# Ability to catabolize sialic acid is present predominately in clinical isolates of *Vibrio vulnificus*

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## Introduction

Sialic acids, also known as neuraminic acids, are a family of nine-carbon amino sugars. Sialic acids are widely distributed in deuterostomes where they perform a number of functions. They are involved in cell-cell interactions, stabilizing glycoconjugates and cell membranes, and acting as chemical messengers (Angata and Varki 2002). Despite being largely absent from most protostomes, plants, fungi, and protists (Angata and Varki 2002), sialic acids are utilized by commensal and pathogenic prokaryotes in a number of ways. Pathogens have been shown to decorate their cell surfaces with sialic acid to avoid an immune response from the host (Vimr *et al.* 2000, 2004). Pathogens obtain the sialic acid primary through de novo biosynthesis or through scavenging from the host (Vimr *et al*. 2000, 2004, Severi *et al.* 2007). Bacteria also have the ability to use sialic acid as a carbon and nitrogen source, and sialic acid catabolism (SAC) is executed by the enzymes encoded by the SAC cluster of genes *nanA, nanE and nanK* (Vimr et al., 2004; Almagro-Moreno and Boyd, 2010; Almagro-Moreno and Boyd 2009a). In *Vibrio cholerae* the SAC cluster is found on Vibrio Pathogenicity Island-2 (VPI-2), and all toxigenic O1 serogroup isolates examined were positive for the cluster, whereas non-toxigenic isolates lacked the SAC genes (Jermyn and Boyd 2002). Furthermore it was demonstrated that the presence of SAC conveys a competitive advantage in the early stage of infection (Almagro-Moreno and Boyd 2009b). *Vibrio vulnificus* is found in estuarine and coastal waters throughout the world (Oliver 2006). *V. vulnificus* causes severe and rapid septicemia, mostly due to oyster consumption, with over a 50% mortality rate within forty-eight hours (Jones and Oliver, 2009). Interestingly, it was also recently demonstrated in a strain of *V. vulnificus* that the ability to catabolize sialic acid is important *in vivo* using a mouse model (Jeong *et al.* 2009).

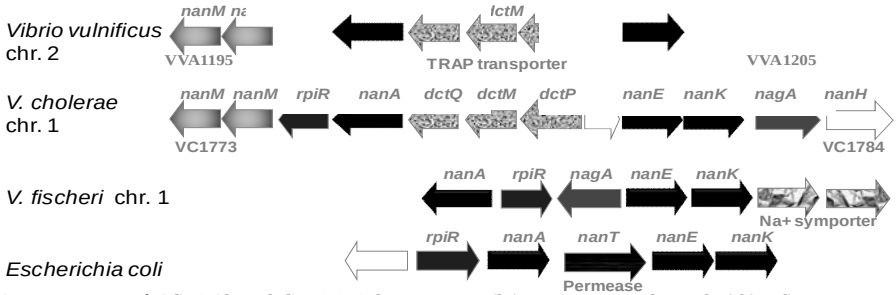


**Figure 1. Structure of Sialic Acid Catabolism (SAC) clusters among *Vibrio* species compared to *Escherichia coli*. Open reading frames (ORFs) are indicated as arrows, the direction of which shows direction of transcription. *V. vulnificus* and *V. cholerae* encode highly homologous SAC genes, however in *V. vulnificus* the genes are on chromosome 2 and no neuraminidase (*nanH*) homology is present. V. *vulnificus* encodes a putative TRAP transporter (*dctPQM*) for uptake of sialic acid into the bacterial cell.**

Whether all *V. vulnificus* strains encode the SAC and can catabolize sialic acid is unknown. We first compared the genome location of SAC in *V. vulnificus* and *V. cholerae* (Fig. 1). We determined the distribution of *nanA* amongst a collection of sixty-seven *V. vulnificus* clinical and environmental isolates. We mapped the distribution of *nanA* to the phylogeny of *V. vulnificus* to determine whether lineage specificity occurred. Next, we confirmed the ability of the *nanA*+ strains to catabolize sialic acid through growth analysis.

## Materials and Methods

### *Bacterial Strains*

A total of 67 isolates, 27 isolates recovered from clinical and 40 from environmental sources, were examined in this study. These isolates represent all three biotypes found in *V. vulnificus* and were collected between 1980-2005, from various locations around the world.

### *Molecular analysis*

Chromosomal DNA was extracted from the isolates using a genome isolation kit. PCR primers for *nanA* were designed based of the sequence of the isolateYJ016. VVA1199F-TTATCGCCGCTCCCCATACA,VVA1199R-GCAACGCCACCATATTCAAC.The amplification of gene fragments was performed by PCR in 25µl reactions.

### *Growth analysis on minimal media supplemented with sialic acid*

Five clinical strains positive for *nanA* and one negative were used. 5 ml of M9 minimal media was inoculated with 100µl of overnight LB 2% NaCl broth culture. The M9 media was supplemented with 1 mg/ml of *N*-acetylneuraminic acid or D-glucose. Growth was detected by measuring the absorbance at 595nm of the cultures.

## Results and Discussion

### *Distribution of the nan cluster*

The SAC cluster in both *V. vulnificus* YJ016 and *V. cholerae* N16961 show high sequence similarity and gene order except for the absence of neuraminidase (*nanH*) in *V. vulnificus* (Fig.1). As such *V. vulnificus* SAC region encodes *nanM, rpiR, nanA, dctPQM, nanE, nank, and nagA*, however these genes are encoded on chromosome 2 (Fig.1). To determine the distribution of SAC amongst our collection of isolates, we designed primers to *nanA*, which encodes the key enzyme in the pathway. The distribution of the SAC cluster

Table1: Distribution of *nanA* amongst *V. vulnificus* isolates

| Strain | Source | Result | Strain | Source | Result | Strain | Source | Result |
|---|---|---|---|---|---|---|---|---|
| 85A667/O | Clinical | + | L-180 | Clinical | - | 99-509 DP-A6 | Oyster | + |
| MO6-24/O | Clinical | + | CIP 81.90 | Clinical | - | b60 | Oyster | + |
| YJ016 | Clinical | + | C7184 | Clinical | - | 99-540 DP-B6 | Oyster | - |
| CMCP6 | Clinical | + | K2637 | Clinical | - | 98-640 DP-B9 | Oyster | - |
| JJ068 | Clinical | + | 90-2-11 | Eel | - | 99-779 DP-D2 | Oyster | - |
| JJ067 | Clinical | + | E86 | Eel | - | 99-609 DP-A4 | Oyster | - |
| JJ072 | Clinical | + | NCIMB 2136 | Eel | - | SS108A-3A | Oyster | - |
| YJ002 | Clinical | + | NCIMB 2137 | Eel | - | JY 1305 | Oyster | - |
| 11028 | Clinical | + | ATCC 33149 | Eel | - | JY 1701 | Oyster | - |
| CDC 900597 | Clinical | + | M-79 | Eel | + | 72M4 | Clam | + |
| CDC 9030-95 | Clinical | + | Env 1 | Environ. | - | 79M4 | Clam | + |
| 313-98 | Clinical | + | L-49 | Environ. | + | CG 62 | Seawater | + |
| CDC 9038-96 | Clinical | + | 345/O | Environ. | + | CG 63 | Seawater | + |
| CDC 9062-96 | Clinical | + | UNCC 913 | Environ. | + | CG 123 | Seawater | + |
| N-87 | Clinical | + | UNCC 1015 | Environ. | + | MLT 364 | Seawater | + |
| MO6 | Clinical | + | MLT 365 | Environ. | + | MLT 362 | Seawater | + |
| KH-03 | Clinical | + | G-83 | Fish | + | SPRC 10215 | Seawater | + |
| K2719 | Clinical | + | 80M4 | Fish | + | IFVv18 | Seawater | + |
| LSU 1866 | Clinical | + | 76M3 | Fish | + | 300-1C1 | Seawater | - |
| SPRC 10143 | Clinical | + | IFVv10 | Mussel | - | MLT 406 | Seawater | - |
| K2667 | Clinical | + | IFVv11 | Mussel | + | 96-9-114s | Sediment | - |
| 6353/O | Clinical | - | CG 27 | Oyster | + | | | |
| IFVv8 | Clinical | - | b122 | Oyster | + | | | |

is consistent with the idea of it as a virulence factor as it was found in 21 out of the 27 clinical isolates studied (Table 1). Of the 40 environmental isolates, 17 were found to contain SAC but only one of the six eel isolates was positive. These findings initially suggest a weak correlation between pathogenic strains and the presence of SAC. To further investigate the relationship between SAC and pathogenicity, the distribution results were mapped to a phylogenetic tree representation of the genetic relationships among the isolates used. The phylogenetic tree constructed divides the isolates in to two lineages I and II (Cohen et al.,

2007). Lineage I contains primarily clinical strains with a B/C genotype previously described by Nilsson et al (2003) and Rosche et al (2005) and Lineage II consists predominately of environmental isolates with the A/E genotype. In this analysis, we found that 34 out of the 37 lineage I clinical isolates were positive for *nanA*, whereas only 5 of the 26 environmental (A/E) lineage II isolates were positive for *nanA*, demonstrating a strong correlation between SAC and clinical *V. vulnificus*. Four isolates which do not fall within either lineage I or II were *nanA* positive.

Table 2: Distribution of *nanA* by isolate type

| | *nanA*+ | *nanA*- |
|---|---|---|
| *V. vulnificus* B/C Type Lineage I isolates | 34 | 3 |
| *V. vulnificus* A/E Type Lineage II isolates | 5 | 21 |

### *Growth analysis of V. vulnificus on minimal media+sialic acid*

The growth analysis conducted on the *nanA*-positive isolate confirms that the presence of the *nanA* gene allows the strains to use sialic acid as the sole carbon source in culture compared to a *nanA* negative strain that was unable to grow M9 plus sialic acid as a sole carbon source.

## Conclusions

The strong correlation of the presence of the *nan* cluster in pathogenic strains would indicate that it is indeed a virulence factor in *Vibrio vulnificus*. As seen in *V. cholerae*, notion that the ability to catabolize sialic acid conveys an advantage in the host could also hold true in *V. vulnificus* infection.

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