微生物学报 *Acta Microbiologica Sinica*
49(3):363 – 371;4 March 2009
ISSN 0001 – 6209;CN 11 – 1995/Q
http://journals.im.ac.cn/actamicrocn

Genetic diversity between two *Vibrio anguillarum* strains exhibiting different virulence by suppression subtractive hybridization

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Abstract: [Objective] and [Methods] *Vibrio anguillarum*, a halophilic Gram-negative bacterium, is the causative agent of vibriosis in fish. *V. anguillarum* strain VIB72 was defined as having high virulence whereas strain CW1 was defined as having low virulence on the basis of their different LD_{50} values to zebra fish. Suppression subtractive hybridization (SSH) was used to identify genetic differences between these two strains. [Results] After screening, 59 subtracted library clones were isolated which were specific for strain VIB72, and the DNA sequences of these clones were determined. Seventeen fragments showed high homology to the genes of known functions in other bacteria. This includes soluble lytic murein transglycosylase, mobilization protein (MobA, MobC), transposase (IS66), resistance-related protein (metallo-beta-lactamase and acetyltransferase family), toxin protein (DT-201 and alveicin A immunity protein), ATP-dependent endonuclease of OLD family like protein, SocE and GTP-binding protein HflX (high frequency of lysogenization). These fragments may represent parts of putative pathogenicity islands (PAIs) in *V. anguillarum*. The remaining fragments showed no significant homology to any known genes. [Conclusion] The results indicated that SSH was successful in identifying genetic differences and putative virulence genes among different strains of *V. anguillarum*.

Keywords: *Vibrio anguillarum*; suppression subtractive hybridization; genetic difference; virulence genes
CLC number: Q933 Document code: A Article ID: 0001-6209(2009)03-0363-09

Vibrio anguillarum, a halophilic Gram-negative bacterium, is the causative agent of vibriosis, which may infect more than 80 different marine and estuarine fish species[1]. This species was separated into 23 serogroups which displayed different pathogenicity and host specificity based on O-antigens[2]. Serogroups O1 and O_2 are by far the most common serogroups. O1 was typically isolated from Pacific salmon (*Oncorhynchus kisutch*), Atlantic salmon (*Salmo salar*), turbot (*Scophthalmus maximus*) and sea bass (*Dicentrarchus labrax*); whereas, serotype O_2 was found in Pacific salmon, Atlantic salmon, turbot, cod (*Gadus morhua*) and European eel (*Anguilla anguilla*), suggesting some correlation between serogroups and virulence[1]. However, all serotypes in which pathogenic strains were found also contained avirulent strains[3].

In marine fish aquaculture, *V. anguillarum* is a primary pathogen that can cause a systemic infection leading to disease and even death, so it is important to elucidate the various virulence factors involved. Extracellular products are believed to play a role in virulence. *V. anguillarum* secrets several exotoxins, which were reported to be correlated to pathogenesis of

Supported by the Major Project of Chinese National Programs for Fundamental Research and Development (2006CB101803) and the National Programs for High Technology Research and Development of China (2007AA09Z434)

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Received: 13 September 2008/Revised: 26 November 2008

vibriosis, such as hemolysins, metalloprotease, polysaccharide, dermatotoxin, hemaglutinin and cytotoxin[4-5]. In addition, a siderophore mediated plasmid-encoded system has been demonstrated to be an important virulence component of this bacterium[6]. However, on the whole, not much is known about the molecular basis of the putative virulence factors of this bacterium.

Comparison of the genome sequences of non-pathogenic and pathogenic strains can provide useful information on genes that are specific for highly virulent isolates. Some of these differences may determine strain-specific characteristics such as virulence factors, which can be used as a valuable tool to establish the nature and severity of disease. Currently, the amount of sequence information available in the database required to perform such analysis is too limited. As an alternative approach suppression subtractive hybridization (SSH) technique[7] is a powerful for analyzing the differences between two complex genomes, and for identifying DNA sequences that are present in one strain (the tester), but absent in another strain (the driver). This technique has been used in different bacterial species to identify genomic differences between virulent and avirulent strains[8-9].

The objective of this study was to identify genetic differences between *V. anguillarum* strains with low virulence and high virulence using SSH, and correlate those differences with virulence. Strain VIB72 was defined as having high virulence whereas strain CW1 was defined as having low virulence on the basis of their differences in LD_{50} values to injected zebra fish (*Brachydanio rerio*). The SSH technique identified presumptive virulence genes. The potential biological functions of these genes will form a foundation for further elucidating how *V. anguillarum* causes disease in fish. In addition, these sets of specific DNA fragments offer a large choice of candidates for the development of new molecular diagnostic and epidemiological tools.

1 MATERIALS AND METHODS

1.1 Bacterial strains, plasmids, culture conditions

Bacterial strains and plasmids used in this study are listed in Table 1. According to our previous work, *V. anguillarum* VIB72 was assessed as a high virulent strain (with a LD_{50} value of $< 10^{3.4}$ to zebrafish), whereas *V. anguillarum* CW1 was assessed as a low virulent strain (with a LD_{50} value of $> 10^{7.5}$ to zebrafish), according to the method of Zhang and Austin[10]. *V. anguillarum* strains were maintained on 2216E medium at 28℃. Luria-Bertani (LB) medium was used for the routine culturing of *E. coli* at 37℃. All strains were stored frozen at −80℃ in 0.9% NaCl solution with 15% (V/V) glycerol. *E. coli* transformants were maintained on LB-Am (LB supplemented with 100 μg of ampicillin ml-1) agar. Plasmid pMD-18T vector kit (TaKaRa, Dalian, China) was used to construct the subtractive library.

Table 1 Bacterial strains and vectors used in this study

Strain or plasmid	Relevant characteristics*	Source or reference
V. anguillarum CW1	having low virulence, serotype O1, isolated from diseased sea perch	[11]
V. anguillarum VIB72	having high virulence, serotype O_2, LMG 4437^T	Norway, cod
E. coli JM109	*recA*1 *endA*1 *gyrA*96 *thi hsdR*17 *supE*44 *relA*1*D* (*lac-proAB*1)	TaKaRa
Plasmids pMD-18T	Cloning vector, Ap^r, LacZ	TaKaRa

* LMG, Laboratorium voor Microbiologie, Ghent, Belgium; ^{T}, indicate type strain; Ap^r, ampicillin resistance.

1.2 DNA extraction

Total bacterial genomic DNA from V. anguillarum strains was prepared using the DNeasy Blood Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

1.3 Suppression subtractive hybridization (SSH)

Bacterial genome subtraction was performed with the PCR-Select Bacterial Genome Subtraction Kit (Clontech, Mountain View, CA, USA) and Advantage cDNA polymerase Kit (Clontech) following the manufacturer's instructions. The PCR cycling condition using the designed primers SSH16SL (5′-GCACAAGCGGTGGAGCAT-3′) and SSH16SR (5′-CCCTGGAGTTCCCGACAT-3′) was: 1 × (94℃,

5 min); 30 × (94℃, 30 s; 65℃, 30 s; 68℃, 12 min). Briefly, the tester (*V. anguillarum* VIB72) and driver (*V. anguillarum* CW1) genomic DNAs were digested with *Rsa* Ⅰ. The tester DNA was then subdivided into two portions, each of which was respectively ligated with different adaptors provided by the subtraction kit. Two hybridizations were performed. In the first, an excess of driver was added to each adaptor-ligated tester sample, and the samples were then heat-denatured and allowed to anneal. In the second hybridization, the two primary hybridization samples were mixed without denaturing. The entire population of molecules was then subjected to PCR to amplify the tester-specifc sequences. The PCR amplification product was purified by DNA Fragment Purification Kit (TaKaRa), and stored at −20℃.

1.4 Screening of the subtracted library

The PCR amplification products were cloned into pMD-18T vector and transformed into *E. coli* JM109 competent cells according to the manual of the pMD-18T vector kit (TaKaRa). Positive clones were screened on LB medium supplemented with 20 mg·ml^{-1} X-Gal (Sigma, USA), 200 mg·ml^{-1} IPTG (Sigma) and 100 μg·ml^{-1} ampicillin (Sigma).

Colony PCR analysis allows duplicated clones to be discarded before proceeding to dot hybridization, and nested primers from the PCR-Select Bacterial Genome Subtraction Kit were used to PCR amplification of individual unique sequences in the subtractive library. The PCR products which have unique sequences were selected and used to prepare DNA dot blots.

1.5 Dot hybridization analysis

Dot blots were performed to identify the subtractive clones that contained VIB72 unique fragments. Probes were prepared by using PCR DIG probe synthesis kit (Roche, Basel, Switzerland). Adaptors 1 and 2R-ligated genomic DNA from tester (VIB72) and driver (CW1) were used as templates respectively, and the nested primers were from the PCR-Select Bacterial Genome Subtraction Kit. After the PCR amplification, the adaptor sequences were removed from the probes by *Rsa* Ⅰ (Clontech). The colony PCR products were transferred to Hybond-N (Amersham, UK) nylon membranes and hybridized with the probes. At the same time, the PCR products were used as positive controls by applying the designed primers (SSH16SLR and SSH16SR). Dot blot analysis was performed with DIG Nucleic Acid Detection Kit following the manufacturer's instructions.

1.6 Sequencing analyses

All DNA sequencing reactions were performed by Biosung CO, LTD (Shanghai, China). The sequences were analyzed for DNA homology using the National Center for Biotechnology Information (NCBI) BLASTN program. Sequences with low DNA homology were analyzed for protein homology using the NCBI BLASTX.

2 RESULTS

2.1 Genomic subtraction between *V. anguillarum* VIB72 and CW1, and identification of DNA fragments specific to strain VIB72

SSH was carried out between the *Rsa* Ⅰ-digested genomic DNA of high virulent strain VIB72 (tester) and low virulent strain CW1 with the aim of isolating VIB72-specific genes (potential virulence genes). From the subtracted library, 384 ampicillin-resistant colonies were randomly picked and subjected to a PCR assay using the nested primers to assess the presence of a DNA insert. A total of 281 recombinant clones yielded a single PCR product, and they were individually tested by dot blotting using DIG-labeled genomic DNA of *V. anguillarum* VIB72 and CW1 digested by *Rsa* Ⅰ. Hybridization results revealed that 187 of these 281 recombinant clones specifically reacted with one or more fragments of the VIB72 strain, while the remainder recognized DNA sequences present in both genomes. Fifty-nine out of 187 clones were selected as distinctive positive clones by dot blot results, and sent to be sequenced (Table 2).

Table 2 Summary of the subtraction and the numbers of subtracted fragments used in dot blot analysis between *V. anguillarum* VIB72 (tester) and CW1 (driver)

Size of subtracted genomic fragments/bp	No. of fragments (or clones)	
	a single PCR product	positive clones (tester only)[a]
150 – 500	192	45
500 – 1000	89	14
Total	281	59

[a] Subtracted fragments that hybridized only to *V. anguillarum* VIB72 (tester).

The sizes of the 59 fragments were ranged from 77 bp to 1000 bp (mean 380 bp), and 76% of the subtracted fragments were less than 500 bp (Table 2). The combined size of the sequenced fragments that were contained in the 59 clones was ~23 kb. Note that the sequences in the unique clones represented only portions of individual genes or genetic elements.

2.2 Sequence analysis of the DNA fragments identified by SSH

Fifty-five subtracted clones of the selected 59 positive clones were successfully sequenced and they were analyzed by using the BLASTN and BLASTX homology search in PAIs (the pathogencity islands)/GenBank[12]. Of the 55 subtracted clones, 30 (55%) demonstrated significant matches with entries in the databases and potentially represented new and novel virulence determinants in *V. anguillarum* (Table 3), and 17 DNA fragments showed high homology to known proteins of other bacteria (Table 3). The VIB72 (tester)-specific DNA sequences were grouped into different categories as shown in Table 3.

Table 3 Summary of BLAST search of clones that were generated by SSH of DNA from strains VIB72 and CW1

Putative virulence factor	Fragment	Insert size/bp	Sequences with similarity[a]	GenBank accession No.	E value[b]	Score[c]
Group 1						
Resistance-related proteins	1C4, 1C0	500	Orf58 [Acetyltransferase (GNAT) family] [*Photorhabdus luminescens*]	AAO17212	6.00E-53	211.0
Toxin proteins	2E1	263	alveicin A immunity protein [*Hafnia alvei*]	YP_025365	4.00E-19	98.0
	2B12	104	toxin protein DT-201 [*E. coli*]	AAA72620	2.00E-08	63.5
Transposase (IS66)	1B6, 2G1	287	transposase (IS66) family [*Photobacterium* sp. SKA34]	ZP_01160223	1.00E-41	173.0
Mobilization proteins	1D4, 2B0	830	mobilization relaxase (MobA) [*Aeromonas salmonicida* subsp. salmonicida A449]	NP_861573	9.00E-92	340.0
Soluble lyticmurein transglycosylase	2F12, 1E7	545	Soluble lytic murein transglycosylase [*Vibrio vulnificus* CMCP6]	NP_760757	8.00E-80	300.0
Phage sequences	4E6	425	GTP-binding protein HflX [*V. parahaemolyticus* RIMD 2210633]	NP_799195	2.00E-72	276.0
Others	2E4	77	catalase [*Campylobacter jejuni*]	CAA59444	3.00E-07	59.7
	1D3, 4A3	672	ATP-dependent endonuclease of the OLD family-like protein (Predicted ATP-binding protein involved in virulence) [*Desulfococcus oleovorans* Hxd3]	YP_01528788.1	2.00E-10	68.6
	4E7,1F5,1F11	320	SocE [*Myxococcus xanthus*]	AAF91388	4.00E-19	98.0
Group 2						
Resistance-related proteins	1C2	185	conserved hypothetical protein (Metallo-beta-lactamase superfamily; pfam00753) [*V. cholerae* AM-19226]	EDN15621	5.00E-07	92.0
Mobilization proteins	1C7, 1C11, 1E2, 1G11	263	putative mobilization protein MobC [*H. alvei*]	YP_025358	1.00E-19	100.0
	2B8,4E8	278	putative mobilization protein MobC [*E. coli*]	YP_052692	1.00E-17	94.4
Plasmid sequences	4B11,	353	hypothetical protein pCollet-06 [*E. coli*].	YP_355421	2.00E-33	140
	2E0	245	hypothetical protein pU302Sp01 [*Salmonella typhimurium*]	YP_194804	5.00E-23	112.0
	4F11, 1D11	380	hypothetical protein pEA28_01 [*Erwinia amylovora*]	NP_758764	3.00E-50	202.0
Others	1E6	317	hypothetical protein NB231_17580 [*Nitrococcus mobilis* Nb-231]	ZP_01128963	7.00E-35	151.0
	1H6	431	hypothetical protein plu0958 [*Ph. luminescens* subsp. *laumondii*]	NP_928292	8.00E-72	174.0
Group 3	Sequences with no significant hits to GenBank (25)					

[a] Sequences with the highest homology to the fragment are listed. The homology is not usually to the entire length of the fragment. [b] The score increases as the level and length of homology increases. c The E value indicates the probability of the match. A match with an E value of 1E-5 and below was taken to be significant. Only the portions of DNA fragment that showed the best hits are listed.

2.2.1 Sequences that are part of genes with previously described functions (Group 1): These include soluble lytic murein transglycosylase, mobilization protein (MobA), acetyltransferase (GNAT) family, transposase (IS66), bacteriocin (alveicin A immunity protein), catalase, toxin protein DT-201, ATP-dependent endonuclease of OLD family like protein, SocE and GTP-binding protein HflX (high frequency of lysogenization).

2.2.2 sequences with homology to genes encoding hypothetical proteins (Group 2): Seven sequences had homology to genes with known functions. They include Metallo-beta-lactamase, mobilization protein (MobC), and fragments with homology to plasmid sequences (pCollet-06). The remaining five fragments have sequences that showed homology to genes encoding conserved hypothetical proteins without a predicted function. These hypothetical proteins have been previously identified during genome sequencing.

2.2.3 sequences that do not show homology to any database sequence (Group 3): This category of 25 clones constituted unknown sequences that did not match any sequence described in the database, or had low level of homology to known sequences. The proteins encoded by these sequences are thus referred to as putative *V. anguillarum* proteins. To date, more than 100 bacterial genomes, including those of several important pathogens, have been sequenced, revealing that around 25% of the ORFs are hypothetical genes without known function.

3 DISCUSSION

A prerequisite to comprehend the pathogenicity mechanisms of an organism is the identification and examination of all its virulence genes. In the last few years, several strategies have been developed for the identification of bacterial genes essential for infection, such as the use of in vivo expression technology, signature-tagged transposon mutagenesis and microarray DNA chips. SSH was initially reported in 1996[7] and was first applied to bacteria in a study of *Helicobacter pylori* in 1998[13]. Because a kit for SSH is now available commercially (PCR-Select Bacterial Genome Subtraction Kit, Clontech), this represents the most accessible version of the technique. The kit has been applied to several different bacterial pathogens including *Aeromonas hydrophila*, *Burkholderia pseudomallei*, *E. coli*, *Klebsiella pneumoniae* and *Photobacterium damselae*[9, 14-17]. The studies described here show that the SSH technique is simple and efficient for identifying genetic differences between strains of different virulence.

In this study, we used SSH to identify genetic differences between *V. anguillarum* strains having high virulence and low virulence. 187 subtracted library clones unique to high virulent strain VIB72 were identified after screening, which means that more than 48% of 384 randomly selected clones obtained were tester specific (that is, present in one strain and absent or substantially different in the other). This ratio is very near to those reported in similar SSH studies, which range between 50% to 60%[13-14]. Thirty out of the 55 subtracted clones demonstrated significant matches with entries in the databases and potentially represented new and novel virulence determinants in *V. anguillarum* (Table 3), and 17 representing DNA fragments showed high homology to known proteins of other bacteria. According to previous studies, some of the putative proteins play a role in virulence of other bacteria, and most of them are described in *V. anguillarum* for the first time. They may well be the potential virulence factors in *V. anguillarum*. Therefore, studying the function of these gene fragments will provide insight to their relative importance.

3.1 Soluble lytic murein transglycosylase

2F12 and 1E7 are highly homologous to the soluble lytic murein transglycosylase. In Gram-negative bacteria, a thin layer of peptidoglycan, which is also called murein, surrounds the cell and is essential for cell viability. Lytic transglycosylases, which are membrane-bound exoenzymes, are believed to function as space makers to allow the insertion of new peptidoglycan material into the cell wall during growth[18]. Moreover, they are involved in making pores in the peptidoglycan layer to allow transport of bulky compounds across the cell wall, such as DNA, toxins, flagella, and fimbrial[19-20]. This indicated that lytic transglycosylase may play an important role in the process of extracellular products secretion, especially the toxin secretion in

pathogenesis.

3.2 Mobilization proteins (MobA, MobC)

Mobilization proteins have the most sequence hits (8 clones) amongst the 55 clones selected, and mobilization relaxase MobA had a highly homology. Consideration of this protein is instructive. Bacterial conjugation is considered as one vehicle of horizontal gene transfer (HGT)[21]. Mobilization proteins (MobABCD) encoded by plasmids, like the plasmids of *A. salmonicida* subsp. *salmonicida* A449, *Pseudomonas aeruginosa* and *E. coli*, are involved in conjugative mobilization[22]. This process can take advantage of the type IV secretion systems (T4SS) involved in the transport of effector proteins active in pathogenesis[23]. Mobilization proteins may be involved in the transfer of virulence plasmids in *V. anguillarum*, or enhance the efficiency and specificity of the process. Also, their existence indicated that many of the sequences identified in this study may have been acquired by the horizontal transfer of DNA. Virulence genes are frequently located on large chromosomal regions of horizontally acquired DNA. Therefore, the large chromosomal regions containing the mob genes need to be further studied.

3.3 Transposase (IS66)

The predicted protein of 1B6 and 2G1 showed similarity to transposase (IS66), which is encoded by insertion sequence (IS). IS is a common mobile element of bacterial genomes, and it is believed to undergo HGT more frequently than other genes, possibly as a consequence of its transfer between the bacterial genome and plasmids[24]. Both transposition and horizontal transfer of ISs may lead to the acquisition and mobilization of host genes in addition to those ISs[25]. Moreover, changes in the expression levels of host genes may occur if ISs integrate in regulatory regions. This suggests that ISs may lead to the change of characteristics in host bacteria, such as antibiotic resistance, pathogenicity and fitness traits. Further work has shown that, by the PCR analysis, the gene which encodes the IS66 only presents in the genome of the *V. anguillarum* strain VIB72, but not CW1 (data not shown). Therefore, the virulence diversity in strains of *V. anguillarum*, like strains VIB72 and CW1, could partly ascribe to the putative transposase genes. In addition, the identification of transposase elements or markers specific to individual strains, has been shown to be an important starting point in the identification of genomic islands implicated in virulence[26]. Therefore, it could be an adequate method for typing virulence genes in *V. anguillarum* by transposase.

3.4 Resistance-related proteins

Bacteria have developed a number of mechanisms to protect them from environmental toxins and antibiotics. The predicted proteins of 1C0, 1C4 and 1C2 showed similarity to acetyltransferase family and metallo-beta-lactamase superfamily, both of which are believed to have antibiotics resistance characteristics. The first group is now termed as the GCN5-related N-acetyltransferase (GNAT) superfamily, and was identified as aminoglycoside acetyltransferases in bacteria which is resistant to the action of the antibiotics gentamicin and kanamycin[27-28]. The other putative resistance-related protein metallo-beta-lactamases are the main cause of resistance of bacteria to beta-lactam antibiotics[29]. The emergence and dissemination of acquired metallo-beta-lactamases was encoded by genes carried on mobile DNA elements among many Gram-negative pathogens, including members of the family Enterobacteriaceae, *P. aeruginosa*, and *Acinetobacter* species[30], which indicated they are potential for horizontal transference.

Until now, the putative mobilization protein, transposase and resistance-related proteins are potential for horizontal transfer. Moreover, similar observations have been made for the putative plasmid sequences (pCollet_ 06) and phage sequences (*hlfX*, high frequency of lysogenization) (Table 3). Therefore, it is necessary to emphasize the role of HGT in pathogen. HGT is especially important in the evolution of pathogenic lifestyles as infection related factors can be transmitted in a single step integration event[31]. Maybe, the result after HGT is a mosaic genome structure where a core *V. anguillarum* VIB72 signature chromosome that maintains its characteristic physiology is punctuated by combinations of islands of genes that confer to each pathogenic strain a characteristic disease-causing lifestyle.

3.5 Toxin protein

2E1, 2B12 and 2C4 showed homology to putative

toxin proteins, which are believed to have been identified in *V. anguillarum* for the first time. The identification of the putative toxin proteins suggests that the encoded gene has a direct relationship with the virulence of *V. anguillarum*. Here, general information about the putative toxin proteins can be observed. (ⅰ) The putative protein DT-201 is a kind of diphtheria toxin (DT). DT is an A-B type protein toxin produced by *Corynebacterium diphtheria*, and the DT-A halts host protein synthesis and causes cell death[32]. (ⅱ) The putative protein Alveicin A immunity protein is a kind of bacteriocins, which are protein toxins produced by bacteria that kill their closest relatives[33]. An additional role has recently been proposed for gram-positive bacteriocins, in which they mediate quorum sensing[34]. So, with their relatively narrow spectrum of killing activity, they can be considered "designer drugs," which target specific bacterial pathogens. The putative toxin protein indentified here should be further studied to determine the presence and distribution in other virulent strains of *V. anguillarum*.

4 CONCLUSIONS

Our results demonstrated that SSH is a successful technique for identifying genetic differences between *V. anguillarum* strains having low virulence and high virulence. This is expected to provide insights into the virulence mechanisms of this fish pathogen. Although from the results described here a direct correlation between a particular sequence and virulence cannot be distinctly inferred; however, the distribution of a particular gene in a bacterial population can provide clues of its implication in virulence, and clearly some of the sequences described here could be involved in the virulence of *V. anguillarum*. The putative mobilization protein, transposase and resistance-related protein are potential for HGT. The correlation of the encoded gene to mobile elements among the subtracted fragments points to the presence of pathogenicity islands (PIs) in *V. anguillarum*, as has been demonstrated in other bacteria. Studies on this possibility are currently under way. In addition, putative toxin protein encoded genes as a part of the subtracted sequences suggests a likely role in virulence. Unfortunately, some putative or hypothetical proteins (Table 3), like ATP-dependent endonuclease of OLD family like protein and SocE, show only a predicted function due to limited information. It is hoped that the identification of critical genetic differences between *V. anguillarum* strains having low virulence and high virulence can provide insights into the pathogenic mechanisms, thus supplying the groundwork for the development of new therapies for *V. anguillarum* infections. Further work of characterizing some of the differentiated genes by deletion mutations and functional studies is going on in our laboratory to elicit novel virulence mechanisms.

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利用抑制性消减杂交(SSH)技术研究不同毒力的鳗弧菌菌株的基因多样性

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摘要:【目的和方法】鳗弧菌是一种嗜盐的革兰氏阴性细菌,也是鱼类弧菌病的主要病原。对斑马鱼的半数致死量研究结果表明,鳗弧菌菌株 VIB72 具有较高的毒力,而菌株 CW1 的毒力较低。本文利用抑制性消减杂交(SSH)技术对这两个菌株的遗传差异进行了研究。【结果】通过对差减文库筛选,分离到 59 个对菌株 VIB72 的阳性克隆,并对这些克隆的 DNA 序列进行了测定。17 个基因片断与其它细菌的已知功能的基因有较高的同源性,其中包括可溶性溶胞壁质转糖基酶、转移蛋白 MobA 和 MobC、转座子 IS66、抑制相关蛋白(金属 β-内酰胺酶和乙酰转移酶家族)、毒素蛋白(DT-201 和 alveicin A 免疫蛋白)、与 OLD 家族相似的 ATP 依赖性核酸内切酶以及 SocE 和 GTP 结合蛋白 HflX(有高频率的溶原化)。这些基因片断有可能是鳗弧菌毒力岛的一部分。其他的基因片断与其它的已知基因没有明显的相关性。【结论】这些结果表明,SSH 技术成功地鉴定了不同致病性的鳗弧菌菌株的基因差异及潜在的毒力基因。

关键词:鳗弧菌;抑制性消减杂交;遗传差异;毒力基因

中图分类号:Q933　　文献标识码:A　　文章编号:0001-6209(2009)03-0371-09

(本文责编:张晓丽)

基金项目:国家"973 项目"(2006CB101803);国家"863 计划"(2007AA09Z434)

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收稿日期:2008-09-13;修回日期:2008-11-26

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