

POTENTIAL OF OCTOPUS INK EXTRACT AS A QUORUM- QUENCHING AGENT TO INHIBIT *Vibrio harveyi* BIOFILM FORMATION IN AQUACULTURE (REVIEW)

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ABSTRACT

Vibrio harveyi is widely recognized as an important bacterial pathogen responsible for diseases in marine and brackish water fish, leading to reduced product quality, risks to food safety, and considerable economic losses on a global scale. A key factor contributing to its pathogenicity is its capacity to form biofilms, a process regulated by the quorum sensing (QS) system, which enhances bacterial virulence and tolerance to antibiotic treatments. Consequently, targeting and disrupting the QS system has emerged as a promising alternative approach for managing *V. harveyi* infections in aquaculture. Among potential natural sources, octopus ink extract has attracted attention due to its rich content of bioactive compounds, particularly alkaloids. This study seeks to evaluate the effectiveness of octopus ink extract as a quorum quenching (QQ) agent in preventing biofilm formation by *V. harveyi* in aquaculture settings. The research was conducted through a systematic literature review, involving the collection and critical examination of relevant scientific publications. An in-depth analysis was performed to elucidate the mechanisms by which octopus ink extract interferes with QS-regulated processes and biofilm development. The findings indicate that active components in octopus ink can interrupt bacterial cell-to-cell communication by blocking QS autoinducers, resulting in reduced expression of virulence genes and inhibition of biofilm formation without directly affecting bacterial viability. This strategy may help minimize the emergence of antibiotic resistance. Overall, octopus ink extract shows strong potential as an effective, environmentally friendly quorum quenching agent and may also be developed as an immunostimulant to enhance fish health and promote sustainable aquaculture practices.

Keywords: Aquaculture, Biofilm, Octopus Ink Extract, Quorum Quenching, *Vibrio harveyi*

ABSTRAK

Vibrio harveyi secara luas dikenal sebagai patogen bakteri penting yang bertanggung jawab atas penyakit pada ikan laut dan air payau, yang menyebabkan penurunan kualitas produk, risiko terhadap keamanan pangan, dan kerugian ekonomi yang cukup besar di tingkat global. Faktor kunci yang berkontribusi terhadap patogenitasnya adalah kemampuannya untuk membentuk biofilm, suatu proses yang diatur oleh sistem quorum sensing (QS), yang meningkatkan virulensi bakteri dan toleransi terhadap pengobatan antibiotik. Akibatnya, menargetkan dan

mengganggu sistem QS telah muncul sebagai pendekatan alternatif yang menjanjikan untuk mengelola infeksi *V. harveyi* dalam akuakultur. Di antara sumber alami potensial, ekstrak tinta gurita telah menarik perhatian karena kandungan senyawa bioaktifnya yang kaya, khususnya alkaloid. Studi ini bertujuan untuk mengevaluasi efektivitas ekstrak tinta gurita sebagai agen peredam quorum sensing (QQ) dalam mencegah pembentukan biofilm oleh *V. harveyi* dalam lingkungan akuakultur. Penelitian ini dilakukan melalui tinjauan literatur sistematis, yang melibatkan pengumpulan dan pemeriksaan kritis publikasi ilmiah yang relevan. Analisis mendalam dilakukan untuk menjelaskan mekanisme bagaimana ekstrak tinta gurita mengganggu proses yang diatur oleh QS dan perkembangan biofilm. Temuan menunjukkan bahwa komponen aktif dalam tinta gurita dapat mengganggu komunikasi antar sel bakteri dengan memblokir autoinducer QS, yang mengakibatkan penurunan ekspresi gen virulensi dan penghambatan pembentukan biofilm tanpa secara langsung memengaruhi viabilitas bakteri. Strategi ini dapat membantu meminimalkan munculnya resistensi antibiotik. Secara keseluruhan, ekstrak tinta gurita menunjukkan potensi yang kuat sebagai agen peredam quorum sensing yang efektif dan ramah lingkungan, dan juga dapat dikembangkan sebagai imunostimulan untuk meningkatkan kesehatan ikan dan mempromosikan praktik akuakultur berkelanjutan.

Kata Kunci: *Akuakultur, Biofilm, Ekstrak Tinta Gurita, Quorum Quenching, Vibrio harveyi*

INTRODUCTION

Vibrio species are important pathogens of aquatic vertebrates and invertebrates. This genus comprises over 140 facultative anaerobic, Gram-negative species. However, diseases in fish and shellfish are most frequently linked to *V. anguillarum*, *V. ordalii*, *V. vulnificus*, *V. alginolyticus*, *V. parahaemolyticus*, *V. harveyi*, and *V. tubiashi* (Vandeputte et al., 2024). *V. harveyi*, the primary bacterium responsible for catastrophic death in fish farming, has led to massive economic loss (Jusidin et al., 2022). The disease caused by the bacterium *Vibrio* sp. is known as vibriosis. The *V. harveyi* strain can kill up to 100% of tiger prawn (*Penaeus monodon*) larvae in hatchery (Ambat et al., 2022). *Vibrio harveyi*, a gram-negative, fermentative rod-shaped bacteria, which require sodium chloride for growth and are motile by polar flagella (Schrama et al., 2025). *Vibrio harveyi* is characterized as a straight to curved, comma-shaped rod bacterium equipped with a sheathed polar flagellum that enables motility. It is primarily transmitted through water, allowing the pathogen to disperse easily within the water column. This bacterium inhabits diverse aquatic environments, including rivers, estuaries, coastal seas, and deep-ocean waters. Its occurrence and abundance are strongly affected by environmental factors such as temperature, pH, salinity, and nutrient availability. *V. harveyi* is considered thermophilic and highly adaptable, with the ability to persist in seawater even under unfavorable conditions. Optimal growth occurs in warm waters above 18 °C, particularly in nutrient-rich seawater and brackish environments. A pH range of 7.5–8.5 is regarded as ideal for its proliferation. Furthermore, *V. harveyi* functions as an opportunistic pathogen, causing disease primarily when host

immunity is compromised by physiological stress or adverse environmental conditions (Kah Sem et al., 2023).

Virulence determines how pathogenic bacteria initiate and sustain infections. It involves diverse factors, including toxins, adhesins, surface polysaccharides, motility structures, siderophores, and secretion systems. Secreted proteins promote biofilm development, which occurs across ecosystems and influences clinical and non-clinical environments worldwide (De Silva & Heo, 2023). A biofilm is a community of microorganisms, such as bacteria, that can live and reproduce as a collective entity known as a colony. In other words, a biofilm is a living biomass with a community structure. The biofilm's structure serves to protect and enable the colony's expansion (Sharma et al., 2023). Biofilm formation is another key factor involved in the pathogenesis of this bacterium and is controlled by a mechanism called quorum sensing (QS). QS is a bacterial communication system that enables this pathogen to express bioluminescence (Guzman et al., 2022).

Quorum sensing (QS), in general, is a sophisticated communication mechanism used by bacteria to coordinate behavior within a population (Neil et al., 2024). QS is communication between microorganism cells (Zhao et al., 2015). Quorum sensing (QS) triggers the formation of biofilms consisting of bacterial cells and an extracellular matrix composed of proteins, polysaccharides, and DNA that can prevent antibiotic penetration, thereby promoting antibiotic tolerance (Munir et al., 2020).

Disruption of the quorum sensing system offers a promising approach to control *V. harveyi* infections in fish. This antivirulence strategy, known as quorum quenching, interferes with bacterial communication rather than killing cells. Consequently, it applies lower selective pressure and reduces the likelihood of resistance development (Santos et al., 2021). One approach to disrupting QS is the use of phytochemical compounds. Phytochemical compounds have been shown to interfere with the expression of pathogenicity by disrupting the bacterial QS system (Samreen et al., 2022). One of the phytochemical compounds that can be used is alkaloids.

Alkaloids have been reported to have inhibitory activity against bacterial biofilms (Ta & Arnason, 2015). Alkaloids are recognized as quorum sensing inhibitors due to their ability to interfere with QS-regulated virulence factors and processes and through direct interactions with specific QS targets (Cushnie et al., 2014). Alkaloids can be found in cephalopod ink such as squid ink (Affandi et al., 2019) and octopus ink (Affandi et al., 2023; Saputra et al., 2025). Previous studies have found that squid (*Loligo duvauceli*) and cuttlefish (*Sepioteuthis lessoniana*) ink have strong antimicrobial activity against biofilm-causing microorganisms (Hamdi et al., 2024; Kumar & Pasha, 2020). Octopus ink has been reported to possess anti-quorum sensing activity capable of inhibiting biofilm formation by *Aeromonas hydrophila* and *Edwardsiella tarda* (Affandi & Setyono, 2025b, 2025a). Building on this evidence, the current study seeks to assess the potential of octopus ink extract as a quorum quenching agent to suppress *Vibrio harveyi* biofilm development in aquaculture environments.

RESEARCH METHODS

Place and Time

This literature review research was conducted in January 2026 in Mataram, West Nusa Tenggara, Indonesia.

Tools and materials

The equipment and resources used in this literature review consisted of a laptop with its charger and mouse, along with scientific articles available in digital format.

Research Procedures

Relevant information for this article was gathered from several academic databases, including Google Scholar, ScienceDirect, Springer, and ProQuest. The sources reviewed consisted of 53 peer-reviewed journal articles and one book chapter were selected based on specific inclusion criteria, such as publication in reputable journals, relevance to quorum sensing inhibition, biofilm control, aquaculture applications, and recency of publication. This study employed a systematic literature review approach to ensure a structured and objective analysis. The review process involved identifying, collecting, reading, documenting, and organizing library-based data in a systematic, analytical, and critical manner. The focus of the review was to evaluate the potential of octopus ink extract as a quorum quenching agent for inhibiting *Vibrio harveyi* biofilm formation in aquaculture environments. An in-depth analysis was conducted to generate objective and reliable conclusions regarding its effectiveness. All data examined were secondary sources derived from previously published research, including scientific journals, books, and other relevant scholarly articles related to quorum sensing inhibition and biofilm control in aquaculture systems (Affandi et al., 2023; Affandi & Setyono, 2023, 2024a, 2024b).

Data Analysis

The data analysis in this article employs a content analysis approach. The process begins by categorizing research findings based on their level of relevance, ranging from highly relevant to moderately relevant. Abstracts are then reviewed to determine alignment with the research objectives, followed by identifying key sections related to the research problem and drawing final conclusions (Affandi & Diamahesa, 2023; Affandi & Diniariwisan, 2024).

RESULTS AND DISCUSSION

Pathogenicity of *Vibrio harveyi*

Pathogenic *Vibrio harveyi* are known to excrete lytic enzymes including haemolysins, proteases (caseinase and gelatinase) and lipase, which cause damage to host tissues, hence allowing them to obtain nutrients (Nurhafizah et al., 2021). *Vibrio harveyi* is a member of the family Vibrionaceae within the class Gammaproteobacteria and is widely recognized as a major bacterial pathogen affecting marine fish and invertebrates, particularly penaeid shrimp, in aquaculture systems. Infected fish can display diverse clinical symptoms, such as eye damage or blindness, gastroenteritis, muscle necrosis, skin ulceration, and tail rot. In shrimp, *V. harveyi* is known as the causative agent of luminous vibriosis, characterized by bioluminescent animals, as well as Bolitas negricans, a condition marked by spherical sloughed tissues in the digestive tract. The mechanisms of pathogenicity

differ between hosts. In shrimp, virulence is associated with lipopolysaccharide endotoxins, extracellular proteases, and interactions with bacteriophages. In fish, pathogenicity involves extracellular hemolysins functioning as phospholipase B, which induce apoptosis through caspase activation. Additionally, *V. harveyi* can persist in a viable but nonculturable state, whose resuscitation may contribute to disease outbreaks in aquaculture (X.-H. Zhang et al., 2020).

Pathogenic bacteria can enter the fish body through various routes and cause infection (Verma et al., 2021). Most microorganisms naturally enter hosts through feeding, allowing ingested pathogens to trigger infection or tissue damage, either directly or by altering other microbial populations. In *Litopenaeus vannamei*, the gastrointestinal tract maintains a balanced microbiota. Disruption of this balance can impair immune function, disturb hemolymph microbial communities, and increase shrimp susceptibility to disease (Gan et al., 2022). *Vibrio* species can invade fish through the skin, gills, or digestive tract. Once inside, they cause infection and tissue damage via various virulence factors. These include extracellular products, toxins, and membrane proteins that promote adhesion, colonization, invasion, and inflammatory responses (Hartawan et al., 2023). *V. harveyi* can pass through the digestive tract of marine fish, withstand digestion, multiply in fecal matter, and subsequently spread into the surrounding seawater (Chrisolite et al., 2008). *V. harveyi* can infect shrimp through oral ingestion, leading to high mortality in aquaculture. To establish infection, the bacterium must successfully compete with the resident intestinal microbiota of the host shrimp (Rungrassamee et al., 2016).

Another potential virulence factors of *V. harveyi*, such as biofilm formation is play important roles in different phases of the disease process (Y. Zhang et al., 2021). Biofilms are defined as multi-layered microbial communities encased in protective matrices that exhibit strong resistance to many antibiotics (Faleye et al., 2021). In *V. harveyi*, the synthesis of virulence factors including caseinase, gelatinase, chitinase, siderophores, motility traits, and biofilm formation is regulated by an AHL-dependent quorum sensing system (Santhakumari et al., 2015). Quorum sensing (QS) is a mechanism through which bacteria regulate collective behaviors by producing, sensing, and responding to small signaling molecules known as autoinducers (Henares et al., 2013).

V. harveyi is a major infectious pathogen of cultured marine fish, shellfish, and crustaceans, causing high mortality and significant losses in global seafood industries. Various virulence factors have been identified in this species that contribute to its pathogenicity. These include quorum sensing-regulated biofilm formation, exopolysaccharide-mediated adhesion, colonization, and immune evasion (Kannan et al., 2019).

Quorum Sensing Mechanism of *Vibrio harveyi*

Intercellular communication through quorum signaling is now a major focus in the study of bacterial sociobiology. Quorum sensing (QS) systems play a role in regulating a variety of cellular activities. Some of the functions controlled by QS include bioluminescence, motility, production of extracellular virulence factors, and biofilm formation (Talagrand-Reboul et al., 2017).

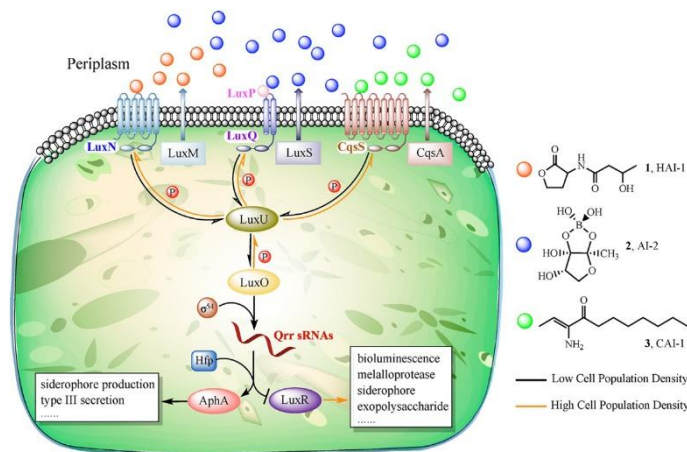


Figure 1. Quorum Sensing Mechanism of *Vibrio harveyi* (Chen et al., 2020)

Vibrio harveyi regulates collective behaviors through a sophisticated quorum sensing (QS) network composed of three interconnected signaling pathways: LuxM/LuxN, LuxS/LuxPQ, and CqsA/CqsS (Figure 1). Together, these systems coordinate key physiological processes, including bioluminescence, metalloproteinase activity, siderophore and exopolysaccharide production, as well as the repression of type III secretion, in response to changes in cell density. The LuxM/LuxN pathway relies on the autoinducer HAI-1, an acyl-homoserine lactone synthesized by LuxM and detected by the sensor kinase LuxN. The LuxS/LuxPQ pathway uses AI-2, a boron-containing signaling molecule generated by LuxS and recognized by LuxPQ; AI-2 is widely conserved and functions as a universal QS signal among diverse bacteria. The third pathway, CqsA/CqsS, involves CAI-1, a long-chain amino ketone produced by CqsA and sensed by CqsS. Although these receptors lack DNA-binding domains, they possess conserved histidine kinase regions that relay signals downstream. Autoinducer production varies with growth phase: HAI-1 and CAI-1 accumulate during late exponential growth, whereas AI-2 appears earlier. At low cell density, receptors act as kinases, triggering phosphorylation cascades via LuxU and LuxO, which induce Qrr sRNAs that activate AphA and repress LuxR. Conversely, at high cell density, receptors function as phosphatases, leading to LuxO inactivation, suppression of Qrr sRNAs, and strong induction of LuxR-dependent population-level behaviors (Chen et al., 2020).

Previous studies show that quorum sensing regulates virulence factor expression, biofilm development, and flagellar motility. It also controls bioluminescence, a vital function of *Vibrio harveyi*, and supports symbiotic interactions. Overall, quorum sensing shapes its lifestyle and phenotypic characteristics (Chen et al., 2020).

Biofilm Formation Mechanism of *Vibrio harveyi*

Biofilm formation by *Vibrio* bacteria goes through five main stages (Haque et al., 2023). (1) Reversible adhesion: initial attachment to surface using pili and flagella. (2) Irreversible adhesion: secretion of extracellular polymeric substances (EPS) and subsequent irreversible attachment. (3) Single layer formation: production of extracellular matrix (ECM) consisting of carbohydrate, DNA, fibrous proteins etc. (4) Microcolony formation and proliferation: generation of a

biocide/antibiotic resistant vibrio due to selection pressure. (5) Maturation: biocide/antibiotic resistant vibrio population and mature biofilm.

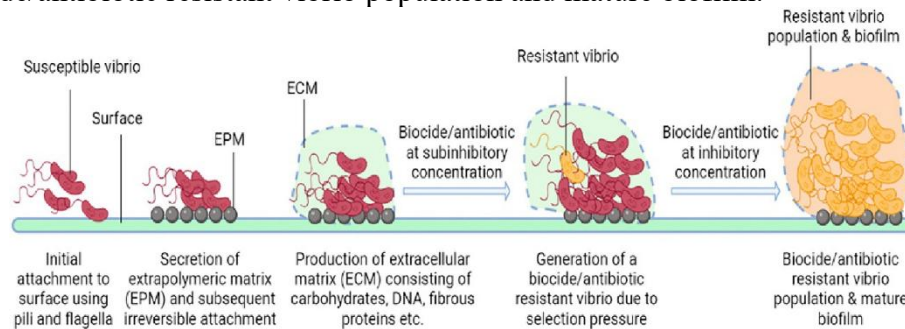


Figure 2. Biofilm Formation Mechanism of *Vibrio harveyi* (Haque et al., 2023)

Quorum sensing (QS) is a bacterial communication system that is essential for controlling and regulating biofilm formation (Che et al., 2024; Markowska et al., 2024; Samrot et al., 2021). This system relies on the presence of signaling molecules that are activated according to bacterial population density. QS also controls various other biological functions, including the secretion of virulence factors, bacterial movement (such as swimming or swarming), and light production (bioluminescence) (M. Zhang et al., 2022).

Quorum sensing (QS) is an intercellular signaling system that plays a vital role in biofilm development by controlling gene expression through small signaling molecules known as autoinducers. Biofilm formation and structural stability depend strongly on this mechanism, which allows bacterial populations to monitor and regulate their density. When autoinducer concentrations reach a critical threshold, they bind to specific receptors and trigger genes responsible for maintaining biofilm architecture and coordinating virulence. Consequently, the persistence and survival of the biofilm community rely heavily on effective QS regulation (Vetrivel et al., 2021).

Intervention Strategy Against *Vibrio harveyi* Biofilm

Inhibition of bacterial biofilm formation can be done through the following approaches (Ghosh et al., 2020):

- a. Preventing the initial adhesion of bacteria to the surface, namely by modifying the surface using coatings such as antibiotics, metal ions, or synthetic compounds to prevent bacterial attachment.
- b. Disrupting the quorum sensing system, by blocking communication between bacteria through inhibition of autoinducer molecules using bioactive compounds that function as quorum sensing inhibitors (QSI), thereby reducing biofilm formation and virulence.
- c. Modulation through second nucleotide signaling molecules, such as (p)ppGpp or c-di-GMP, which can suppress biofilm formation and increase bacterial sensitivity to antibiotics.
- d. Inhibiting the biofilm maturation process chemically, for example by using compounds such as synthetic glycolipids or deacylated lipopolysaccharides to disrupt the cell wall structure and prevent biofilm strengthening.
- e. Disrupting mature biofilms, through enzymatic strategies such as the use of Dispersin B or DNase which are capable of destroying biofilm matrix components such as exopolysaccharides and extracellular DNA.

Interventions against bacterial biofilms can be carried out using two main approaches, namely preventing biofilm maturation and destroying biofilms that have already formed (Kang et al., 2023). Maturation prevention includes:

- Inhibiting bacterial adhesion, namely by modifying the surface using antibacterial materials or antifouling structures to prevent bacteria from adhering in the early stages of biofilm formation.
- Inhibits extracellular matrix (ECM) formation, by targeting components such as exopolysaccharides and eDNA to increase biofilm sensitivity to antibiotics and reduce bacterial adhesion.
- Disrupting communication between bacteria, through secondary metabolite compounds that inhibit the quorum sensing system, thereby preventing coordination of biofilm formation.
- Interfering with bacterial metabolism, by modifying metabolic pathways such as purine biosynthesis to inhibit biofilm growth based on specific metabolic needs.

Meanwhile, the destruction of mature biofilms can be done through:

- Chemical methods, such as the use of antibiotics, antimicrobial peptides, DNase enzymes, or the formation of reactive oxygen species (ROS) that damage the biofilm structure through photodynamic therapy or catalytic enzymes.
- Physical methods, including photothermal therapy (PTT) which produces local heat to destroy biofilms without damaging healthy tissue, and the use of sharp or magnetic nanomotors that penetrate biofilms and produce ROS.
- Biological methods, namely using specific bacteriophages that attack bacteria in biofilms and release matrix-destroying enzymes, or utilizing probiotics that compete with pathogenic bacteria to reduce colonization and prevent the formation of new biofilms.

Mechanism of Alkaloids in Inhibiting *Vibrio harveyi* Biofilm

Alkaloids are heterocyclic, nitrogen-containing compounds that occur naturally in a wide range of plant and animal sources. Numerous alkaloids have been identified, and many display broad-spectrum antibacterial properties while causing relatively few side effects. These compounds exert their antimicrobial effects through multiple mechanisms, including interference with bacterial cell wall synthesis, disruption of cell membrane integrity, inhibition of metabolic pathways, and suppression of nucleic acid and protein synthesis. Alkaloids are also effective in preventing biofilm development. Their antibiofilm activity is primarily associated with the downregulation of quorum sensing (QS) regulatory genes, such as *agrA*, and the inhibition of other QS-related genes, including *luxS*, *pfS*, *sdiA*, *hflX*, *motA*, and *fliA*, particularly in antibiotic-resistant bacteria. Furthermore, alkaloids can decrease the activity of the QS signaling molecule autoinducer-2 (AI-2), which further impairs bacterial communication and biofilm formation. Through these combined actions, alkaloids represent promising agents for controlling bacterial virulence and biofilm-associated infections (M. Zhang et al., 2022).

Alkaloids have a strong effect on bacterial biofilms by inhibiting adhesion processes due to impaired cell motility. Furthermore, alkaloids have the potential to reduce the amount of exopolysaccharides (EPS), a key component of biofilm structure. Observations indicate that alkaloids can inhibit biofilm development from the early stages by targeting adhesin proteins, disrupting established biofilms, and inhibiting EPS production (Mishra et al., 2020).

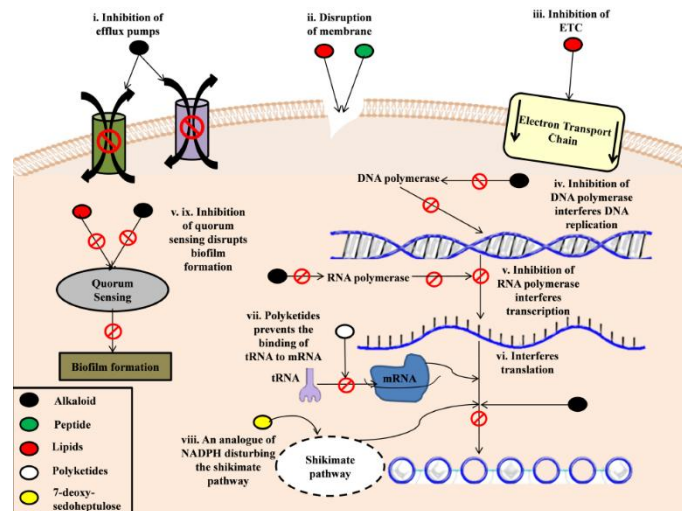


Figure 3. Mechanism of Alkaloids in Inhibiting *Vibrio* Biofilm (Kar et al., 2022)

The mechanism of alkaloids in inhibiting *Vibrio* bacterial biofilms is shown in Figure 3, with several ways of working as follows: (i) alkaloids inhibit the activity of bacterial efflux pumps; (ii) peptides and lipids disrupt the structure of the cell membrane, thus causing leakage of cell contents; (iii) lipids also disrupt the electron transport chain, which has an impact on disrupting cell function; (iv) alkaloids inhibit the work of DNA polymerase, disrupting the DNA replication process; (v) inhibit RNA polymerase, thus disrupting the transcription process; (vi) alkaloids also inhibit the translation process; (vii) polyketides prevent the binding of tRNA to mRNA, which stops translation; (viii) the compound 7-deoxy-sedoheptulose acts as a NADPH analog in the Shikimate pathway and inhibits translation; and (ix) inhibition of the quorum sensing system by alkaloids also disrupts the biofilm formation process (Kar et al., 2022).

Mechanism of Octopus Ink Extract as Quorum Quenching in *Vibrio harveyi* Biofilm Formation

Cephalopod ink, such as squid, contains betaine, cinnamic acid, and choline. Betaine and choline are classified as alkaloids, while cinnamic acid is a carboxylic acid. These three compounds are known to possess various biological activities, such as antibacterial, antioxidant, antiviral, antifungal, and other properties (Affandi et al., 2019). Octopus ink contains various key compounds, including alkaloids, melanin, amino acids, and carboxylic acids. These compounds give octopus ink a variety of biological functions, such as antimicrobial, antioxidant, antibacterial, antiretroviral, anticancer, antiulcerogenic, anti-inflammatory, antiviral, antifungal, and antiproliferative activities (Affandi et al., 2023). Octopus ink extract has strong antioxidant activity with an IC_{50} value of 94.4661 ppm. Octopus ink extract also contains alkaloids, saponins, phenols, and steroids, according to phytochemical and FTIR tests. These compounds have numerous roles, including immunostimulant, antibacterial, antiviral, antiparasitic, antifungal, antioxidant, and more (Affandi, Azhar, et al., 2025). Octopus ink is known to contain alkaloid compounds. Alkaloids play a role in damaging bacterial cell membranes, which contributes to enhancing the non-specific immune response in fish and inhibiting bacterial growth. Furthermore, alkaloids also have various

properties, one of which is antimicrobial activity (Affandi, Scabra, et al., 2025; Saputra et al., 2025).

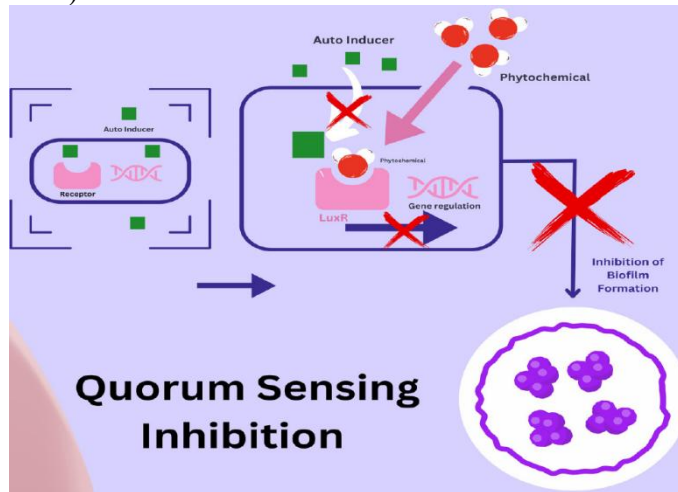


Figure 4. Mechanism of Octopus Ink Extract as Quorum Quenching in *Vibrio* Biofilm Formation (Arya & Usha, 2024)

The mechanism of quorum sensing inhibition by octopus ink extract containing alkaloids is carried out by blocking autoinducer molecules such as AHL, general autoinducers, and type 2 autoinducers. Communication between bacterial cells occurs through the synthesis and release of autoinducers which then bind to receptor proteins. Octopus ink extract works by competitively binding autoinducers to their receptors, thereby inhibiting natural binding and causing a decrease in the expression of target genes controlled by the quorum sensing system (Arya & Usha, 2024). The quorum sensing inhibition process is schematically presented in Figure 4.

CLOSING

Conclusion

Octopus ink extract shows promise as a quorum quenching agent for inhibiting *Vibrio harveyi* biofilm formation in aquaculture. Bioactive compounds, particularly alkaloids, can disrupt bacterial cell-to-cell communication and reduce virulence factor expression. This approach provides an innovative and eco-friendly strategy to lower infection risks in cultured fish while limiting antibiotic resistance. Additionally, octopus ink extract has potential application as an immunostimulant in aquaculture systems.

Suggestion

Suggestions for further research are needed to identify specific active compounds in octopus ink that play a role in quorum quenching activity. Field application trials are also needed to assess the effectiveness and safety of octopus ink extract on a cultivation scale. Furthermore, developing product formulations based on octopus ink extract as antipathogenic and immunostimulant agents could be a strategic step in supporting sustainable aquaculture.

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REFERENCES

- Affandi, R. I., Azhar, F., Scabra, A. R., Muahiddah, N., Dwiyantri, S., Saputra, M. A., Diniariwisan, D., Rahmadani, T. B. C., Asri, Y., & Setyono, B. D. H. (2025). Bioactive compounds identification of octopus (*Octopus sp.*) ink extract as candidates for aquaculture immunostimulants: a preliminary study. *AAFL Bioflux*, *18*(1), 500–511.
- Affandi, R. I., & Diamahesa, W. A. (2023). POTENSI TANAMAN BROLOWALI (*Tinospora cordifolia*) SEBAGAI IMUNOSTIMULAN PADA IKAN. *LEMURU: Jurnal Ilmu Perikanan Dan Kelautan*, *5*(3), 453–463. <https://doi.org/10.36526/jl.v5i3.2967>
- Affandi, R. I., & Diniariwisan, D. (2024). POTENTIAL USE OF FENNEL (*FOENICULUM VULGARE*) AS FISH IMMUNOSTIMULANT: ARTICLE REVIEW. *Jurnal Perikanan Unram*, *14*(2), 657–672. <https://doi.org/10.29303/jp.v14i2.816>
- Affandi, R. I., Fadjar, M., & Ekawati, A. W. (2019). Active Compounds on Squid (*Loligo sp.*) Ink Extract Powder as Immunostimulant Candidate to Against Shrimp Disease. *Research Journal of Life Science*, *6*(3), 150–161. <https://doi.org/10.21776/ub.rjls.2019.006.03.1>
- Affandi, R. I., Fadjar, M., Muahiddah, N., & Setyono, B. D. H. (2023). POTENSI TINTA GURITA (*OCTOPUS SP.*) SEBAGAI IMUNOSTIMULAN PADA UDANG VANAME (*LITOPENAEUS VANNAMEI*). *GANEC SWARA*, *17*(1), 318–325. <https://doi.org/10.35327/gara.v17i1.403>
- Affandi, R. I., Scabra, A. R., & Saputra, M. A. (2025). Effect of Octopus (*Octopus sp.*) Ink Extract on Haematological of the Catfish (*Clarias sp.*) Infected with *Aeromonas hydrophila*. *Egyptian Journal of Aquatic Biology & Fisheries Zoology*, *29*(3), 3375–3391.
- Affandi, R. I., & Setyono, B. D. H. (2023). Potensi Tanaman Sambiloto (*Andrographis paniculata*) Sebagai Imunostimulan Pada Ikan. *JURNAL VOKASI ILMU-ILMU PERIKANAN (JVIP)*, *4*(1), 131–141. <https://doi.org/10.35726/jvip.v4i1.7109>
- Affandi, R. I., & Setyono, B. D. H. (2024a). Potensi Tanaman Lempuyang (*Zingiber zerumbet*) Sebagai Imunostimulan Pada Ikan. *JURNAL VOKASI ILMU-ILMU PERIKANAN (JVIP)*, *4*(2), 182–193. <https://doi.org/10.35726/jvip.v4i2.7246>
- Affandi, R. I., & Setyono, B. D. H. (2024b). Potensi Tanaman Sambiloto (*Andrographis paniculata*) Sebagai Imunostimulan Pada Udang. *JURNAL VOKASI ILMU-ILMU PERIKANAN (JVIP)*, *5*(1), 09–21. <https://doi.org/10.35726/jvip.v5i1.7333>
- Affandi, R. I., & Setyono, B. D. H. (2025a). POTENSI EKSTRAK TINTA GURITA SEBAGAI QUORUM QUENCHING UNTUK MENCEGAH

- PEMBENTUKAN BIOFILM *Edwardsiella tarda* PADA AKUAKULTUR (REVIEW). *Jurnal Perikanan Pantura (JPP)*, 8(2), 809–823. <https://doi.org/10.30587/jpp.v8i2.10511>
- Affandi, R. I., & Setyono, B. D. H. (2025b). Potential of Octopus Ink Extract as Anti-Quorum Sensing to Prevent *Aeromonas hydrophila* Biofilm in Aquaculture. *Jurnal Biologi Tropis*, 25(1), 459–470. <https://doi.org/10.29303/jbt.v25i1.8438>
- Ambat, K. N., Abida, I. W., & Maherlina, R. (2022). ABUNDANCE OF *Vibrio* sp. IN POND WATER SAMPLE AT THE UPT LABORATORY OF FISH HEALTH AND THE ENVIRONMENT OF PASURUAN, EAST JAVA. *Juvenil*, 3(3), 66–72.
- Arya, S., & Usha, R. (2024). Bioprospecting and Exploration of Phytochemicals as Quorum Sensing Inhibitors against Cariogenic Dental Biofilm. *Journal of Pure and Applied Microbiology*, 18(1), 100–117. <https://doi.org/10.22207/JPAM.18.1.10>
- Che, J., Shi, J., Fang, C., Zeng, X., Wu, Z., Du, Q., Tu, M., & Pan, D. (2024). Elimination of Pathogen Biofilms via Postbiotics from Lactic Acid Bacteria: A Promising Method in Food and Biomedicine. *Microorganisms*, 12(4). <https://doi.org/10.3390/microorganisms12040704>
- Chen, J., Lu, Y., Ye, X., Emam, M., Zhang, H., & Wang, H. (2020). Current advances in *Vibrio harveyi* quorum sensing as drug discovery targets. *European Journal of Medicinal Chemistry*, 207, 112741. <https://doi.org/10.1016/j.ejmech.2020.112741>
- Chrisolite, B., Thiyagarajan, S., Alavandi, S. V., Abhilash, E. C., Kalaimani, N., Vijayan, K. K., & Santiago, T. C. (2008). Distribution of luminescent *Vibrio harveyi* and their bacteriophages in a commercial shrimp hatchery in South India. *Aquaculture*, 275(1–4), 13–19. <https://doi.org/10.1016/j.aquaculture.2007.12.016>
- Cushnie, T. P. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, 44(5), 377–386. <https://doi.org/10.1016/j.ijantimicag.2014.06.001>
- De Silva, L. A. D. S., & Heo, G.-J. (2023). Biofilm formation of pathogenic bacteria isolated from aquatic animals. *Archives of Microbiology*, 205(1), 36. <https://doi.org/10.1007/s00203-022-03332-8>
- Faleye, O. S., Sathiyamoorthi, E., Lee, J.-H., & Lee, J. (2021). Inhibitory Effects of Cinnamaldehyde Derivatives on Biofilm Formation and Virulence Factors in *Vibrio* Species. *Pharmaceutics*, 13(12), 2176. <https://doi.org/10.3390/pharmaceutics13122176>
- Gan, L., Zheng, J., Xu, W.-H., Lin, J., Liu, J., Zhang, Y., Wu, Z., Lv, Z., Jia, Y., Guo, Q., Chen, S., Liu, C., Defoirdt, T., Qin, Q., & Liu, Y. (2022). Deciphering the virulent *Vibrio harveyi* causing spoilage in muscle of aquatic crustacean *Litopenaeus vannamei*. *Scientific Reports*, 12(1), 16296. <https://doi.org/10.1038/s41598-022-20565-1>
- Ghosh, A., Jayaraman, N., & Chatterji, D. (2020). Small-Molecule Inhibition of Bacterial Biofilm. *ACS Omega*, 5(7), 3108–3115. <https://doi.org/10.1021/acsomega.9b03695>
- Guzman, J. P. M. D., Yatip, P., Soowannayan, C., & Maningas, M. B. B. (2022).

- Targeting quorum sensing and biofilm formation in the control of *Vibrio harveyi* infections in *Penaeus vannamei*. *Aquaculture Research*, 53(14), 4919–4930. <https://doi.org/10.1111/are.15995>
- Hamdi, S. A. H., El-Shazly, M. A. M., Fol, M. F., Mossalem, H. S., Ghareeb, M. A., Ibrahim, A. M., Aloufi, A. ., El-Ghany, M. N. A., & Korany, S. M. (2024). Octopus vulgaris ink chemical profiling and validation of its potential as antioxidant, antimicrobial, anti-cancer as well as anti-Schistosomal drug in vitro. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 76(5), 1–11. <https://doi.org/10.1590/1678-4162-13217>
- Haque, E., Hussain, J., Shankar, S., Haldar, S., & Chatterjee, S. (2023). Implication of *Vibrio* biofilms in human and seafood sector. In S. Das & N. A. Kungwani (Eds.), *Understanding Microbial Biofilms: Fundamentals to Applications* (pp. 247–260). Academic Press. <https://doi.org/10.1016/C2020-0-02522-3>
- Hartawan, G. S., Yanuhar, U., Musa, M., Baihani, A., Wahyudi, Y. A., Huda, C., & Caesar, N. R. (2023). Viral Nervous Necrosis and Vibriosis in Grouper Fish: A Case Study. *Journal of Aquaculture and Fish Health*, 12(3), 334–345. <https://doi.org/10.20473/jafh.v12i3.40256>
- Henares, B., Xu, Y., & Boon, E. (2013). A Nitric Oxide-Responsive Quorum Sensing Circuit in *Vibrio harveyi* Regulates Flagella Production and Biofilm Formation. *International Journal of Molecular Sciences*, 14(8), 16473–16484. <https://doi.org/10.3390/ijms140816473>
- Jusidin, M. R., Othman, R., Shaleh, S. R. M., Ching, F. F., Senoo, S., & Oslan, S. N. H. (2022). In Vitro Antibacterial Activity of Marine Microalgae Extract against *Vibrio harveyi*. *Applied Sciences*, 12(3), 1148. <https://doi.org/10.3390/app12031148>
- Kah Sem, N. A. D., Abd Gani, S., Chong, C. M., Natrah, I., & Shamsi, S. (2023). Management and Mitigation of Vibriosis in Aquaculture: Nanoparticles as Promising Alternatives. *International Journal of Molecular Sciences*, 24(16), 12542. <https://doi.org/10.3390/ijms241612542>
- Kang, X., Yang, X., He, Y., Guo, C., Li, Y., Ji, H., Qin, Y., & Wu, L. (2023). Strategies and materials for the prevention and treatment of biofilms. *Materials Today Bio*, 23(June). <https://doi.org/10.1016/j.mtbio.2023.100827>
- Kannan, S., Krishnamoorthy, G., Kulanthaiyesu, A., & Marudhamuthu, M. (2019). Effect of biosurfactant derived from *Vibrio natriegens* MK3 against *Vibrio harveyi* biofilm and virulence. *Journal of Basic Microbiology*, 59(9), 936–949. <https://doi.org/10.1002/jobm.201800706>
- Kar, J., Ramrao, D. P., Zomuansangi, R., Lalbiaktluangi, C., Singh, S. M., Joshi, N. C., Kumar, A., Kaushalendra, Mehta, S., Yadav, M. K., & Singh, P. K. (2022). Revisiting the role of cyanobacteria-derived metabolites as antimicrobial agent: A 21st century perspective. *Frontiers in Microbiology*, 13(November), 1–12. <https://doi.org/10.3389/fmicb.2022.1034471>
- Kumar, A., & Pasha, Y. (2020). Isolation and Characterization of Microorganisms From Squid *Loligoduvaulceliand* Generation of Microbe Free Crude Ink. *International Journal of Pharmaceutical, Chemical & Biological Sciences*, 10(3), 80–87.
- Markowska, K., Szymanek-Majchrzak, K., Pituch, H., & Majewska, A. (2024).

- Understanding Quorum-Sensing and Biofilm Forming in Anaerobic Bacterial Communities. *International Journal of Molecular Sciences*, 25(23). <https://doi.org/10.3390/ijms252312808>
- Mishra, R., Panda, A. K., De Mandal, S., Shakeel, M., Bisht, S. S., & Khan, J. (2020). Natural Anti-biofilm Agents: Strategies to Control Biofilm-Forming Pathogens. *Frontiers in Microbiology*, 11(October). <https://doi.org/10.3389/fmicb.2020.566325>
- Munir, S., Shah, A. A., Shahid, M., Manzoor, I., Aslam, B., Rasool, M. H., Saeed, M., Ayaz, S., & Khurshid, M. (2020). Quorum Sensing Interfering Strategies and Their Implications in the Management of Biofilm-Associated Bacterial Infections. *Brazilian Archives of Biology and Technology*, 63. <https://doi.org/10.1590/1678-4324-2020190555>
- Neil, B., Cheney, G. L., Rosenzweig, J. A., Sha, J., & Chopra, A. K. (2024). Antimicrobial resistance in aeromonads and new therapies targeting quorum sensing. *Applied Microbiology and Biotechnology*, 108(1), 205. <https://doi.org/10.1007/s00253-024-13055-z>
- Nurhafizah, W. W. I., Lee, K. L., Laith A., A. R., Nadirah, M., Danish-Daniel, M., Zainathan, S. C., & Najiah, M. (2021). Virulence properties and pathogenicity of multidrug-resistant *Vibrio harveyi* associated with luminescent vibriosis in Pacific white shrimp, *Penaeus vannamei*. *Journal of Invertebrate Pathology*, 186, 107594. <https://doi.org/10.1016/j.jip.2021.107594>
- Rungrassamee, W., Klanchui, A., Maibunkaew, S., & Karoonuthaisiri, N. (2016). Bacterial dynamics in intestines of the black tiger shrimp and the Pacific white shrimp during *Vibrio harveyi* exposure. *Journal of Invertebrate Pathology*, 133, 12–19. <https://doi.org/10.1016/j.jip.2015.11.004>
- Samreen, Qais, F. A., & Ahmad, I. (2022). Anti-quorum sensing and biofilm inhibitory effect of some medicinal plants against gram-negative bacterial pathogens: in vitro and in silico investigations. *Heliyon*, 8(10), e11113. <https://doi.org/10.1016/j.heliyon.2022.e11113>
- Samrot, A. V., Mohamed, A. A., Faradjeva, E., Jie, L. S., Sze, C. H., Arif, A., Sean, T. C., Michael, E. N., Mun, C. Y., Qi, N. X., Mok, P. L., & Kumar, S. S. (2021). Mechanisms and impact of biofilms and targeting of biofilms using bioactive compounds—a review. *Medicina (Lithuania)*, 57(8), 1–28. <https://doi.org/10.3390/medicina57080839>
- Santhakumari, S., Kannappan, A., Pandian, S. K., Thajuddin, N., Rajendran, R. B., & Ravi, A. V. (2015). Inhibitory effect of marine cyanobacterial extract on biofilm formation and virulence factor production of bacterial pathogens causing vibriosis in aquaculture. *Journal of Applied Phycology*, 28(1), 313–324. <https://doi.org/10.1007/s10811-015-0554-0>
- Santos, R. A., Monteiro, M., Rangel, F., Jerusik, R., Saavedra, M. J., Carvalho, A. P., Oliva-Teles, A., & Serra, C. R. (2021). *Bacillus* spp. Inhibit *Edwardsiella tarda* Quorum-Sensing and Fish Infection. *Marine Drugs*, 19(11), 602. <https://doi.org/10.3390/md19110602>
- Saputra, M. A., Scabra, A. R., & Affandi, R. I. (2025). Effectiveness of Octopus (*Octopus* sp.) Ink Extract on the Growth of Catfish (*Clarias* sp.) Infected with *Aeromonas hydrophila*. *Journal of Fish Health*, 5(1), 1–14. <https://doi.org/10.29303/jfh.v5i1.6102>

- Schrama, D., Ribeiro, M. C., Sousa, R., Livramento, M., Gamboa, M., Ovelheiro, A., Candeias-Mendes, A., Silva, I. A. L., Pousão-Ferreira, P., Lourenço-Marques, C., & Soares, F. (2025). Infection by *Vibrio harveyi* in Broodstock Senegalese sole (*Solea senegalensis*) in South Portugal. *Aquaculture Reports*, 45(August). <https://doi.org/10.1016/j.aqrep.2025.103097>
- Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Aalinkel, R. (2023). Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. *Microorganisms*, 11(6), 1614. <https://doi.org/10.3390/microorganisms11061614>
- Ta, C., & Arnason, J. (2015). Mini Review of Phytochemicals and Plant Taxa with Activity as Microbial Biofilm and Quorum Sensing Inhibitors. *Molecules*, 21(1), 29. <https://doi.org/10.3390/molecules21010029>
- Talagrand-Reboul, E., Jumas-Bilak, E., & Lamy, B. (2017). The social life of *Aeromonas* through biofilm and quorum sensing systems. *Frontiers in Microbiology*, 8(JAN). <https://doi.org/10.3389/fmicb.2017.00037>
- Vandeputte, M., Kashem, M. A., Bossier, P., & Vanrompay, D. (2024). *Vibrio* pathogens and their toxins in aquaculture: A comprehensive review. *Reviews in Aquaculture*, 16(4), 1858–1878. <https://doi.org/10.1111/raq.12926>
- Verma, V. K., Prakash, O., Kumar, R. S. R., Rani, K. V., & Sehgal, N. (2021). Water hyacinth (*Eichhornia crassipes*) leaves enhances disease resistance in *Channa punctata* from *Vibrio harveyi* infection. *The Journal of Basic and Applied Zoology*, 82(1), 6. <https://doi.org/10.1186/s41936-020-00203-w>
- Vetrivel, A., Ramasamy, M., Vetrivel, P., Natchimuthu, S., Arunachalam, S., Kim, G.-S., & Murugesan, R. (2021). *Pseudomonas aeruginosa* Biofilm Formation and Its Control. *Biologics*, 1(3), 312–336. <https://doi.org/10.3390/biologics1030019>
- Zhang, M., Han, W., Gu, J., Qiu, C., Jiang, Q., Dong, J., Lei, L., & Li, F. (2022). Recent advances on the regulation of bacterial biofilm formation by herbal medicines. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.1039297>
- Zhang, X.-H., He, X., & Austin, B. (2020). *Vibrio harveyi*: a serious pathogen of fish and invertebrates in mariculture. *Marine Life Science & Technology*, 2(3), 231–245. <https://doi.org/10.1007/s42995-020-00037-z>
- Zhang, Y., Deng, Y., Feng, J., Hu, J., Chen, H., Guo, Z., Gao, R., & Su, Y. (2021). ToxR modulates biofilm formation in fish pathogen *Vibrio harveyi*. *Letters in Applied Microbiology*, 74(2), 288–299. <https://doi.org/10.1111/lam.13606>
- Zhao, J., Chen, M., Quan, C. S., & Fan, S. D. (2015). Mechanisms of quorum sensing and strategies for quorum sensing disruption in aquaculture pathogens. *Journal of Fish Diseases*, 38(9), 771–786. <https://doi.org/10.1111/jfd.12299>