insulin (33). To circumvent the potential effect of IDE on glucagon, we performed *V. vulnificus* infection experiments using mice on which glucose was orally administered (Figs. 7 and 8).

We hypothesized that the lower glucose levels in mice infected by wild type *V. vulnificus* is due to the consumption of the sugar by the proliferating pathogen. To explore this, we employed the Δcrr strain harboring prK-sidC, a strain that cannot utilize glucose due to an inability to transport sugar. The reason for the introduction of an extra copy of *sidC* under the vector promoter is that *sidC* is located on the chromosome cannot be expressed because the sugar group translocation system (34) regulates the expression of *sidC* (Fig. 9). Therefore, the chromosomal *sidC* under its own cognate promoter would not be expressed without Crr. Our assumption was that, if the reduced level of glucose was caused not by bacterial consumption but by some other factors (for instance, degradation of glucagon by SidC), the glucose levels in mice infected by Δcrr (prK-sidC) would also be as low as those infected by wild type. The results clearly showed that the blood glucose level in mice infected by Δcrr (prK-sidC) is even higher than in mice infected by wild type bacteria. It is noteworthy that the presence of *V. vulnificus* strains expressing SidC led to a reduction in blood insulin levels, and that the amount of blood glucose is inversely proportional to the level of proliferation of the infecting pathogen (Figs. 7 and 8).

It also is noteworthy that the proliferation of MO6–24/O (prK-sidC) is higher than that of wild type, even if the consumption of blood glucose by these two strains are not significantly distinct (Fig. 8, B and C). SidC may have additional functions for the pathogenesis of *V. vulnificus*. Human IDE has multiple roles in the cell, including degradation of both small molecular weight proteins and β-amyloid (5, 6, 27), and therefore SidC may affect host physiology in multiple ways as well. Using a mouse cytokine antibody array, we eliminated the possibility that SidC degrades host cytokines (data not shown). Other possible roles for SidC-associated pathogenicity remain to be explored.

Regardless of the underlying mechanism for the pathogenicity of SidC, our results may explain why there is an increased risk of *V. vulnificus* infections in patients with diabetes mellitus (45), and may explain the higher risk of other bacterial infections as well (46). SidC homologs are present in other pathogenic *Vibrio* species, such as *Vibrio cholerae* and *Vibrio parahaemolyticus* (identity %/similarity %; 71/84 and 74/86, respectively) (data not shown). The SidC homolog in *V. cholerae* is up-regulated during the late stages of infection, as measured using a recombination-based in vivo expression technology (47), suggesting a role for SidC in the pathogenicity of this species.
FIGURE 9. The transcription of the *sid* operon is repressed by glucose via CRP-cAMP. A, transcription levels of the *sid* operon. Luciferase activity from *V. vulnificus* harboring a *sidA-luxAB* fusion at 28 (open) or 37 °C (closed) in AB (square), LB (triangle), or brain heart infusion medium (circle). Relative luminescence units (RLU) represent the luminescence values normalized to cell density (A$_{600}$. Data are the average of three independent experiments and error bars denote S.D. B, transcription levels of the *sid* operon using a *sidA-luxAB* fusion in wild type *V. vulnificus* grown in brain heart infusion broth. The arrow indicates the time at which 0.2% glucose (square), 0.2% sucrose (triangle), or no supplement (circle) was added. C, expression of *sidC* is repressed in a crp-deletion mutant. Luciferase activities of MO6–24/O(pBBR1-MCS2) (empty bar), Δcrp (pBBR1-MCS2) (black bar), and Δcrp (pBBR1-crP) (gray bar) harboring *sid-luxAB* (upper figure). The relative luminescence units represent the luminescence values normalized to cell density (A$_{600}$. The data are the average of three independent experiments, and error bars denote the S.D. and p values are shown. Western hybridization using polyclonal antisera against SidC is shown in the lower panel. Total protein was extracted when cells reached an A$_{600}$ of 2.2, and 40 μg of the total protein was loaded into each lane. D, nucleotide sequence upstream of the *sid* operon containing a consensus CRP-cAMP binding site. Sequences homologous to the consensus CRP-binding sites are indicated by arrows, and mutated bases are indicated below. E, gel shift assays using purified CRP-cAMP and either the region upstream of the wild type *sid* operon (upper panel) or the same region containing mutations in the CRP binding site (lower panel); lanes 1–4, CRP concentrations of 0, 100, 200, and 400 nM, respectively, with 1 mM CAMP each; lanes 5–7, 400 nM CRP with unlabeled probe as a competitor at 0.5, 5, and 50 ng, respectively, with 1 mM CAMP each; lane 8, 400 nM CRP alone. F, luciferase activities of MO6–24/O(pBBR1-MCS2) (circle), Δcrp (pBBR1-MCS2) (square), and Δcrp (pBBR1-crP) (triangle) harboring a wild type *sid* promoter region fused to the *luxAB* reporter (pPsid-lux), and MO6–24/O (pBBR1-MCS2) harboring the same region with a mutated CRP binding site fused to *luxAB* (pPsid<-lux) (open circle). Relative luminescence units represent the luminescence values normalized to cell density (A$_{600}$. Error bars denote the S.D.
species as well. More in-depth study of this novel pathogenic mechanism could lead to new options for both treating and preventing infections caused by pathogenic Vibrio species. Likewise, further understanding of other previously unrecognized catabolite-scavenging capabilities used by pathogenic microorganisms could address the increasing challenges in treating infectious diseases.

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References

1. Horseman, M. A., and Surani, S. (2011) A comprehensive review of Vibrio vulnificus: an important cause of severe sepsis and skin and soft-tissue infection. Int. J. Infect. Dis. 15, e157–166
2. Jones, M. K., and Oliver, J. D. (2009) Insulin degradative process and potential. Endocr. Rev. 19, 608–624
3. Duckworth, W. C., Bennett, R. G., and Hamel, F. G. (1998) Insulin degradation: progress and potential. FEBS Lett. 453, 77–84
4. Haeque, R., and Nazir, A. (2014) Insulin-degrading enzyme: a link between Alzheimer’s and type 2 diabetes mellitus. CNS Neurol. Drug. Disc. Targets 13, 35–44
5. Cheng, Y. S., and Zipser, D. (1979) Purification and characterization of protease III from Escherichia coli. J. Biol. Chem. 254, 4698–4706
6. Swamy, K. H., and Goldberg, A. L. (1982) Subcellular distribution of various proteases in Vibrio cholerae. J. Bacteriol. 149, 1027–1033
7. Becker, A. B., and Roth, R. A. (1992) An unusual active site identified in a family of zinc metalloendopeptidases. Proc. Natl. Acad. Sci. U.S.A. 89, 3835–3839
8. Shen, Y., Joachimiak, A., Rosner, M. R., Safavi, A., Hersh, L. B., and Selkoe, D. J. (1998) Insulin-degrading enzyme regulates extracellular levels of amyloid β-protein by degradation. J. Biol. Chem. 273, 32730–32738
9. Glebov, K., Schütze, S., and Walter, J. (2011) Functional relevance of a novel SlyX motif in non-conventional secretion of insulin-degrading enzyme. J. Biol. Chem. 286, 22711–22715
10. Shen, Y., Joachimiak, A., Rosner, M. R., and Tang, W. J. (2006) Structures of human insulin-degrading enzyme reveal a new substrate recognition mechanism. Nature 443, 870–874
11. Bellia, F., Pietropaolo, A., and Grasso, G. (2013) Formation of insulin fragments by insulin-degrading enzyme: the role of zinc(II) and cystine bridges. J. Mass Spectrom. 48, 135–140
12. Aden, D. P., Fogel, A., Plotkin, S., Damjanov, I., and Knowles, B. B. (1979) Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line. Nature 282, 615–616
13. Lee, T., Jeong, C. S., An, Y. J., Lee, H. I., Park, S. J., Seok, Y. J., Kim, P., Lee, J. H., Lee, K. H., and Cha, S. S. (2011) FrsA functions as a cofactor-independent decarboxylase to control metabolic flux. Nat. Chem. Biol. 7, 434–436
14. Maiani, J. T., McFedries, A., Foda, Z. H., Kleiner, R. E., Du, X. Q., Leissinger, M. A., Tang, W., and Reedy, R., Seligman, A. M., Saghatelian, A., and Liu, D. R. (2014) Anti-diabetic activity of insulin-degrading enzyme inhibitors mediated by multiple hormones. Nature 511, 94–98
15. Kimata, K., Takahashi, H., Inada, T., Postma, P., and Aiba, H. (1997) cAMP receptor protein-cAMP plays a crucial role in glucose-lactose diauxie by activating the major glucose transporter gene in Escherichia coli. Proc. Natl. Acad. Sci. U.S.A. 94, 12914–12919
16. Busby, S., and Ebright, R. H. (1999) Transcription activation by catabolite activator protein (CAP). J. Biol. Chem. 273, 199–203
17. Ribet, D., and Cossart, P. (2010) Post-translational modifications in host cells during bacterial infection. FEBS Lett. 584, 2748–2758
18. Wu, S. I., Lim, K. C., Huang, J., Saidi, R. F., and Sears, C. L. (1998) Bacteroides fragilis enterotoxin cleaves the zonula adherens protein, E-cadherin. Proc. Natl. Acad. Sci. U.S.A. 95, 14979–14984
19. Popoff, M. R., and Bouvet, P. (2009) Clostridial toxins. Future Microbiol. 4, 1021–1026

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39. McArdle, J., Moorman, N. J., and Munger, J. (2012) HCMV targets the metabolic stress response through activation of AMPK whose activity is important for viral replication. PLoS Pathog. 8, e1002502
40. Terry, L. J., Vastag, L., Rabinowitz, J. D., and Shenk, T. (2012) Human kinome profiling identifies a requirement for AMP-activated protein kinase during human cytomegalovirus infection. Proc. Natl. Acad. Sci. U.S.A. 109, 3071–3076
41. Bowden, S. D., Rowley, G., Hinton, J. C., and Thompson, A. (2009) Glucose and glycolysis are required for the successful infection of macrophages and mice by Salmonella enterica serovar typhimurium. Infect. Immun. 77, 3117–3126
42. Xavier, M. N., Winter, M. G., Spees, A. M., den Hartigh, A. B., Nguyen, K., Roux, C. M., Silva, T. M., Astfurl, V. L., Kerrinnes, T., Keestra, A. M., Monack, D. M., Luciw, P. A., Eigenheer, R. A., Bäumler, A. J., Santos, R. L., and Tsolis, R. M. (2013) PPARγ-mediated increase in glucose availability sustains chronic Brucella abortus infection in alternatively activated macrophages. Cell Host Microbe 14, 159–170
43. Brunton, J., Steele, S., Ziehr, B., Moorman, N., and Kawula, T. (2013) Feeding uninvited guests: mTOR and AMPK set the table for intracellular pathogens. PLoS Pathog. 9, e1003552
44. Kingsbury, J. M., Goldstein, A. L., and McCusker, J. H. (2006) Role of nitrogen and carbon transport, regulation, and metabolism genes for Saccharomyces cerevisiae survival in vivo. Eukaryot. Cell 5, 816–824
45. Koenig, K. L., Mueller, J., and Rose, T. (1991) Vibrio vulnificus: hazard on the half shell. West. J. Med. 155, 400–403
46. Gan, Y. H. (2013) Host susceptibility factors to bacterial infections in type 2 diabetes. PLoS Pathog. 9, e1003794
47. Schild, S., Tamayo, R., Nelson, E. J., Qadri, F., Calderwood, S. B., and Camilli, A. (2007) Genes induced late in infection increase fitness of Vibrio cholerae after release into the environment. Cell Host Microbe 2, 264–277
48. Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599
49. 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
50. Milton, D. L., O’Toole, R., Horstedt, P., and Wolf-Watz, H. (1996) Flagellin A is essential for the virulence of Vibrio anguillarum. J. Bacteriol. 178, 1310–1319
51. Kovach, M. E., Elzer, P. H., Hill, D. S., Robertson, G. T., Farris, M. A., Roop, R. M., 2nd, and Peterson, K. M. (1995) Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. Gene 166, 175–176