Bile acids activate the antibacterial T6SS1 in the gut pathogen *Vibrio parahaemolyticus*

Sarah Schiffmann[1], Shir Mass[1], and Dor Salomon[1,*]

[1] Department of Clinical Microbiology and Immunology, School of Medicine, Faculty of Medical and Health Sciences, Tel Aviv University, Israel

[*] For correspondence: dorsalomon@mail.tau.ac.il

Abstract

The marine bacterium *Vibrio parahaemolyticus* is a major cause of seafood-borne gastroenteritis in humans and of acute hepatopancreatic necrosis disease in shrimp. Bile acids, produced by the host and modified into secondary bile acids by commensal bacteria in the gastrointestinal tract, induce the virulence factors leading to disease in humans and shrimp. Here, we show that secondary bile acids also activate this pathogen's type VI secretion system 1 (T6SS1), a toxin-delivery apparatus mediating interbacterial competition. This finding implies that *Vibrio parahaemolyticus* exploits secondary bile acids to activate its virulence factors and identify the presence of commensal bacteria that it needs to outcompete in order to colonize the host.

Importance

Bacterial pathogens often manipulate their host and cause disease by secreting toxic proteins. However, to successfully colonize a host, they must also remove commensal bacteria that reside in it and may compete with them over resources. Here, we find that the same host-derived molecules that activate the secreted virulence toxins in a gut bacterial pathogen, *Vibrio parahaemolyticus*, also activate an antibacterial toxin delivery system that targets such commensal bacteria. These findings suggest that a pathogen can use one cue to launch a coordinated, trans-kingdom attack that enables it to colonize a host.

Keywords

Regulation, deoxycholate, secondary bile acid, type VI secretion system, Vibrio parahaemolyticus

Observation

Vibrio parahaemolyticus (*Vpara*) is a Gram-negative bacterium common in marine and estuarine environments. It is a leading cause of seafood-borne gastroenteritis (1) and acute hepatopancreatic necrosis disease (AHPND), which globally affects the shrimp farming industry (2). *Vpara* virulence in mammals is dependent on a toxin delivery apparatus named type III secretion system 2 (T3SS2) (3, 4), which is encoded within a horizontally acquired genomic island, VPaI-7 (5), in clinical isolates. AHPND in shrimp is caused by the PirAB binary toxin, encoded on a conjugative plasmid (6).

Although mutually exclusive in *Vpara* genomes (7), both T3SS2 and PirAB are induced by bile acids, an important component in the gastrointestinal tract of animals promoting lipid absorption, protein cleavage, and antimicrobial toxicity (8–11). Whereas bile-mediated activation of T3SS2 occurs via the VtrA/C receptor, which is also encoded within VPaI-7 (10, 11), AHPND-causing strains lack VtrA/C (7), and it is unclear how they sense bile acids to activate PirAB (9).

We previously reported that pathogenic *Vpara* strains employ a toxin delivery apparatus, named type VI secretion system 1 (T6SS1), to outcompete rival bacteria (12, 13). We reasoned that T6SS1 plays an indirect role during host infection by enabling *Vpara* to combat gut commensal bacteria and colonize the host. Interestingly, another gastrointestinal pathogen, *Salmonella* Typhimurium, was shown to use the host bile acids as a cue to activate its antibacterial T6SS and establish a niche inside the host gut (14). Although environmental factors that regulate the *Vpara* T6SS1 have been investigated in several *Vpara* strains (12, 13), little is known about *Vpara* T6SS regulation by host factors. Nevertheless, a subset of *Vpara* T6SS1 genes was found to be transcriptionally induced during infection of an infant rabbit model (15), suggesting that host gut factors can regulate this system.

The abovementioned observations led us to hypothesize that *Vpara* uses bile sensing to induce its antibacterial T6SS1 in addition to its major virulence factors, thus gaining a competitive advantage during host infection. To test this hypothesis, we first set out to investigate the effect of bile acids on T6SS1 transcriptional regulation. Based on our previous delineation of the *Vpara* T6SS1 regulatory cascade in the type strain RIMD 2210633 (16), we constructed a reporter plasmid (pT6SS1^{report}) in which the predicted promoter region of *vp1400*, the first gene in an operon encoding several conserved T6SS1 structural components, is fused to a promoterless *cat* and *gfp* reporter cassette. To verify that this reporter reliably represents T6SS1, we monitored GFP expression in *Vpara* strain RIMD 2210633 under conditions known to induce or repress T6SS1, in defined M9 media containing 3% NaCl (wt/vol) at 30°C. As expected, GFP fluorescence was detected when *hns*, encoding a histone-like nucleoid structuring protein previously shown to repress T6SS1 (17), was deleted (**Fig. 1A**). Fluorescence was also detected upon the addition of phenamil, an inhibitor of the polar flagella motor shown to induce T6SS1 by mimicking surface sensing activation (12), to the media of wild-type *Vpara* (**Fig. 1A**). In contrast, phenamil addition did not induce GFP fluorescence in a strain in which we deleted the T6SS1 positive regulator, *tfoY* (18). Similar results were obtained when we monitored the expression of the *cat* reporter gene, providing chloramphenicol resistance, as growth on agar plates containing chloramphenicol (**Fig. 1B**). Therefore, we conclude that pT6SS1^{report} reliably represents the status of T6SS1 activation.

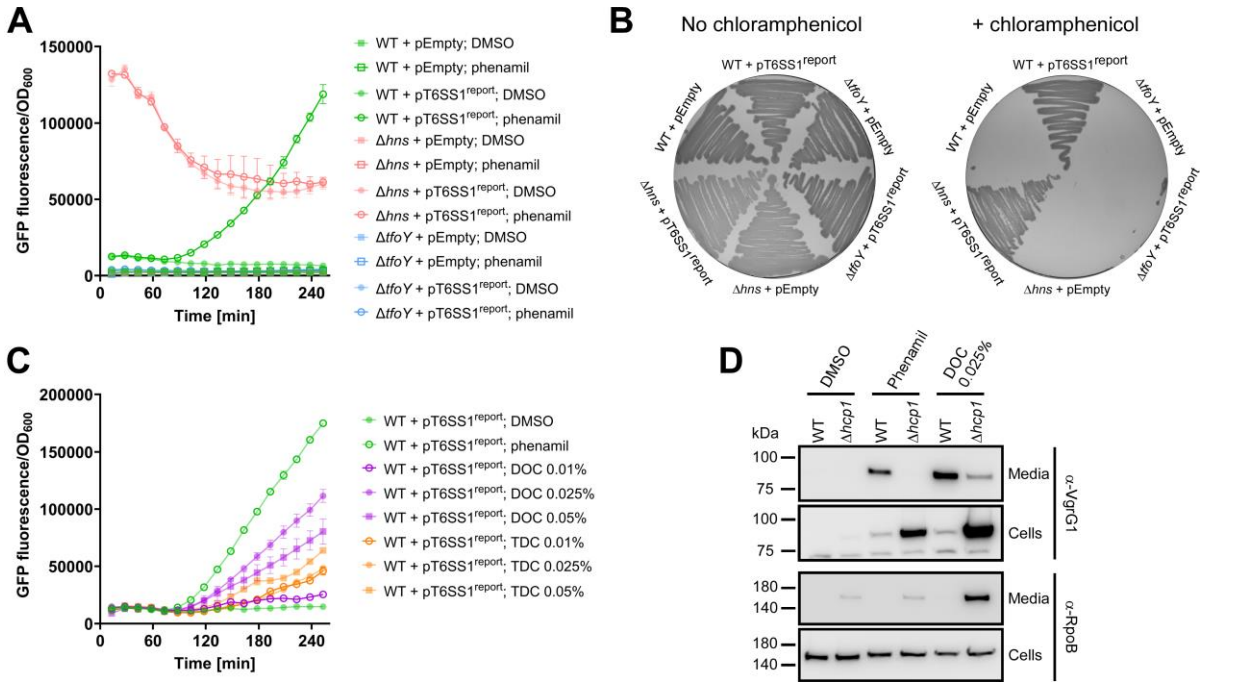

Fig. 1. Bile acids activate *Vibrio parahaemolyticus* T6SS1. A, C) Fluorescence intensity over time in the indicated *V. parahaemolyticus* RIMD 2210633 strains carrying an empty plasmid (pEmpty) or a plasmid containing a *gfp* and *cat* reporter cassette fused to the promoter of *vp1400* (pT6SS1^{report}), measured as GFP fluorescence to OD_{600} readings (arbitrary units). Bacteria were grown at 30°C in M9 media supplemented with 3% (wt/vol) NaCl and kanamycin (250 µg/mL), to maintain the plasmids. Where indicated, the media were supplemented with phenamil (20 µM), DMSO (20% [vol/vol], added to the media as a control at the same volume of the phenamil solution), or the indicated concentrations of DOC or TDC (wt/vol). Data are shown as the mean ± SD, n = 3 independent replicates. **B)** Growth of the indicated *V. parahaemolyticus* RIMD 2210633 strains at 30°C on MLB (LB with 3% [wt/vol] NaCl) agar plates containing kanamycin (250 µg/mL) to maintain the plasmids, with or without chloramphenicol (10 µg/mL) to monitor *cat* expression. **D)** Expression (cells) and secretion (media) of VgrG1 from the indicated *V. parahaemolyticus* RIMD 2210633 strains grown for 4 hours in M9 media supplemented with 3% (wt/vol) NaCl and either phenamil, DMSO (as detailed for A,C), or DOC at 30°C. RNA polymerase β subunit (RpoB) was used as a loading and lysis control. In A-D, results from a representative experiment out of at least three independent experiments are shown. WT, wild-type.

To investigate the effect of bile acids on T6SS1 transcription, we chose two secondary bile acids, which are bile acids modified in the host gut by commensal bacteria (8): taurodeoxycholate (TDC) and deoxycholate (DOC), which are strong and intermediate inducers of the *Vpara* T3SS2, respectively (19). Remarkably, the addition of DOC to the growth media at concentrations previously shown to activate T3SS2 (i.e., 0.025-0.05% [wt/vol]) (19) resulted in induction of GFP fluorescence from the T6SS1 reporter to levels comparable to those of the known inducer, phenamil (**Fig. 1C**). Surprisingly, TDC, which is a strong activator of T3SS2, had only a mild effect on the T6SS1 reporter (**Fig. 1C**). Neither bile acid hampered *Vpara* growth at these concentrations (**Fig. S1**). Moreover, neither DOC nor phenamil induced expression from the promoter of a T6SS1-unrelated gene, *vpa1270*, thus indicating that the observed

phenotypes do not result from global effects on *Vpara* transcription or translation (**Fig. S2**). Taken together, these results suggest that bile acids induce T6SS1.

Next, we sought to determine whether the observed bile acid-induced T6SS1 transcriptional activation translates to the assembly of a functional T6SS1 apparatus. To this end, we investigated the effect of DOC on T6SS1 activity by monitoring the expression and secretion of the hallmark T6SS structural secreted protein, VgrG1 (20). In agreement with the results obtained for the transcriptional reporter, DOC induced VgrG1 expression and secretion to a level comparable to that of phenamil (**Fig. 1D**). Moreover, intracellular accumulation of VgrG1 was evident upon inactivation of T6SS1 by deleting the conserved structural component Hcp1 (Δ*hcp1*) (21). Notably, although inactivation of T6SS1 hampered the DOC-induced secretion of VgrG1 compared to the wild-type strain, a low level of VgrG1 signal was detected in the "media" fraction of the DOC-treated Δ*hcp1* strain; this observation can be explained by cell lysis apparent in the Δ*hcp1* strains (see the α-RpoB panel in **Fig. 1D**), which was exacerbated in presence of DOC. These results demonstrate that bile acids activate *Vpara* T6SS1 in the clinical strain RIMD 2210633.

Conclusions

Our results suggest that *Vpara* use host-derived molecules, indicative of the presence of commensal bacteria, as a signal that activates virulence factors and antibacterial determinants playing a role in interbacterial competition. Therefore, bile acids may be exploited by *Vpara* to establish a niche inside the host. Future in-vivo competition assays using animal models are required to test this prediction. Notably, the bile acid receptor that regulates T6SS1 remains to be identified. Moreover, secondary bile acids were shown to repress the T6SS in another gut pathogen, *V. cholerae* (22), indicating that exploiting commensal bacteria-made molecules to activate antibacterial determinants in the host gut may not be a universal bacterial strategy.

Acknowledgments

We thank members of the Salomon lab for helpful discussions. This study was partly funded by a research grant awarded by the Tel Aviv University Center for Combatting Pandemics and by the Israel Science Foundation (grant number 1362/21) to DS.

References

1. Baker-Austin C, Oliver JD, Alam M, Ali A, Waldor MK, Qadri F, Martinez-Urtaza J. 2018. Vibrio spp. infections. Nat Rev Dis Prim 4:1–19.
2. Kumar V, Roy S, Behera BK, Bossier P, Das BK. 2021. Acute hepatopancreatic necrosis disease (Ahpnd): Virulence, pathogenesis and mitigation strategies in Shrimp aquaculture. Toxins (Basel) 13:524.
3. Yang H, Santos M de S, Lee J, Law HT, Chimalapati S, Verdu EF, Vallance BA, Orth K. 2019. A novel mouse model of enteric vibrio parahaemolyticus infection reveals that the type iii secretion system 2 effector vopc plays a key role in tissue invasion and gastroenteritis. MBio 10:e02608-19.

4. Ritchie JM, Rui H, Zhou X, Iida T, Kodoma T, Ito S, Davis BM, Bronson RT, Waldor MK. 2012. Inflammation and Disintegration of Intestinal Villi in an Experimental Model for Vibrio parahaemolyticus-Induced Diarrhea. PLoS Pathog 8:e1002593.

5. Boyd EF, Cohen AL V., Naughton LM, Ussery DW, Binnewies TT, Stine OC, Parent MA. 2008. Molecular analysis of the emergence of pandemic Vibrio parahaemolyticus. BMC Microbiol 30:110.

6. Lee C-T, Chen I-T, Yang Y-T, Ko T-P, Huang Y-T, Huang J-Y, Huang M-F, Lin S-J, Chen C-Y, Lin S-S, Lightner D V., Wang H-C, Wang AH-J, Wang H-C, Hor L-I, Lo C-F. 2015. The opportunistic marine pathogen Vibrio parahaemolyticus becomes virulent by acquiring a plasmid that expresses a deadly toxin. Proc Natl Acad Sci 112:10798–10803.

7. Vandeputte M, Coppens S, Bossier P, Vereecke N, Vanrompay D. 2024. Genomic mining of Vibrio parahaemolyticus highlights prevalence of antimicrobial resistance genes and new genetic markers associated with AHPND and tdh + /trh + genotypes. BMC Genomics 25:1–14.

8. Hofmann AF, Hagey LR. 2008. Bile acids: Chemistry, pathochemistry, biology, pathobiology, and therapeutics. Cell Mol Life Sci 65:2461-2483.

9. Kumar R, Ng TH, Chang CC, Tung TC, Lin SS, Lo CF, Wang HC. 2020. Bile acid and bile acid transporters are involved in the pathogenesis of acute hepatopancreatic necrosis disease in white shrimp Litopenaeus vannamei. Cell Microbiol 22:e13127.

10. Li P, Rivera-Cancel G, Kinch LN, Salomon D, Tomchick DR, Grishin N V., Orth K. 2016. Bile salt receptor complex activates a pathogenic type III secretion system. Elife 5:1–26.

11. Marcela de Souza Santos DS, Peng Li, Krachler Anne-Marie KO. 2015. Vibrio parahaemolyticus virulence determinants. The comprehensive sourcebook of bacterial protein toxins, p. 230–260. *In* Joseph Alouf DL, PMR (ed.), Fourth ed. Elsevier.

12. Salomon D, Gonzalez H, Updegraff BL, Orth K. 2013. Vibrio parahaemolyticus Type VI secretion system 1 Is activated in marine conditions to target bacteria, and is differentially regulated from system 2. PLoS One 8:e61086.

13. Li P, Kinch LN, Ray A, Dalia AB, Cong Q, Nunan LM, Camilli A, Grishin N V, Salomon D, Orth K. 2017. Acute hepatopancreatic necrosis disease-causing Vibrio parahaemolyticus strains maintain an antibacterial type VI secretion system with versatile effector repertoires. Appl Environ Microbiol 83:e00737-17.

14. Sana TG, Flaugnatti N, Lugo KA, Lam LH, Jacobson A, Baylot V, Durand E, Journet L, Cascales E, Monack DM. 2016. *Salmonella* Typhimurium utilizes a T6SS-mediated antibacterial weapon to establish in the host gut. Proc Natl Acad Sci 113:E5044–E5051.

15. Livny J, Zhou X, Mandlik A, Hubbard T, Davis BM, Waldor MK. 2014. Comparative RNA-Seq based dissection of the regulatory networks and environmental stimuli underlying Vibrio parahaemolyticus gene expression during infection. Nucleic Acids Res 42:12212-12223.

16. Jana B, Keppel K, Salomon D. 2021. Engineering a customizable antibacterial T6SS-based platform in Vibrio natriegens. EMBO Rep 22:e53681.

17. Salomon D, Klimko JA, Orth K. 2014. H-NS regulates the Vibrio parahaemolyticus type VI secretion system 1. Microbiol (United Kingdom) 160:1867–1873.

18. Ben-Yaakov R, Salomon D. 2019. The regulatory network of Vibrio parahaemolyticus type VI secretion system 1. Environ Microbiol 21:2248–2260.

19. Gotoh K, Kodama T, Hiyoshi H, Izutsu K, Park K-S, Dryselius R, Akeda Y, Honda T, Iida T. 2010. Bile Acid-Induced Virulence Gene Expression of Vibrio parahaemolyticus Reveals a Novel Therapeutic Potential for Bile Acid Sequestrants. PLoS One 5:e13365.

20. Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, Heidelberg JF, Mekalanos JJ. 2006. Identification of a conserved bacterial protein secretion system in Vibrio cholerae using the Dictyostelium host model system. Proc Natl Acad Sci 103:1528–1533.

21. Jana B, Fridman CM, Bosis E, Salomon D. 2019. A modular effector with a DNase domain and a marker for T6SS substrates. Nat Commun 10:3595.

22. Bachmann V, Kostiuk B, Unterweger D, Diaz-Satizabal L, Ogg S, Pukatzki S. 2015. Bile Salts Modulate the Mucin-Activated Type VI Secretion System of Pandemic Vibrio cholerae. PLoS Negl Trop Dis 9:e0004031.