Indole signaling, a promising target to control vibriosis in aquaculture

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Abstract

Diseases caused by pathogenic vibrios result in major losses in aquaculture. The use of antibiotics in order to control these infections has led to the development and spread of antibiotic-resistant pathogens, rendering antibiotic treatments ineffective and causing problems for food safety. Therefore, novel methods to control vibriosis are needed. A promising alternative is the interference with quorum sensing, bacterial cell-to-cell communication. Indole is one of the cell-to-cell signaling molecules produced by vibrios. Recent research has shown that indole controls virulence-related phenotypes such as motility and biofilm formation, and virulence towards various aquatic organisms. Consequently, interfering with indole signaling is a valid strategy for the control of vibriosis in aquaculture. Several indole analogues have been investigated in order to identify more potent virulence inhibitors. These included both natural indoles (such as auxins) and synthetic compounds (such as halogenated indoles). Several of these compounds have been shown to protect aquatic organisms against vibriosis without affecting the growth of the vibrios. The latter is important as it implies that the selective pressure for resistance development will be lower than for antibiotics.

Key words: Aquaculture; *Vibrio*; quorum sensing; indole signaling; antivirulence therapy

1. The need for new disease control strategies in aquaculture

For a few decades, aquaculture is one of the fastest-growing food-producing sectors worldwide. In 2020, global aquaculture production reached a record of 122.6 million tonnes worth USD 281.3 billion (FAO, 2022). However, aquaculture production will need to further increase in the future in order to meet the increasing demand due to the still-growing human population. The World Bank has stated that "Beyond 2030, aquaculture will likely dominate future global fish supply. Consequently, ensuring successful and sustainable development of global aquaculture is an imperative agenda for the global economy." (Word Bank, 2013). However, a number of factors currently limit the further expansion of aquaculture, amongst which disease is one of the major ones. Indeed, diseases are causing major losses in aquaculture, especially in the early life stages of aquatic animals (Vadstein et al., 2013).

Vibrios, including strains belonging to species like *Vibrio anguillarum*, *Vibrio campbellii, Vibrio harveyi, Vibrio parahaemolyticus* and *Vibrio splendidus*, are the most important bacterial pathogens of aquatic animals, causing up to 100% mortality (Ruwandeepika et al., 2012; Hickey and Lee, 2018; Kumar et al., 2020; Zhang and Li, 2021). Vibrios are ubiquituous in the marine environment, both free-living and in association with eukaryotes –either beneficial, neutral or pathogenic (Thompson et al., 2004). It is important to note that pathogenicity is a strain characteristic rather than a species characteristic as within a given species (e.g. *V. parahaemolyticus*) some strains are pathogenic, whereas others are not (Defoirdt, 2014). Further, vibrios are opportunistic pathogens, i.e. non-obligate and/or non-specialised pathogens of a focal host (Brown et al., 2012). Indeed, vibrios can reproduce outside their host and are often able to infect various host types. These characteristics contribute to the widespread problems caused by vibrios as they can reach high densities in the environment surrounding the cultured animals.

In order to control bacterial diseases in aquaculture, farmers have been and are using antibiotics (Lulijwa et al., 2020). Sixty-seven different antibiotics have been used in aquaculture in the top-15 producing countries between 2008 and 2018 in order to prevent and treat bacterial infections. The use of antibiotics in aquaculture varies between countries, and in recent years, the use has been drastically decreased in some countries (Lulijwa et al., 2020). The frequent use of antibiotics causes a number of problems, amongst which the development and spread of resistance is one of the most important (Cabello et al., 2016; Loo et al., 2020). The major reason why antibiotic resistance is spreading rapidly is that it imposes a high selective pressure on

bacteria (Davies and Davies, 2010). Indeed, resistant mutants can multiply in the presence of antibiotic, whereas the sensitive wild types will be killed or inhibited in their growth. As a consequence, all resources become available to the resistant mutant and competition by other (sensitive) bacteria is eliminated. Bacteria from aquaculture systems have been found to show multiple resistance to antibiotics belonging to different classes (Defoirdt et al., 2011), and recently, a rise in the presence of antibiotic-resistant *Vibrio* in the environment has been documented (Loo et al., 2020). This is problematic in two ways. First, it renders antibiotics ineffective in treating infections caused by the resistant vibrios in aquaculture, which causes a problem for food security (Loo et al., 2020). Second, antibiotic resistance genes can often be horizontally transmitted between bacteria, which ultimately leads to antibiotic-resistant human pathogens and thus causing a problem for food safety (Cabello et al., 2016). In order to tackle the problem of antibiotic resistance, several organizations have launched action plans. One of the actions proposed in the recent action plan of FAO, is the development of safe and efficacious alternatives to antibiotics (FAO, 2021).

2. Antivirulence therapy as an alternative to antibiotics

A couple of years ago, antivirulence therapy was proposed as an alternative to antibiotics for the control of bacterial disease in aquaculture (Defoirdt, 2014). In contrast to antibiotics, antivirulence therapy does not aim at killing or growth inhibition. Rather than that, it aims at inhibiting the production of molecules and cell structures that pathogens need to infect their host, i.e. virulence factors (Dickey et al., 2017). Antivirulence therapy imposes less selective pressure for resistance development than antibiotics because it does not kill or inhibit growth of sensitive bacteria (Rasko and Sperandio, 2010), and consequently, resistant mutants still have to compete for resources with sensitive wild types.

Antivirulence therapy can focus on inhibiting the production of specific virulence factors such as adhesion or secretion systems (Escaich, 2008). However, a far more intensively studied target is quorum sensing, bacterial cell-to-cell communication (Defoirdt, 2018). In quorum sensing, bacteria produce, release and respond to small signal molecules. Hence, quorum sensing is a gene regulation mechanism in which bacteria coordinate certain activities in accordance to the presence of signal molecules. Interestingly, quorum sensing systems have been documented to control virulence factor production in various bacterial pathogens of plants, animals and humans (Defoirdt, 2018; Whiteley et al., 2017), including vibrios (Milton, 2006). As a

consequence, quite some research these days is devoted to the inhibition of quorum sensing in order to control bacterial diseases. A major advantage of targeting quorum sensing systems is that they often control the production of multiple virulence factors and thus, interfering with it can block the production of several virulence factors at once, which will likely increase the efficacy. One of the signal molecules controlling virulence in vibrios is indole (Defoirdt, 2019). In the following paragraphs I will discuss indole signaling in vibrios and the interference with indole signaling to control vibriosis.

3. Indole signaling as a target for antivirulence therapy against vibrios

3.1. Indole signaling in vibrios

More than 85 bacterial species, including several vibrios, are known to produce indole from tryptophan by the action of the tryptophanase enzyme encoded by the *tnaA* gene (Lee and Lee, 2010), and indole has been proposed as an interspecies and interkingdom signaling molecule (Lee et al., 2015). The production of indole by TnaA is best understood in *E. coli*, for which it has been documented that *tnaA* expression is repressed at low tryptophan levels (Yanofsky et al., 1991). Further, in *E. coli*, tryptophanase is repressed by glucose through catabolite repression (Isaacs et al., 1994), and this was also documented in *V. splendidus* (Zhang et al., 2017). In vibrios, indole is mainly produced during stationary phase (Li et al., 2014; Yang et al., 2017; Zhang et al., 2022d). This is also reflected in increased *tnaA* mRNA levels in stationary phase cultures of *V. anguillarum* and *V. campbellii* (Li et al., 2014; Yang et al., 2017). The mechanism by which indole reaches the extracellular environment is yet unknown; in *E. coli* there is evidence for both protein-mediated transport and passive diffusion through membranes (Zarkan et al., 2020). In the extracellular environment, indole levels will accumulate depending on the presence of tryptophan, the growth phase of the vibrios, the cell density, and diffusion. Indole levels in stationary phase cultures of marine vibrios in Luria-Bertani broth vary between 50 and 250 μM, whereas indole levels in *V. cholerae* cultures are higher (up to 600 μM) (Table 1). An indole receptor has not yet been definitively identified in vibrios, although recent research indicated that the membrane regulator ToxR is involved in indole sensing in *V. cholerae* (Howard et al., 2019). ToxR is also present in other vibrios

(Ruwendeepika et al., 2010) and thus it might be involved in indole sensing in various *Vibrio* species.

Indole controls several virulence-related phenotypes in vibrios; most notably biofilm formation and swimming and swarming motility (Figure 1). Further, bioluminescence experiments indicated that indole also inhibits the three channel quorum sensing system in *V. campbellii*, another quorum sensing system controlling the virulence of the bacterium (Yang et al., 2017). Indeed, indole decreased quorum sensing-regulated bioluminescence in wild type *V. campbellii*, whereas it had no effect on the constitutive bioluminescence of an engineered strain (thus excluding the possibility that indole inhibited bioluminescence biochemistry). Further experiments using quorum sensing mutants revealed that indole interferes with quorum sensing signal transduction, and reverse transcriptase qPCR showed that it decreases the mRNA levels of the quorum sensing master regulator LuxR (Yang et al., 2017). In *V. anguillarum* and *V. campbellii*, indole signaling has also been shown to be connected with the stress sigma factor RpoS since indole increased *rpoS* mRNA levels, and indole and *tnaA* mRNA levels were increased in an *rpoS* deletion mutant (Li et al., 2014; Yang et al., 2017). This made us hypothesise that indole is a stress (starvation) signal. Indeed, the TnaA enzyme converts the amino acid tryptophan into indole, ammonia and pyruvate (Lee and Lee, 2010), the latter of which can feed the TCA cycle. Hence, indole can serve as a reliable cue informing the vibrios that energy is being derived from protein. During passage through the gastrointestinal tract of a host, bacteria experience a shift in fermentation pattern, with easily degradable carbohydrates being metabolised in the proximal part, and more refractory compunds (including proteins) in the distal part (Windey et al., 2012). Indole could thus enable vibrios to sense the shift to protein fermentation towards the distal part of the gastrointestinal tract, thereby facilitating the metabolic changes that are required to survive in the external environment (e.g. turning off virulence gene expression).

Importantly, elevated indole levels have been shown to decrease virulence to aquatic organisms in all marine vibrios studied thus far, including *V. anguillarum* (in sea bass), *V. campbellii* (in brine shrimp and giant river prawn), *V. crassostreae* (in blue mussel), *V. parahaemolyticus* (in brine shrimp and whiteleg shrimp) and *V. tasmaniensis* (in blue mussel) (Li et al., 2014; Yang et al., 2017; Paopradit et al., 2022; Zhang et al., 2022c; Zhang et al., 2022d). At the concentrations that decreased virulence factor production and virulence, indole did not affect the growth of the vibrios. This is important because it indicates that there will be a lower risk for the spread of resistance against the virulence-decreasing effect of indole signaling when

compared to antibiotics. Indeed, in contrast to antibiotics, indole sensitive wild types are not killed or inhibited in their growth and thus a resistant mutant still has to compete for resources with the sensitive bacteria. As a consequence, resistance will not spread as rapidly as it does for antibiotics (for which sensitive competitors of a resistant mutant are eliminated).

3.2. The impact of indole analogues on the virulence of vibrios

Importantly, at concentrations that optimally decrease virulence-related phenotypes (200 μM), indole is toxic to aquatic animals such as brine shrimp and blue mussel larvae (Yang et al., 2017). Given the fact that indole signaling controls the virulence of *Vibrio* species, several research groups have been studying the application of indole analogues to control vibriosis. Natural indole analogues are widely present in nature (Lee et al., 2015). Most notably are the auxin plant hormone indole-3-acetic acid and its precursors such as indole-3-acetamide and indole-3-acetonitrile (Figure 2) (Zhao, 2010). Indole-3-acetic acid has been shown to decrease biofilm formation and motility of *V. campbellii, V. harveyi* and *V. parahaemolyticus* strains at 200 μM and to protect brine shrimp larvae from these pathogens when added at 400 μM to the rearing water (Zhang et al., 2023). At this concentration, indole-3-acetic acid had no effect on growth of the vibrios in the rearing water. However, higher concentrations were shown to inhibit the growth of *V. campbellii* (Zhang et al., 2023). Furthermore, indole-3-acetic acid (at 30 mg/l = 171 μM) and undecanoic acid (at 10 mg/l) have been shown to have a synergistic activity against biofilm formation of *V. harveyi* and virulence towards brine shrimp larvae (Salini et al., 2019). The auxin precursors indole-3-acetamide and indole-3-acetonitrile were shown to have a similar effect as indole-3-acetic acid, with indole-3-acetonitrile being active at a relatively low concentration of 10 μM (Yang et al., 2017; Zhang et al., 2022b). Interestingly, auxins are not only produced by terrestrial plants, but also by algae and seaweeds (Stirk et al., 2004; Lin et al., 2022). Hence, micro-algae and seaweeds could be interesting sources of auxins to control vibriosis in aquaculture. Further research will be needed in order to investigate whether these sources contain sufficiently high levels of auxins to protect aquatic animals from vibrios. In this research, one will need to focus on concentrated samples in order to mimic algal or seaweed levels (and thus also auxin concentrations) in the digestive tract of animals that were feeding on these sources of auxin.

In addition to natural indole analogues, synthetic derivatives have also been studied. A first group of derivatives are indene, 2,3-benzofuran and thianaphthene, in which the N atom of indole is replaced by C, O and S, respectively. All three of these compounds have been shown

to increase the survival of brine shrimp larvae challenged with *V. campbellii* to around 80% or more when added to the rearing water at 200 µM, the concentration at which indole is toxic to brine shrimp (Zhang et al., 2022b). At this concentration, the compounds had no effect on the density of *V. campbellii* in the rearing water. Further, they significantly decreased swimming motility, but had no effect on biofilm formation. Indene, 2,3-benzofuran and thianaphthene had no effect on the survival of challenged brine shrimp when added 1 day after the pathogen, suggesting that interfering with indole signaling has no curative effect. However, in a field situation in which some individuals are affected by vibriosis, whereas others are not, these compounds might still be valuable in order to stop transmission of disease from affected to unaffected animals.

A second interesting group of indole analogues are halogenated indoles. Sathiyamoorthi et al. (2021) investigated the impact of 16 halogenated indoles on *V. parahaemolyticus*. They reported that 4-chloroindole and 7-chloroindole inhibited biofilm formation of *V. parahaemolyticus* without affecting growth at 20 mg/l (132 µM), whereas growth was completely inhibited at 50 mg/l and 200 mg/l, respectively. Both compounds also inhibited swimming and swarming motility at 50 mg/l and protease activity at 10 mg/l. Zhang et al. (2022a) investigated the impact of 31 halogenated indoles on *V. campbellii*. None of the compounds affected growth of *V. campbellii* for concentrations up to 200 µM, whereas 10 compounds increased the survival of brine shrimp challenged with *V. campbellii* to over 80% when added to the rearing water at 20 µM, and 5 compounds (6-bromoindole, 7-bromoindole, 4-fluoroindole, 5-iodoindole and 7-iodoindole) did so when added at 10 µM. All of these compounds decreased swimming motility of *V. campbellii* at 10 µM and most of them inhibited biofilm formation at 100 µM. Finally, the compounds had little effect on protease and hemolytic activity at 100 µM.

4. Conclusions and further perspectives

Indole has been shown to decrease the virulence of all marine vibrios that are pathogenic to aquatic organisms studied thus far. Furthermore, more potent indole analogues with an antivirulence effect have been identified. These include both natural indoles (such as auxins and auxin precursors) as well as synthetic compounds (such as halogenated indoles). Together, this research has indicated that indole signaling is a valid target for the control of vibriosis in aquaculture, and indole analogues or natural sources of indoles are interesting novel virulence

inhibitors for aquaculture. Thus far, studies have been limited to laboratory scale experiments, and thus, larger scale experiments would be a logic next step. Furthermore, in addition to applying indoles to the rearing water of aquatic organisms, further studies should also investigate the efficacy of incorporation into feed. Finally, it could be interesting to synthesise novel indole analogues in order to identify compounds that are even more potent than the currently known indoles. In this regard, it would be interesting to identify the indole receptor in marine vibrios in order to design agonists based on structural properties of the receptor. Before indoles can be applied in practice, toxicity of the indole analogues to be used will need to be established for the cultured organisms, and possible side effects towards non-target bacteria (e.g. bacteria in biofilters, or probiotics) will need to be investigated.

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Conflict of interest

The author declares no conflict of interest. The funders had no role in the writing of the manuscript, or in the decision to publish it.

Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

References

Brown, S.P., Cornforth, D.M., Mideo, N., 2012. Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. Trends Microbiol. 20, 336-342.

Cabello, F., Godfrey, H.P., Buschmann, A.H., Dölz, H.J., 2016. Aquaculture as yet another environmental gateway to the development and globalisation of antimicrobial resistance. Lancet Infect. Dis. 16, e127-e133.

Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev. 74, 417-433.

Defoirdt, T., 2019. Amino acid-derived quorum sensing molecules controlling the virulence of vibrios (and beyond). PLoS Path. 15, e1007815.

Defoirdt, T., 2018. Quorum-sensing systems as targets for antivirulence therapy. Trends Microbiol. 26, 313-328.

Defoirdt, T., 2014. Virulence mechanisms of bacterial aquaculture pathogens and antivirulence therapy for aquaculture. Rev. Aquacult. 6, 100-114.

Dickey, S.W., Cheung, G.Y.C., Otto, M., 2017. Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance. Nat. Rev. Drug Discov. 16, 457-471.

Escaich, S., 2008. Antivirulence as a new antibacterial approach for chemotherapy. Curr. Opin. Chem. Biol. 12, 400-408.

FAO, 2021. The FAO Action Plan on Antimicrobial Resistance 2021–2025. Rome.

FAO., 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome.

Hickey, M.E., Lee, J.L., 2018. A comprehensive review of *Vibrio (Listonella) anguillarum*: ecology, pathology and prevention. Rev. Aquacult. 10, 585-610.

Howard, M.F., Bina, X.R., Bina, J.E., 2019. Indole inhibits ToxR regulon expression in *Vibrio cholerae*. Infect. Immun. 87, e00776-18.

Isaacs, H., Chao, D., Yanofsky, C., Saier, M.H., 1994. Mechanism of catabolite repression of tryptophanase synthesis in *Escherichia coli.* Microbiology 140, 2125-2134.

Kumar, R., Ng, T.H., Wang, H.C., 2020. Acute hepatopancreatic necrosis disease in penaeid shrimp. Rev. Aquacult. 12, 1867-1880.

Lee, J.H., Lee, J., 2010. Indole as an intercellular signal in microbial communities. FEMS Microbiol. Rev. 34, 426-444.

Lee, J.H., Wood, T.K., Lee, J., 2015. Roles of indole as an interspecies and interkingdom signaling molecule. Trends Microbiol. 23, 707-718.

303 Li, X., Yang, Q., Dierckens, K., Milton, D.L., Defoirdt, T., 2014. RpoS and indole signaling
304 control the virulence of *Vibrio anguillarum* towards gnotobiotic sea bass (*Dicentrarchus labrax*)
305 larvae. PLoS One 9, e111801.

306 Lin, H., Li, Y., Hill, R.T., 2022. Microalgal and bacterial auxin biosynthesis: implications for
307 algal biotechnology. Curr. Opin. Biotechnol. 73, 300-307.

308 Loo, K.Y., Letchumanan, V., Law, J.W.F., Pusparajah, P., Goh, B.H., Ab Mutalib, N.S., He,
309 Y.W., Lee, L.H., 2020. Incidence of antibiotic resistance in *Vibrio* spp. Rev. Aquacult. 12,
310 2590-2608.

311 Lulijwa, R., Rupia, E.J., Alfaro, A.C., 2020. Antibiotic use in aquaculture, policies and
312 regulation, health and environmental risks: a review of the top 15 major producers. Rev.
313 Aquacult. 12, 640-663.

314 Milton, D.L., 2006. Quorum sensing in vibrios: Complexity for diversification. Int. J. Med.
315 Microbiol. 296, 61-71.

316 Mueller, R.S., Beyhan, S., Saini, S.G., Yildiz, F.H., Bartlett, D.H., 2009. Indole acts as an
317 extracellular cue regulating gene expression in *Vibrio cholerae*. J. Bacteriol. 191, 3504-3516.

318 Rasko, D.A., Sperandio, V., 2010. Anti-virulence strategies to combat bacteria-mediated
319 disease. Nat. Rev. Drug Discov. 9, 117-128.

320 Ruwandeepika, HA.D., Defoirdt, T., Bhowmick, P.P., Shekar, M., Bossier, P., Karunasagar, I.,
321 2010. Presence of typical and atypical virulence genes in vibrio isolates belonging to the
322 Harveyi clade. J. Appl. Microbiol. 109, 888-899.

323 Ruwandeepika, H.A.D, Jayaweera, T.S.P., Bhowmick, P.P., Karunasagar, I., Bossier, P.,
324 Defoirdt, T., 2012. Pathogenesis, virulence factors and virulence regulation of vibrios
325 belonging to the Harveyi clade. Rev. Aquacult. 4, 59-74.

326 Salini, R., Santhakumari, S., Ravi, A.V., Pandian, S.K., 2019. Synergistic antibiofilm efficacy
327 of undecanoic acid and auxins against quorum sensing mediated biofilm formation of
328 luminescent *Vibrio harveyi*. Aquaculture. 498, 162-170.

329 Sathiyamoorthi, E., Faleye, O.S., Lee, J.H., Raj, V., Lee, J., 2021. Antibacterial and antibiofilm
330 activities of chloroindoles against *Vibrio parahaemolyticus*. Front. Microbiol. 12, 714371.

Stirk, W.A., Arthur, G.D., Lourens, A.F., Novak, O., Strnad, M., van Staden, J., 2004. Changes in cytokinin and auxin concentrations in seaweed concentrates when stored at an elevated temperature. J. Appl. Phycol. 16, 31-39.

Thompson, F.L., Iida, T., Swings, J., 2004. Biodiversity of vibrios. Microbiol. Mol. Biol. Rev. 68, 403-431.

Vadstein, O., Bergh, Ø., Gatesoupe, F.J., Galindo-Villegas, J., Mulero, V., Picchietti, S., Scapigliati, G., Makridis, P., Olsen, Y., Dierckens, K., Defoirdt, T., Boon, N., De Schryver, P., Bossier, P., 2013. Microbiology and immunology of fish larvae. Rev. Aquacult. 5, S1-S25.

Whiteley, M., Diggle, S.P., Greenberg, E.P., 2017. Progress in and promise of bacterial quorum sensing research. Nature 551, 313-320.

Windey, K., De Preter, V., Verbeke, K., 2012. Relevance of protein fermentation to gut health. Mol. Nutr. Food Res. 56, 184-196.

World Bank., 2013. Fish to 2030 : Prospects for Fisheries and Aquaculture. Agriculture and environmental services discussion paper;no. 3. Washington, DC

Yang, Q., Pande, G.S.J., Wang, Z., Lin, B., Rubin, R.A., Vora, G.J., Defoirdt, T., 2017. Indole signalling and (micro)algal auxins decrease the virulence of *Vibrio campbellii*, a major pathogen of aquatic organisms. Environ. Microbiol. 19, 1987-2004.

Yanofsky, C., Horn, V., Gollnick, P., 1991. Physiological studies of tryptophan transport and tryptophanase operon induction in *Escherichia coli.* J. Bacteriol. 173, 6009-6017.

Zarkan, A., Liu, J., Matuszewska, M., Gaimster, H., Summers, D.K., 2020. Local and universal action: the paradoxes of indole signaling in bacteria. Trends Microbiol. 28, 566-577.

Zhang, S., Shao, Y., Zhao, X., Li, C., Guo, M., Lv, Z., Zhang, W., 2020. Indole contributes to tetracycline resistance via the outer membrane protein OmpN in *Vibrio splendidus*. World J. Microbiol. Biotechnol. 36, 36.

Zhang, S., Yang, Q., Defoirdt, T., 2022a. Halogenated indoles decrease the virulence of *Vibrio campbellii* in a gnotobiotic brine shrimp model. Microbiol. Spectrum 10, e02689-22.

Zhang, S., Yang, Q., Defoirdt, T., 2022b. Indole analogues decreasing the virulence of *Vibrio campbellii* towards brine shrimp larvae. Microbial Biotechnol. 15, 2917-2928.

Zhang, S., Yang, Q., Defoirdt, T., 2022c. Indole decreases the virulence of pathogenic vibrios belonging to the Harveyi clade. J. Appl. Microbiol. 132, 167-176.

Zhang, S., Yang, Q., Fu, S., Janssen, C.R., Eggermont, M., Defoirdt, T., 2022d. Indole decreases the virulence of the bivalve model pathogens *Vibrio tasmaniensis* LGP32 and *Vibrio crassostreae* J2-9. Sci. Rep. 12, 5749.

Zhang, S., Van Haesebroeck, J., Yang, Q., Defoirdt, T., 2023. Indole-3-acetic acid increases the survival of brine shrimp challenged with vibrios belonging to the Harveyi clade. J. Fish Dis. in press.

Zhang, S., Zhang, W., Liu, N., Song, T., Liu, H., Zhao, X., Xu, W., Li, C., 2017. Indole reduces the expression of virulence related genes in *Vibrio splendidus* pathogenic to sea cucumber *Apostichopus japonicus*. Microbial Pathog. 111, 168-173.

Zhang, W.W., Li, C.H., 2021. Virulence mechanisms of vibrios belonging to the Splendidus clade as aquaculture pathogens, from case studies and genome data. Rev. Aquacult. 13, 2004-2026.

Zhao, Y.D., 2010. Auxin biosynthesis and its role in plant development. Annu. Rev. Plant Biol. 61, 49-64.

Tables

Table 1. Indole production in vibrios and phenotypes affected by indole.

Species	[indole] produced (μM)	Phenotypes affected by high [indole][1]				References
		Motility	Biofilm	Virulence	Other	
Vibrio anguillarum	50	=	↓	↓	EPS[2] ↓	Li et al., 2014
Vibrio campbellii	60	↓ = [3]	↓	↓	EPS[2] ↓	Yang et al., 2017; Zhang et al., 2022c
Vibrio cholerae	600	↓	↑	?	VPS[2] ↑, CT[2]↓	Mueller et al., 2009; Howard et al., 2019
Vibrio crassostreae	250	↓	↓	↓	-	Zhang et al., 2022d
Vibrio harveyi	?	↓	↓ = [3]	↓	-	Zhang et al., 2022c
Vibrio parahaemolyticus	?	↓	↓	↓	*pirAB*[2]↓	Paopradit et al., 2022; Zhang et al., 2022b
Vibrio splendidus	150	?	?	?	*vsm*[2]↓ *vsh*[2]↓	Zhang et al., 2017
Vibrio tasmaniensis	200	↓	=	↓	-	Zhang et al., 2022d

[1] The impact of indole is represented by the following symbols: ↑ means that the activity increases with increased levels of indole, ↓ means that the activity decreases with increased levels of indole, = means that indole has no effect on the phenotype, ? means unknown

[2] EPS: Extracellular polysaccharide; VPS: *Vibrio* polysaccharide; CT: cholera toxin; *pirAB: Photorhabdus* insect related toxin mRNA; *vsm*: *V. splendidus* metalloprotease mRNA; *vsh: V. splendidus* hemolysin mRNA

[3] different for different strains

Figures

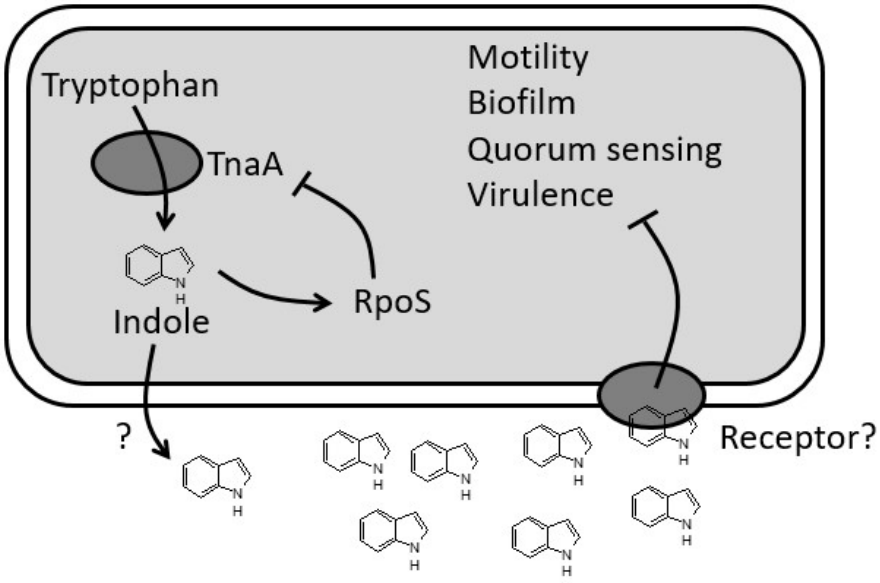
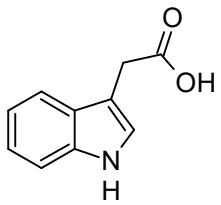
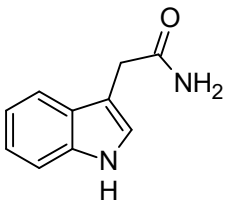


Figure 1. Indole signaling in marine vibrios, with unknown mechanisms indicated with a "?". Indole is produced from tryptophan by tryptophanase (TnaA). It is not yet known whether indole passively diffuses through the membranes of vibrios or is actively transported. Indole accumulates extracellularly dependent on tryptophane levels, growth stage, cell density, and diffusion. High levels of indole result in a decrease in motility, biofilm formation, three channel quorum sensing, and virulence. Indole increases the expression of *rpoS*, and RpoS blocks *tnaA* expression. A receptor for indole in vibrios has not yet been definitively identified, and the signal transduction cascade is not known.

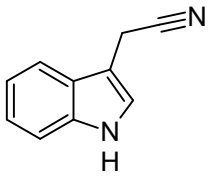
Natural indoles



Indole-3-acetic acid

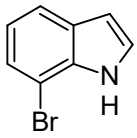


Indole-3-acetamide

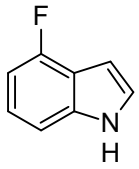


Indole-3-acetonitrile

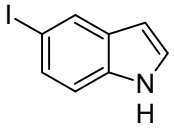
Halogenated indoles



7-bromoindole



4-fluoroindole



5-iodoindole

404

405 **Figure 2.** Chemical structures of examples of indole analogues with an antivirulence effect
406 towards vibrios.

407

408