Functional characterization of VC1929 of *Vibrio cholerae* El Tor: role in mannose-sensitive haemagglutination, virulence and utilization of sialic acid

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The nonadhesive mutant CD11 of *Vibrio cholerae* El Tor, defective in expression of mannose-sensitive haemagglutinin, lacks a protein when compared with its parent strain. Determination of the amino acid sequence revealed the identity of the protein as the product of VC1929, which is annotated to encode a protein, DctP, involved in the transport of C4-dicarboxylates. We cloned the *dctP* gene in pUC19 vector and expressed it in mutant CD11. Expression of DctP in the resulting complemented strain restored virulence, adhesive and colonizing capabilities, mannose-sensitive haemagglutination (MSHA) and ability to grow in medium containing sialic acid as a sole carbon source. The mutation in CD11 was caused by insertion of an adenine nucleotide in the reading frame of *dctP*. Recombinant purified DctP protein showed MSHA of human red blood cells, and protected rabbits against infection by *V. cholerae*. The protein was localized in membrane and cell wall fractions. The mutant, recombinant CD11 expressing DctP and parent strains were grown in M9 minimal medium in the presence of various carbohydrates (glucose, malate, fumarate, succinate or N-acetylneuraminic acid). The mutant was unable to grow in minimal medium containing N-acetylneuraminic acid (sialic acid) as the sole carbon source whereas the recombinant and parent strains utilized all the sugars tested. It is concluded that DctP is a mannose-sensitive haemagglutinin and a virulence factor and is involved in the utilization of sialic acid.

INTRODUCTION

*Vibrio cholerae* is an enteropathogen, which causes the human diarrhoeal disease cholera. Pathogenesis of cholera begins with infection by bacteria resulting from ingestion of contaminated food and/or water, and colonization of the intestinal epithelial surface aided by various adhesins present on the bacterial surface. A number of antigens of *V. cholerae* have been identified as having roles in adherence to the intestine (Kaper *et al.*, 1995). Adherence is followed by the bacteria releasing a toxin, which causes rapid fluid loss from the body leading to diarrhoea and dehydration (Field *et al.*, 1989). Some 30 years ago, a mutant of *V. cholerae* El Tor was described which, unlike the parent strain, neither adhered efficiently to intestinal mucosa of rabbits nor expressed mannose-sensitive haemagglutinins (Bhattacharjee & Srivastava, 1978). The mutant failed to efficiently colonize the gut of infant mice and was found to be lacking in a protein (Jacob *et al.*, 1993). Amino acid sequencing of the protein revealed homology to VC1929 of the *V. cholerae* genome (Heidelberg *et al.*, 2000). VC1929 is predicted to be a protein, DctP, whose role in transport of C4-dicarboxylates across the membrane has been reported for *V. cholerae* (http://www.ncbi.nlm.nih.gov). C4-dicarboxylates are tricarboxylic acid cycle (TCA) intermediates, and the transport of the C4-dicarboxylates succinate, fumarate and malate across the cell membrane is controlled by a common C4-dicarboxylate transport system that has been described for various bacteria (Janausch *et al.*, 2002).

In this study, we show that purified VC1929 protein of *V. cholerae* can agglutinate human erythrocytes, a process which is inhibited in the presence of D-mannose. DctP appears to be involved in utilization of sialic acid, as the DctP-deficient mutant CD11 was unable to grow in media containing N-acetylneuraminic acid as sole carbon source.

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**Abbreviations:** HA, haemagglutination; MSHA, mannose-sensitive haemagglutination; RBC, red blood cells; RT-qPCR, real-time quantitative PCR.

A supplementary figure showing the nucleotide sequence of the mutant *dctP* gene of strain CD11 is available with the online version of this paper.
DctP is a virulence factor, as it mediates adherence and colonization of V. cholerae in the infant mouse model, and the purified protein as a subunit vaccine protected rabbits from experimental cholera.

**METHODS**

**Bacterial strains, plasmids and media.** *Vibrio cholerae* strain KB207 is wild-type El Tor Ogawa, which is highly pathogenic in experimental models of cholera. CD11 is an isogenic nonadhesive, noncolonizing attenuated strain (Bhattacharjee & Srivastava, 1978; Jacob et al., 1993). CD11RO is a complemented strain in which the gene encoding DctP has been introduced into CD11 (see below). *Escherichia coli* strains DH5α and BL21(DE3)PlySs were used for cloning and expression, respectively. pGEMT-easy vector was used to clone the amplified gene, pET14b for expression of the cloned gene in *E. coli* and pUC19 for expression in *V. cholerae*. *E. coli* strains and plasmids were purchased from Novagen/Invitrogen/Biolabs. Bacteria were normally grown in Luria–Bertani (LB) broth at 37 °C with aeration. Plates were made by adding 1.5 % Difco agar. Antibiotics were added to the following concentrations: streptomycin, 50 μg ml⁻¹; ampicillin, 50 μg ml⁻¹. Compositions of PBS, LB and M9 minimal medium were as described by Miller (1972).

**Cloning the dctP gene.** The 999 bp DNA sequence of VC1929 was amplified by PCR from *V. cholerae* genomic DNA using the forward primer 5′-CCGAAGTTGAAAGTTAGC-3′ and reverse primer 5′-GCTTGTGACAGCGAGGA-3′. PCR cycling parameters were: (1) initial denaturation at 95 °C for 5 min (1 cycle), (2) denaturation at 94 °C for 45 s, (3) annealing at 50 °C for 45 s, (4) extension at 72 °C for 45 s, (5) repetition of steps 2–4 for 30 cycles, and (6) extension at 72 °C for 5 min (1 cycle). The amplified DNA fragment was excised from the gel and purified using a Qiagen gel extraction kit. The amplified DNA was ligated into pGEMT-easy vector following the standard protocol and sequenced to confirm the identity of the gene. The dctP gene was released from the cloning vector by digestion with BanHI and subcloned into expression vector pET14b to give pET14b::dctP, which was transformed into *E. coli* BL21(DE3)PlySs. Expression in this vector is driven by the T7 promoter. Transformations and all DNA manipulations were carried out according to Hanahan (1983) and Sambrook et al. (1989).

Mutant dctP and 300 bp of its upstream region were amplified from the genomic DNA of CD11 using the same protocol as described above. Primers for the former were the same as used for wild-type dctP, whereas for the latter the forward and reverse primers were 5′-GGATCCCAGTATGAAAGGCGCTTACC-3′ and 5′-GGATCCCTTACTTCTGGTACGCTGC-3′, respectively.

**Expression and purification of DctP protein.** A single colony of transformed *E. coli* BL21(DE3)PlySs from a freshly streaked LB agar plate was inoculated into LB broth containing ampicillin and grown overnight at 37 °C. The culture was 100-fold diluted in fresh LB broth containing antibiotic and incubated to OD₆₀₀ 0.6. The culture was induced by the addition of 1 mM IPTG and further incubated at 37 °C for 2–3 h. The cells were harvested by centrifugation at 6000 g in a Sorvall GSA rotor for 10 min at 4 °C. After discarding the supernatant, the pellet was suspended in 10 mM Tris/HCl (pH 7.5) and sonicated at 12 db/10 s for 10 cycles with 1 min intervals on ice. Since DctP protein occurs in the insoluble fraction, the sonicate was centrifuged at 12 000 g for 30 min at 4 °C to separate the pellet containing DctP. The pellet was incubated in binding buffer (20 mM Tris/HCl, pH 7.5, 300 mM NaCl) containing 6 M urea for 30 min. The His-tagged fusion protein His-6–DctP was purified using a Ni²⁺ NTA affinity column according to standard procedures. His-6–DctP protein was recovered from the column by elution with 300 mM imidazole.

**Complementation of mutant CD11 with the dctP gene.** The pUC19 vector was digested with BanHI and dephosphorylated by calf intestinal alkaline phosphatase treatment. The vector was then ligated to dctP and transformed into *E. coli* DH5α. The clones with inserts were identified by restriction digestion of plasmids with BanHI and agarose gel electrophoresis. This was followed by restriction analysis with SacI to choose the correctly oriented insert, which yielded two fragments of 180 and 3506 bp. The recombinant plasmid pUC19::dctP was isolated from the correctly oriented clone and electroporated into the CD11 mutant according to a previously described protocol (Marcus et al., 1990). The complemented strain was designated CD11RO.

**Haemagglutination (HA) assay.** Human red blood cells (RBC) were collected, washed three times in PBS and resuspended to 2 % (v/v). Serial double dilutions of bacteria in PBS were made in microtitre plates using 0.05 ml microdiluters. An equal volume of RBC was added and mixed, and the plates were left at room temperature for 1 h. The highest dilution giving HA was recorded. HA by purified DctP protein was performed by adding protein instead of bacteria. The effect of D-mannose on HA was examined by including D-mannose (100 μg ml⁻¹) in the PBS (Bhattacharjee & Srivastava, 1978).

**Western blotting.** Western blotting was performed to examine expression and localization of DctP protein in *V. cholerae* strains using rabbit anti-DctP antibodies prepared by two subcutaneous injections of purified DctP protein as described above. Bacterial lysates were separated by SDS-PAGE (12.5 % acrylamide); separated proteins were transferred onto a nitrocellulose membrane in a Trans-Blot apparatus (Bio-Rad Mini Protein III) and probed with anti-DctP antibody (1 : 4000 dilution) followed by incubation with horseradish-peroxidase-linked anti-rabbit IgG (1 : 5000 dilution). The blot was developed by dipping in a colour development solution containing hydrogen peroxide and 3,3′-diaminobenzidine tetrahydrochloride (Jacob et al., 1993).

**Rabbit ileal loop assay.** The rabbit ileal loop assay was carried out to determine the immunogenicity of the DctP protein as described previously (Jacob et al., 1993). Six Swiss albino rabbits of 1 kg were used in both the control and the immunized group. The immunized group received two doses of purified DctP protein (50 μg protein in 300 μl PBS) subcutaneously within a 21 day interval. The control unimmunized rabbits received 300 μl PBS. All rabbits were bled before immunization as well as 48 h before stavation and challenge. Protection against challenge by *V. cholerae* was determined 30 days after the second dose of vaccine. Loops (8–9 cm) of small intestine were ligated from the caecal end and challenged with 10⁵ c.f.u. KB207. Rabbits were killed after 18 h. The volume (ml) of fluid per cm accumulating in each loop was measured and protection expressed as described by Jacob et al. (1993).

**Vibriocidal antibody titration.** Titration of vibriocidal antibodies in the sera of unimmunized and DctP-immunized rabbits was carried out essentially as described by us (Finkelstein, 1962; Jacob et al., 1993) using KB207 as standard strain and 5 % guinea pig complement. Sera were inactivated by incubation at 56 °C for 30 min.

**Suckling mouse assay.** The 5-day-old suckling mouse model was used to determine the enteropathogenicity and colonization of strains KB207, CD11 and (complemented) CD11RO (Jacob et al., 1993). Briefly, a suspension of the respective strain (10⁶ c.f.u. ml⁻¹) was prepared. A 0.2 ml volume containing 0.01 % Evans blue dye was orally inoculated into each mouse and the mice were returned to their
mothers. Three to five mice from each group were killed each day. Intestines were removed and homogenized in 10 ml PBS. Viable counts were made on selective agar medium containing antibiotics.

**Real-time PCR analysis.** RNA was extracted from mid-exponential-phase cultures of strains KB207, CD11 and CD11RO using the RNAeasy mini kit (Qiagen). To remove genomic DNA contamination, the isolated RNA samples were treated with DNase I and confirmed by PCR amplification. The cDNA was synthesized using a first-strand cDNA synthesis kit (Fermentas) according to the manufacturer’s instructions. After normalization with 16s rRNA, real-time PCR was performed in a LightCycler 480 II (Roche) using SYBR GREEN I Master mix (Roche). The primer pair 5'-CTAATT-GCCGCTTCTCAGTT-3'/5'-CATCGTACACGGTTAGTTA-3' was used for real-time quantitative PCR (RT-qPCR) gene expression analysis. RT-qPCR cycling conditions were as follows: (1) preincubation at 95°C for 10 min (1 cycle), (2) amplification at 95°C for 10 s, 53°C for 20 s and 72°C for 20 s (40 cycles), (3) melting curve analysis at 95°C for 10 s, 65°C for 1 min, and 97°C until acquisition of the frequency of acquired fluorescence data, and then cooling at 40°C for 10 s.

All the data analysis was done by using the LightCycler 480 II software version 1.5. Student’s t test was performed to determine the significance of the relative expression ratio. P<0.05 was considered significant. The protocol followed was as described by Gupta et al. (2010).

**Localization of DctP protein.** The presence of DctP was determined in various cellular fractions of V. cholerae strain KB207 by SDS-PAGE and Western blot analysis. Preparation of subcellular fractions was carried out as described by Lee et al. (1992). β-Galactosidase served as a marker protein, which was monitored in each fraction (Miller, 1972) and found only in the cytosolic fraction. Briefly, KB207 cells were grown in 50 ml LB broth and centrifuged at 6000 g for 10 min. The supernatant was filtered through a 0.2 μm syringe filter and kept as the extracellular fraction. The pellet was washed twice with chilled PBS and finally suspended in PBS. Cells were sonicated at 4°C. Unbroken cells were removed by centrifugation (6000 r.p.m. for 10 min, Sorvall SL-50T rotor) and the supernatant was centrifuged at 20000 g for 1 h. The pellet was kept as the cell wall fraction and the supernatant was centrifuged at 100000 g for 1 h. The pellet was kept as the cell membrane fraction, and the supernatant as the cytosolic fraction. Concentration of protein was estimated in the different fractions (Bradford, 1976).

**Growth of V. cholerae strains with different carbon sources.** Growth of V. cholerae in M9 minimal medium containing glucose, fumarate, malate, succinate or N-acetylneuraminic acid (sialic acid) as a sole source of carbon and energy was determined by measuring OD_{600} for 20 h by Bioscreen growth analyser. The final concentration of all carbon sources was 20 mM. M9 medium with no carbon source was grown in parallel lane (Fig. 1c). Protein expression was validated by expression of DctP-specific RNA by RT-qPCR. Relative expression was nearly 10-fold and 20-fold higher in CD11RO and KB207, respectively, compared with CD11 (Fig. 2). These data confirmed that CD11RO is a genuine dctP-complemented strain expressing DctP.

**Haemagglutination**

Mannose-sensitive haemagglutination (MSHA) by KB207 has been reported previously (Bhattacharjee & Srivastava, 1978), whereas HA by CD11 was unaffected by the presence of D-mannose. It was therefore concluded that CD11, which is a nonadhesive mutant, was deficient in expression of an antigen involved in MSHA. Complemented strain CD11RO resembled the parent strain KB207 in that it agglutinated human RBC, and this could be inhibited by D-mannose. Since CD11RO expressed DctP protein, and MSHA could be attributed to DctP protein, the haemagglutinating property of the purified protein was tested. When the protein was added in place of bacteria, it showed MSHA with human RBC.

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### RESULTS

**Expression of recombinant DctP protein in E. coli**

The DctP protein was expressed in E. coli. Total proteins of uninduced and IPTG-induced cultures were separated by SDS-PAGE. The His-tagged DctP protein was purified by Ni^{2+}-NTA affinity column chromatography (Fig. 1a). A high level of protein expression was observed; however, most of the protein was found in inclusion bodies. With the addition of urea (6.0 M) in buffer during cell lysis, the solubility improved considerably. The recombinant protein of about 37 kDa appeared to be the product of a 999 bp fragment as predicted by bioinformatics using the NCBI database.

**Complementation of CD11 with cloned dctP gene**

CD11, which does not express DctP protein, was transformed with recombinant plasmid pUC19::dctP, and a clone with the dctP gene in the correct orientation with respect to the promoter was selected and designated CD11RO. Empty pUC19 vector was also electroporated separately into CD11 for comparison. Expression of DctP protein was observed in the parent strain KB207 and CD11RO but not in CD11 mutant or CD11 harbouring empty plasmid (Fig. 1b). Hence the observed protein appeared to be DctP. This was confirmed by Western blotting using rabbit anti-DctP antibodies. DctP protein was detected in the cell lysates of KB207 and CD11RO but not in CD11. For comparison, purified DctP was loaded in a parallel lane (Fig. 1c). Protein expression was validated by expression of DctP-specific RNA by RT-qPCR. Relative expression was nearly 10-fold and 20-fold higher in CD11RO and KB207, respectively, compared with CD11 (Fig. 2). These data confirmed that CD11RO is a genuine dctP-complemented strain expressing DctP.
agglutination of RBC occurred which was inhibited by D-mannose (Table 1). Therefore DctP protein may be defined as a mannose-sensitive haemagglutinin.

Colonization and enteropathogenicity in suckling mice

Colonization of intestine by KB207, CD11 and complemented strain CD11RO was analysed using a suckling mouse model. Determination of c.f.u. suggested that KB207 and CD11RO multiplied and increased in number, suggesting colonization of the intestine, whereas c.f.u. of CD11 were lower and did not increase (Fig. 3); hence this strain did not colonize, as also observed previously (Jacob et al., 1993). When mice were left beyond 48 h, death of the animals started in groups inoculated with KB207 and CD11RO and all mice died in the next 48 h. However, no or very few deaths were recorded in mice infected with CD11. The observations with CD11 and KB207 are consistent with earlier observations on the attenuation of virulence of CD11 compared with KB207. It is evident from the present data that DctP-complemented CD11RO has acquired the colonizing and enteropathogenic characteristics of the parent strain KB207. Hence DctP appears to be a virulence factor of *V. cholerae*.

**Table 1.** Haemagglutination assay

<table>
<thead>
<tr>
<th>Strain/protein</th>
<th>Mean HA titre (reciprocal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without D-mannose</td>
</tr>
<tr>
<td>KB207</td>
<td>64</td>
</tr>
<tr>
<td>CD11</td>
<td>32</td>
</tr>
<tr>
<td>CD11RO</td>
<td>64</td>
</tr>
<tr>
<td>DctP protein</td>
<td></td>
</tr>
<tr>
<td>1.0 µg per well</td>
<td>HA−</td>
</tr>
<tr>
<td>2.5 µg per well</td>
<td>HA+</td>
</tr>
<tr>
<td>BSA</td>
<td>1.0 µg per well</td>
</tr>
<tr>
<td></td>
<td>2.5 µg per well</td>
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</tbody>
</table>

http://mic.sgmjournals.org
DctP can protect against *V. cholerae*

When rabbits were immunized with purified DctP protein and challenged with KB207 in the ileal loop assay, significant protection was observed, with a significant rise in vibriocidal antibody titre (Table 2). Unimmunized control rabbits were not protected and significant accumulation of fluid was observed. This result further suggests that DctP protein is a virulence factor of *V. cholerae*.

Cellular localization of DctP

Bioinformatic analysis (NCBI database) suggested that DctP may be a periplasmic protein. Localization of DctP in major cellular fractions was checked by Western blotting with anti-DctP antibodies. The protein was found in the membrane and cell wall fractions (Fig. 4).

Bioinformatic analysis

It was found that DctP of *V. cholerae* has the greatest homology (50 %) to the probable C4-dicarboxylate-binding periplasmic protein of *Pseudomonas aeruginosa* and less than 2 % to its *E. coli* counterpart. Interestingly, the amino acid sequence of DctP exhibits 54 % homology to sialic-acid-binding protein (SiaP) of *Aggregatibacter aphrophilus* (NCBI database).

Growth in the presence of different carbon sources

Given the homologies of DctP of *V. cholerae* to a C4-dicarboxylate-binding periplasmic protein and a sialic-acid-binding periplasmic protein (see above), growth of KB207, CD11 and DctP-complemented CD11RO was tested in minimal medium containing glucose, fumarate, malate, succinate or N-acetylneuraminic acid. Whereas KB207 and CD11RO grew in medium with N-acetylneuraminic acid as the sole carbon source, CD11 could not grow in this medium (Fig. 5). However, no difference was found between the three strains with respect to growth on the other carbon sources tested (results not shown).

**The mutation in the dctP gene of CD11**

The *dctP* coding sequence and the region upstream of this gene were sequenced and compared with the published sequence of the *V. cholerae* genome. No difference was found between parent and mutant sequences from −300 to +1bp of *dctP* (contig 2082387–2082085). A striking difference in the *dctP* coding sequence of CD11 (contig 2082085–2081087) compared with the parent sequence was found in the middle of the reading frame. At position +402 insertion of one adenine nucleotide was detected, which caused a shift in the reading frame resulting in creation of a number of stop codons (see Supplementary Fig. S1, available with the online version of this paper).

**DISCUSSION**

Three major conclusions have emerged from this study. First, DctP protein is a haemagglutinin that can agglutinate RBC in a mannose-sensitive manner, as do intact bacteria expressing this antigen on their surface. Furthermore, the haemagglutinating phenotype was sensitive to inhibition by D-mannose. Second, DctP is a virulence factor of *V. cholerae*, which contributes to virulence by aiding colonization of the intestine by *V. cholerae*. Third, DctP appears to be involved in the utilization of sialic acid.

Adherence of bacteria to mucosal cells of the intestine is important for colonization. An array of antigens are elaborated by *V. cholerae*, which are involved in adherence to and colonization of intestine (see Kaper et al., 1995). Surface antigens facilitating colonization of host intestine by other pathogenic bacteria and correlation between haemagglutination and adherence to mucosal cells have been reported (Burrows et al., 1976; Elwell & Shipley, 1980; Jones & Rutter, 1974; Koransky et al., 1975). We have earlier

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**Table 2. Protection by DctP antigen against *V. cholerae* in rabbit ileal loop assay**

<table>
<thead>
<tr>
<th>Group</th>
<th>Fluid (ml per cm loop)</th>
<th>Protection from challenge (%)</th>
<th>Vibriocidal titre (mean ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± sd</td>
<td></td>
</tr>
<tr>
<td>DctP vaccinated</td>
<td>0.0–0.5</td>
<td>0.26 ± 0.125</td>
<td>78</td>
</tr>
<tr>
<td>Control unvaccinated</td>
<td>0.9–1.6</td>
<td>1.175 ± 0.234*</td>
<td>0</td>
</tr>
</tbody>
</table>

*Fluid volume in the unvaccinated group was significantly higher (*P*<0.01) compared with the vaccinated group, by Student’s *t*-test.
†Vibriocidal antibody titre in the vaccinated group was significantly higher (*P*<0.01) compared with the unvaccinated group, by Mann–Whitney *U* test.
reported a correlation between haemagglutination and adherence of *V. cholerae* to mucosal cells. Using the nonadhesive mutant CD11 of *V. cholerae* El Tor we demonstrated that mannose-sensitive haemagglutinins mediated adherence to RBC as well as to intestine (Bhattacharjee & Srivastava, 1978). Enteropathogenic *E. coli* has been reported to adhere to human mucosal cells via mannose receptors (Ofek et al., 1977). Comparison of total proteins of mutant CD11 and the parent strain by SDS-PAGE revealed that the mutant was lacking in expression of a protein (Jacob et al., 1993). The amino acid sequence of the protein disclosed its identity to VC1929 (DctP), which is a mannose-sensitive haemagglutinin. Since complementation of the CD11 mutant with *dctP* restored the ability to colonize the intestine and to display MSHA, it may be concluded that CD11 was mutated within the *dctP* locus. The mannose-sensitive haemagglutinin from El Tor biotypes reported by Jonson et al. (1991, 1994), a ~17 kDa pilin, appears to be different from DctP on the basis of molecular mass. However, both these antigens have common functional properties as haemagglutinin, colonization factor and protective antigen. The calculated molecular mass of DctP on the basis of amino acid residues appears to be close to 37 kDa; hence the previous report of 33 kDa (Jacob et al., 1993) stands corrected. Agglutination of RBC by CD11 lacking DctP protein was not inhibited by D-mannose, which shows that haemagglutinins other than mannose-sensitive ones, such as L-fucose-sensitive haemagglutinins, might be involved in agglutination of RBC (Attridge et al., 1996; Jones & Freter, 1976).

A number of proteins associated with virulence of *V. cholerae* have been listed, including various types of toxin, pili, adhesins and enzymes (Kaper et al., 1995), and certainly DctP qualifies to be added to the list of virulence factors. The nonadhesive, noncolonizing and relatively apathogenic strain CD11 behaved like a wild-type enteropathogenic strain when complemented with *dctP*.

Sialic acid is ubiquitously found in mucus-rich areas of gut and lung and it can be utilized as a sole carbon and energy source by pathogenic and commensal bacteria (Severi et al., 2007; Almagro-Moreno & Boyd, 2009). A cluster of genes involved in scavenging, transport and catabolism of sialic acid have been identified in *V. cholerae*; one of the genes of this cluster encodes neuraminidase (NanH), which plays an important role in making sialic acid available to bacteria as a carbon source and in binding of cholera toxin to sialogangliosides (Galen et al., 1992). A nanA deletion in this cluster caused deficiency in intestinal colonization (Almagro-Moreno & Boyd, 2009). Inability of the *dctP* mutant CD11 to grow with sialic acid as the sole source of carbon suggests that DctP protein, with a periplasmic location, may be involved in binding to and transport of sialic acid into the cell. Interestingly, DctP shows homology to sialic-acid-binding protein SiaP of *Aggregatibacter aphrophilus*. Hence, DctP is expected to provide selective advantage to *V. cholerae*, as the gut environment is rich in sialic acid.

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