Mining of the biosynthetic mechanisms of *Vibrio spp.* polysaccharides and potential role in biofilm formation

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Introduction

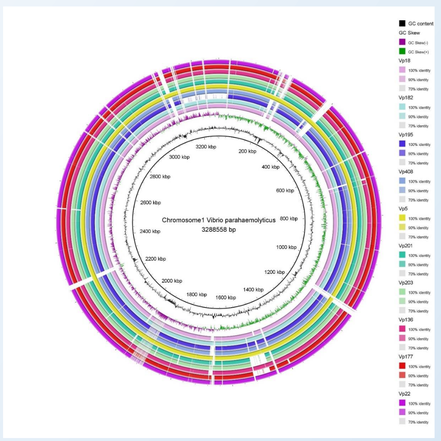
Vibrio spp. are ubiquitous marine bacteria, they encompass the well-studied human pathogen, *Vibrio cholerae*, and two other human pathogens, *V. vulnificus* and *V. parahaemolyticus,* as well as some characterized animal pathogens. Virulence depends on different mechanisms including contact and host colonization, ability to survive and to grow . The main factors of virulence, excreted or present on the bacteria cell surface are extracellular vesicles, capsular polysaccharide (CPS), and hemolysins and urease production in *V. parahaemolyticus*.
Furthermore, biofilm assuring survival and dissemination of pathogen microorganisms in the environment is considered as virulence factor. Polysaccharides are major components of extracellular matrix synthetized upon biofilm growth and therefore may play major roles in virulence. Bacterial polysaccharides include expolysaccharides (EPSs) which are released to the surrounding medium, and two surface polysaccharides: lipopolysaccharides (LPS) with an O-antigen polysaccharide linked to the Lipid A core complex, and already known to give endotoxins and capsular polysaccharides (CPS) with K-antigen.
The results presented here are part of a study on growth inhibition and virulence attenuation of *V. parahaemolyticus* by the use of lactic bacteria and on a better comprehension of the pathogenicity and virulence mechanisms.

Genome and carbohydrate-active enzymes diversity in *V. parahaemolyticus*

Complete genome sequencing was performed on different *Vibrio parahaemolyticus* strains isolated from shellfish

Species name	Total CDS number	Total genome length	Replicon		
			Sequence length	%GC	CDS number
Vibrio parahaemolyticus RIMD 2210633	5131	5165770	3288558	45.39	3229
			1877212	45.35	1902
Vibrio parahaemolyticus Vp5	4780	5103880	3211751	45,3	2987
			1864737		1732
Vibrio parahaemolyticus Vp18	4787	5115131	3297266	45,28	3095
			1803990		1636
Vibrio parahaemolyticus Vp22	4957	5211050	3281076	45,23	3081
			1896632		1740
Vibrio parahaemolyticus Vp136	4742	5080133	3299078	45,27	3076
			1848690		1694
Vibrio parahaemolyticus Vp177	5094	5185645	3247594	45,23	3084
			1875030		1705
Vibrio parahaemolyticus Vp182	5122	5237328	3263214	45,17	3072
			1894036		1769
Vibrio parahaemolyticus Vp195	4945	5099906	2997941	45,22	2767
			1847971		1703
Vibrio parahaemolyticus Vp201	4849	5131162	3258275	45,34	3070
			1847159		1710
Vibrio parahaemolyticus Vp203	4861	5140450	3251638	45,34	3062
			1845016		1710
Vibrio parahaemolyticus Vp408	4950	5119409	3178214	45,21	2947
			1848645		1703

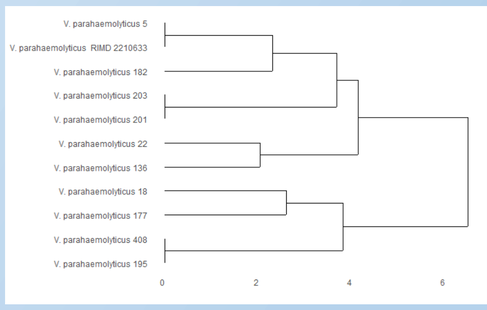
Genomes consisting in two chromosomes were assembled and compared to *Vibrio parahaemolyticus* type strain RIMD 2210633



Composition of Carbohydrate-Active enzymes (CAZymes) including carbohydrate esterase (CE), glycoside hydrolase (GH), glycosyl transferase (GT) and polysaccharide lyase (PL) involved in synthesis and degradation of complex carbohydrates was analysed through *in silico* identification of genes encoding CAZYmes. In total, among the 11 *V. parahaemolyticus* strains studied, 8, 23, 17 and 2 families were found for CE, GH, GT and PL, respectively.

Strain	CAZYMES				
	CE	GH	GT	PL	Total
Vibrio parahaemolyticus RIMD 2210633	12	43	33	4	92
Vibrio parahaemolyticus Vp5	12	43	33	4	92
Vibrio parahaemolyticus Vp18	13	46	33	4	96
Vibrio parahaemolyticus Vp22	14	48	34	4	100
Vibrio parahaemolyticus Vp136	13	43	33	4	93
Vibrio parahaemolyticus Vp177	15	51	37	4	107
Vibrio parahaemolyticus Vp182	14	48	35	4	101
Vibrio parahaemolyticus Vp195	15	48	37	4	104
Vibrio parahaemolyticus Vp201	15	51	37	4	107
Vibrio parahaemolyticus Vp203	15	51	37	4	107
Vibrio parahaemolyticus Vp408	15	48	37	4	104

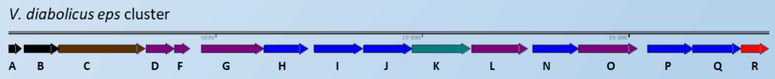
V. parahaemolyticus strains were clustered using hierarchical clustering and Euclidean distance on the basis of CaZymes (CE, GH, GT and PL) families using R software.



Comparative genomic and diversity of biosynthetic pathways involved in glycopolymers biosynthesis in *Vibrio sp. strains*

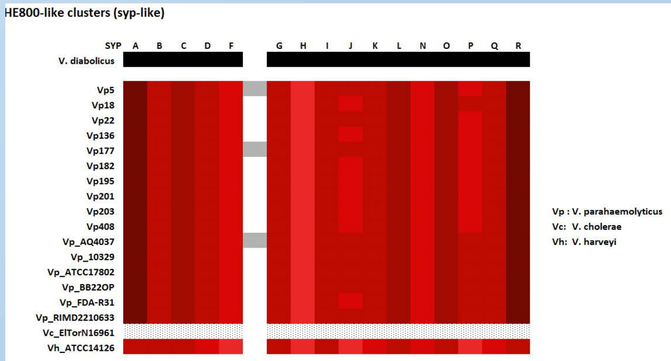
The *eps* locus in *Vibrio diabolicus* described in Goudenège et al.(2014) is involved in the production of the biotechnologically valuable HE800 EPS. The presence of several GT within the locus indicates the capacity to generate diversity in the glycosidic structure. In this study, proteins orthologous to *V. diabolicus* HE800 biosynthetic cluster were searched into the genomes using BLAST+ 2.2.3.0 package (Camacho et al. 2009).
Results show that *Vibrio parahaemolyticus* (Vp) and *V. harveyi* (Vh_ATCC14126) sequences display such an *eps* locus with a high level of synteny, particularly in the 5' end of the cluster (A gene) and in the 3' end encompassing R gene. *Vibrio cholerae* (Vc_ElTorN16961) genome does not display such *eps* cluster.

V. diabolicus eps cluster



SYP Protein	Function
A	Anti-sigma factor antagonist
B	Outer membrane protein
C	Periplasmic protein involved in polysaccharideexport (Wza)
D	Putative protein with a nucleotide domain
F	Hybrid sensor kinase (truncated gene)
G	Response regulator with σ54 interaction domain
H	Glycosyltransferase (GT4)
I	Glycosyltransferase (GT4)
J	Glycosyltransferase (GT4)
K	Oligosaccharide translocase (Wzx)
L	Polymerase (Wzy)
N	Glycosyltransferase (GT4)
O	Putative polysaccharide biosynthesis chain length regulator (Wzc)
P	Glycosyltransferase (GT4)
Q	Glycosyltransferase (GT2)
R	Phosphoglycosyltransferase (pGT)
	CAZYMES

HE800-like clusters (syp-like)

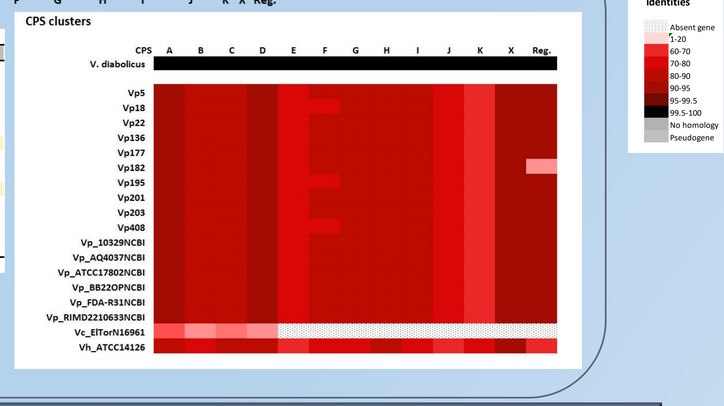


The *cps* locus in *Vibrio diabolicus* presents synteny with a locus designated as an exopolysaccharide cluster related to the rugose colony phenotype in *V. parahaemolyticus (*Chen et al., 2010), but was previously suggested by Guvener et al. (2003) to be responsible of the capsular polysaccharide synthesis in *V. parahaemolyticus*.
Results show that the global synteny of *cps* locus is highly conserved in all *V. parahaemolyticus* strain studied, whereas it is absent from *Vibrio cholera*e and *V. harveyi*.

V. diabolicus cps cluster

CPS Protein	Function
A	undecaprenyl-phosphate glycosyl phosphotransferase
B	uncharacterized, conserved
C	polysaccharide export, Wza
D	3 domaines
E	uncharacterized
F	glycosyltransferase(GT4)
G	Lipid A core - O-antigen ligase
H	uncharacterized
I	glycosyltransferase(GT4)
J	Export, Wzx
K	uncharacteri antibiotic resistance protein VanZ [Vibrio harveyi]
X	ASCH domain-containing protein, RNA-binding domain
Reg.	TetR transcriptional regulator, Tetracyclin repressor-like

CPS clusters



Future work

Clusterisation on the basis of CaZymes composition of *V. parahaemolyticus* strains has now to be compared with virulence tests which are ongoing *in vivo* on the model *Caenorhabiditis elegans*.
Comparative genomics reveals a high range of homology of *eps* and *cps* biosynthesis gene clusters in *V. parahaemolyticus*. Extracellular matrix composition study is also ongoing on biofilm. The biofilm biochemical composition will allow to understand if these extracellular components are virulence and/or antimicrobial resistance factors.

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