EDITORIAL

T3SS effectors in Vibrios: Homology in sequence, diversity in biological functions?

Carlos R. Osorio

Departamento de Microbiología e Parasitoloxía, Instituto de Acuicultura, Universidade de Santiago de Compostela, Santiago de Compostela, Spain

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Vibrio alginolyticus is a Gram-negative bacterium that thrives in marine environments, and is also an important pathogen that causes vibriosis in a variety of marine animals. Infections in cultivated fish and invertebrates result in significant financial losses for aquaculture industries worldwide [1-3]. Furthermore, this bacterium can also be an opportunistic pathogen for humans, causing diarrhea as well as extraintestinal infections such as otitis and wound infections [4-6]. The mechanism of pathogenesis of this bacterium has not been completely elucidated but a number of virulence factors have been described, including an alkaline serine protease [7-8], hemolysins [9], siderophores used as host iron scavengers [10] and a type III secretion system [11].

The type III secretion system (T3SS) is a conserved apparatus among several Gram-negative bacteria that delivers effector proteins directly into host cells [12]. Effector proteins can behave as virulence factors that manipulate host cell physiology and cause a diversity of cellular responses and cell damage. T3SS have been characterized in a number of Vibrionaceae species. The first report of a T3SS in Vibrio was in V. parahaemolyticus where two sets of T3SS gene clusters, T3SS1 and T3SS2, were discovered [13]. T3SS1 is found in all V. parahaemolyticus strains, while T3SS2 is present only in KP-positive strains. Although initially thought to be absent from V. cholerae T3SS gene clusters were subsequently reported in non-O1, non-O139 strains [14,15] and in non-toxigenic O1 V. cholerae [16]. Similar clusters were identified in other Vibrio species, including V. alginolyticus [11,17], V. mimicus [18], V. harveyi [19] and V. tubiashii [17], among others. Recently, a T3SS was also reported in the type strain of another member of the Vibrionaceae family: the fish and human pathogen Photobacterium damselae subsp. damselae [20].

The best studied Vibrio T3SS so far is T3SS1 from V. parahaemolyticus, a pathogen for humans as well as for marine animals. This system induces death of mammalian cell lines in a multifaceted process that starts with autophagy, followed by cell rounding, and culminating with cell death in a caspase-independent process [21,22]. The effector protein VopQ is necessary and sufficient to induce autophagy in HeLa cells through its interaction with the VopS domain of the V-type H+-ATPase in lysosomal membranes [23,24]. Another effector, VopS, is involved in cell rounding and in the collapse of the actin cytoskeleton by inhibiting Rho GTPases [25].

The V. alginolyticus genome encodes a system homologous to the V. parahaemolyticus T3SS1, including homologues of VopQ and VopS. Interestingly, it was shown that the V. alginolyticus T3SS was responsible for causing rapid apoptosis, cell rounding and osmotic lysis in the fish cell line EPC [11]. These findings were in clear contrast with the effect of V. parahaemolyticus on mammalian cells which is characterized by the activation of autophagy but not of apoptosis [21,22]. Interestingly, when the same V. alginolyticus strain was used to infect the human HeLa cell line apoptosis was not induced but activation of autophagy occurred instead [26].

In the current issue of Virulence, Zhe Zhao and colleagues [27] uncovered the role of two V. alginolyticus T3SS effector proteins, Val1686 and Val1680, in apoptosis, cell rounding and osmotic lysis in the fish cell line FHM. The authors report the singularities of these two V. alginolyticus proteins, which are homologues of the V. parahaemolyticus T3SS1 VopS (91% id.) and VopQ (88% id.) effectors, respectively. Zhao and colleagues show here that V. alginolyticus VopS not only contributes to cell rounding of fish cells by inhibiting Rho GTPases but that it also is essential for the induction of
apoptosis. A deletion mutant unable to produce VopS is severely impaired in its ability to induce these two cell responses and transfection of VopS into the fish cell line FHM is sufficient to cause cell rounding and apoptosis. The authors also show that the V. alginolyticus VopQ contributes to fish cell lysis but does not induce autophagy, a characteristic that distinguishes it from its V. para-haemolyticus homologue VopQ.

The findings reported by Zhao and colleagues in the present issue of Virulence provide new insights into the mechanisms of V. alginolyticus pathogenesis for fish cells. These data unequivocally demonstrate that this marine pathogen is capable of inducing apoptosis in fish cells and this effect is dependent on the T3SS effector VopS. Importantly, these results suggest that the type of host cell line used in the experiments is a variable of the maximal importance when drawing conclusions about the biological effects caused by a T3SS effector protein. The inherent differences between fish and mammalian cells may constitute the main reason why the V. alginolyticus and V. para-haemolyticus VopS and VopQ proteins induce distinct cellular responses. Very elegant experiments have contributed to unveil the roles of V. para-haemolyticus VopS and VopQ using mammalian cell lines and yeasts as host cell models [24,25,28,29], but information on the effects of these effectors in fish cells is scarce. In the light of the novel findings reported by Zhao and colleagues [27], it would be of high interest to compare the cellular effects caused by pairs of homologous T3SS effector proteins from different Vibrio species using the same host cell line. Future studies are needed in order to determine whether the different cellular responses elicited by V. alginolyticus and V. para-haemolyticus T3SS effectors are due to amino acid substitutions in homologous proteins or to inherent differences between fish and mammalian cells.

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No potential conflicts of interest were disclosed.

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**ORCID**

Carlos R. Osorio http://orcid.org/0000-0002-3099-4064

**References**

[1] Lee KK, Yu SR, Chen FR, et al. Virulence of Vibrio alginolyticus isolated from diseased tiger prawn, Penaeus monodon. Curr Microbiol. 1996;32:229–31.

[2] Liu CH, Cheng W, Hsu JP, et al. Vibrio alginolyticus infection in the white shrimp Litopenaeus vannamei confirmed by polymerase chain reaction and 16S rDNA sequencing. Dis Aquat Org. 2004;61:169–74.

[3] Xie ZY, Ke SW, Hu CQ, et al. First characterization of bacterial pathogen, Vibrio alginolyticus, for Litopenaeus vannamei white syndrome in the south China sea. PLoS One. 2013;8:e75425.

[4] Chien JY, Shih JT, Hsueh PR, et al. Vibrio alginolyticus as the cause of pleural empyema and bacteremia in an immunocompromised patient. Eur J Clin Microbiol Infect Dis. 2002;21:401–3.

[5] Austin B. Vibrios as causal agents of zoonoses. Vet Microbiol. 2010;140:310–7.

[6] Jacobs SK, Newto AE, Mahon BE. Vibrio alginolyticus infections in the USA, 1988–2012. Epidemiol Infect. 2017;145:1491–9.

[7] Lee KK, Yu SR, Liu PC. Alkaline serine protease is an exotoxin of Vibrio alginolyticus in Kuruma prawn, Penaeus japonicus. Curr Microbiol. 1997;34:110–7.

[8] Rui H, Liu Q, Wang Q, et al. Role of alkaline serine protease, Asp, in Vibrio alginolyticus virulence and regulation of its expression by LuxO-LuxR regulatory system. J Microbiol Biotechnol. 2009;19:431–8.

[9] Cai SH, Wu ZH, Jian JC, et al. Cloning and expression of gene encoding the thermostable direct hemolysin from Vibrio alginolyticus strain HY9901, the causative agent of vibriosis of crimson snapper (Lutjanus erythopterus). J Appl Microbiol. 2007;103:496–9.

[10] Wang Q, Liu Q, Cao X, et al. Characterization of two TonB systems in marine fish pathogen Vibrio alginolyticus: their roles in iron utilization and virulence. Arch Microbiol. 2008;190:595–603.

[11] Zhao Z, Chen C, Hu CQ, et al. The type III secretion system of Vibrio alginolyticus induces rapid apoptosis, cell rounding and osmotic lysis of fish cells. Microbiology. 2010;156:2864–72.

[12] Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol Mol Biol Rev. 1998;62:379–433.

[13] Makino K, Oshima K, Kurokawa K, et al. Genome sequence of Vibrio parahaemolyticus: a pathogenic mechanism distinct from that of V. cholerae. Lancet. 2003;361:743–9.

[14] Dziejman M, Serraturo D, Tam VC, et al. Genomic characterization of non-O1, non-O139 Vibrio cholerae reveals genes for a type III secretion system. Proc Natl Acad Sci USA. 2005;102:3465–70.

[15] Tam VC, Serraturo D, Dziejman M, et al. A type III secretion system in Vibrio cholerae translocates a formin/spire hybrid-like actin nucleator to promote intestinal colonization. Cell Host Microbe. 2007;1:95–107.

[16] Mahmoud J, Rashed SM, Islam T, et al. Type three secretion system in non-toxigenic Vibrio cholerae O1, Mexico. J Med Microbiol. 2014;63:1760–2.

[17] Park KS, Ono T, Rokuda M, et al. Functional characterization of two type III secretion systems of Vibrio parahaemolyticus. Infect Immun. 2004;72:6659–65.
[18] Okada N, Matsuda S, Matsuyama J, et al. Presence of genes for type III secretion system 2 in Vibrio mimicus strains. BMC Microbiol. 2010;10:302.

[19] Henke JM, Bassler BL. Quorum sensing regulates type III secretion in Vibrio harveyi and Vibrio parahaemolyticus. J Bacteriol. 2004;186:3794–805.

[20] Vences A, Rivas AJ, Lemos ML, et al. Chromosome-encoded hemolysin, phospholipase, and collagenase in plasmidless isolates of Photobacterium damselae subsp. damselae contribute to virulence for fish. Appl Environ Microbiol. 2017;83:pii: e00401–17.

[21] Burdette DL, Yarbrough ML, Orvedahl A, et al. Vibrio parahaemolyticus orchestrates a multifaceted host cell infection by induction of autophagy, cell rounding, and then cell lysis. Proc Natl Acad Sci USA. 2008;105:12497–502.

[22] Burdette DL, Yarbrough ML, Orth K. Not without cause: Vibrio parahaemolyticus induces acute autophagy and cell death. Autophagy. 2009;1:100–2.

[23] Sreelatha A, Orth K, Starai VJ. The pore-forming bacterial effector, VopQ, halts autophagic turnover. Autophagy. 2013;9:2169–70.

[24] Sreelatha A, Bennett TL, Zheng H, et al. Vibrio effector protein, VopQ, forms a lysosomal gated channel that disrupts host ion homeostasis and autophagic flux. Proc Natl Acad Sci USA. 2013;110:11559–64.

[25] Yarbrough ML, Li Y, Kinch LN, et al. AMPylation of Rho GTPases by Vibrio VopS disrupts effector binding and downstream signaling. Science. 2009;323:269–72.

[26] Zhao Z, Zhang L, Ren C, et al. Autophagy is induced by the type III secretion system of Vibrio alginolyticus in several mammalian cell lines. Arch Microbiol. 2011;193:53–61.

[27] Zhao Z, Liu J, Deng Y, et al. The Vibrio alginolyticus T3SS effectors, Val1686 and Val1680, induce cell rounding, apoptosis and lysis of fish epithelial cells. Virulence. 2018;

[28] Burdette DL, Seemann J, Orth K. Vibrio VopQ induces PI3-kinase-independent autophagy and antagonizes phagocytosis. Mol Microbiol. 2009;73:639–49.

[29] Sreelatha A, Bennett TL, Carpinone EM, et al. Vibrio effector protein VopQ inhibits fusion of V-ATPase containing membranes. Proc Natl Acad Sci USA. 2015;112:100–5.