

OCTOPUS INK EXTRACT AS A POTENTIAL QUORUM- QUENCHING CANDIDATE FOR *Staphylococcus aureus* BIOFILM IN AQUACULTURE (REVIEW)

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ABSTRACT

Staphylococcus aureus is a Gram-positive opportunistic pathogen detected in seafood, cultured aquatic animals, aquaculture water, and fish-handling environments. Its persistence is supported by multiple virulence determinants and biofilm formation, which enhances tolerance to environmental stress, host defenses, disinfectants, and antimicrobial treatment. The accessory gene regulator (*agr*) quorum-sensing system coordinates density-dependent expression of several virulence-associated traits, making bacterial communication a potential antivirulence target. Octopus ink contains alkaloids, phenolic compounds, steroids, melanin-associated components, peptides, and other bioactive constituents with reported antimicrobial, antioxidant, and immunomodulatory activities. This review evaluated the potential of octopus ink extract to interfere with quorum sensing and biofilm development in *S. aureus*. A systematic literature review and content analysis were conducted using 67 scientific articles, primarily from Scopus-indexed Q1-Q4 journals published during 2017-2026. Importantly, evidence specifically supporting antibiofilm and quorum-sensing interference in *S. aureus* derives mainly from studies of alkaloids and related bioactive compounds, rather than from direct experimental testing of octopus ink extract against *S. aureus*. These studies indicate possible mechanisms involving reduced adhesion, interference with *ica*-associated matrix production, modulation of Agr signaling, membrane disruption, and metabolic inhibition. However, Agr inhibition does not consistently suppress biofilm formation because it may also reduce dispersal in some strains. Therefore, octopus ink extract should be regarded as a promising multi-target quorum-quenching and antibiofilm candidate, not a confirmed agent against *S. aureus*. Direct mechanistic studies are required to validate its efficacy and safety in aquaculture.

Keywords: Alkaloids, Biofilm, Octopus Ink Extract, Quorum Quenching, *Staphylococcus aureus*

ABSTRAK

Staphylococcus aureus merupakan bakteri Gram-positif oportunistik yang ditemukan pada produk perikanan, organisme akuatik budidaya, air budidaya, dan lingkungan penanganan ikan. Persistensinya didukung oleh berbagai faktor

virulensi dan pembentukan biofilm yang meningkatkan toleransi terhadap tekanan lingkungan, pertahanan inang, disinfektan, dan perlakuan antimikroba. Sistem quorum sensing accessory gene regulator (agr) mengatur ekspresi berbagai faktor virulensi secara bergantung pada kepadatan sel, sehingga komunikasi bakteri menjadi target potensial untuk strategi antivirulensi. Tinta gurita mengandung alkaloid, senyawa fenolik, steroid, komponen terkait melanin, peptida, dan senyawa bioaktif lain yang memiliki aktivitas antimikroba, antioksidan, dan imunomodulator. Kajian ini mengevaluasi potensi ekstrak tinta gurita dalam mengganggu quorum sensing dan perkembangan biofilm *S. aureus*. Tinjauan literatur sistematis dan analisis isi dilakukan terhadap 67 artikel ilmiah, terutama dari jurnal terindeks Scopus Q1-Q4 yang diterbitkan pada 2017-2026. Penting untuk ditegaskan bahwa bukti yang mendukung aktivitas antibiofilm dan gangguan quorum sensing pada *S. aureus* terutama berasal dari penelitian mengenai alkaloid dan senyawa bioaktif terkait, bukan dari pengujian langsung ekstrak tinta gurita terhadap *S. aureus*. Studi tersebut menunjukkan mekanisme potensial melalui penurunan adhesi, gangguan produksi matriks terkait ica, modulasi sistem Agr, kerusakan membran, dan penghambatan metabolisme. Namun, penghambatan Agr tidak selalu menekan biofilm karena pada beberapa strain juga dapat mengurangi proses dispersi. Oleh karena itu, ekstrak tinta gurita lebih tepat diposisikan sebagai kandidat quorum-quenching dan antibiofilm multitarget, bukan sebagai agen yang telah terkonfirmasi terhadap *S. aureus*. Penelitian mekanistik langsung masih diperlukan untuk memvalidasi efektivitas dan keamanannya dalam akuakultur.

Kata Kunci: Alkaloid, Biofilm, Ekstrak Tinta Gurita, Quorum Quenching, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is a non-motile, catalase-positive, Gram-positive coccus that can persist as a commensal organism but also acts as an important opportunistic pathogen. Its relevance to aquaculture is increasingly viewed through a One Health perspective because *S. aureus* and methicillin-resistant *S. aureus* (MRSA) have been recovered from fish, shrimp farms, seafood products, aquaculture water, and fish handlers. Molecular evidence suggests that *S. aureus* detected in fish may often originate from repeated human or environmental spillover rather than from a long-established fish-adapted lineage. Even so, aquaculture production and post-harvest handling can create interfaces where contaminated water, equipment, workers, and aquatic products allow the organism to persist and circulate (Derke *et al.*, 2025; El-Ashker *et al.*, 2026; Matuszewska *et al.*, 2023; Murugadas *et al.*, 2017; Okafor *et al.*, 2025; Rajan *et al.*, 2021; Sivaraman *et al.*, 2021, 2022). These findings justify attention to *S. aureus* not only as a food-safety organism but also as a biofilm-forming contaminant that may persist in aquaculture-associated environments.

The pathogenicity of *S. aureus* depends on a broad repertoire of adhesins, immune-evasion proteins, enzymes, toxins, and regulatory systems. Surface proteins such as clumping factors and fibronectin-binding proteins promote attachment to host or abiotic surfaces, while secreted cytotoxins, leukocidins,

hemolysins, proteases, and phenol-soluble modulins contribute to tissue damage and dissemination. The organism can also acquire antimicrobial resistance determinants, most notably *mecA*-associated methicillin resistance, which further complicates disease control and increases the public-health importance of contaminated aquatic products (Ahmad-Mansour *et al.*, 2021; Cheung *et al.*, 2021; Foster, 2019; Lakhundi & Zhang, 2018; Touaitia *et al.*, 2025). In intensive aquaculture, where high organic loads and repeated contact with surfaces can support microbial persistence, biofilm-associated survival is particularly relevant to sanitation and pathogen management.

Biofilm formation enables *S. aureus* cells to shift from a planktonic lifestyle into structured communities embedded in an extracellular matrix containing polysaccharides, proteins, extracellular DNA, and other components. The process involves attachment, accumulation, maturation, and dispersal, and it is regulated through interacting networks that include the *icaADBC* locus, *SarA*, *SigB*, *Agr*, and *LuxS*-related signaling. Biofilm cells often show increased tolerance to antibiotics, disinfectants, oxidative stress, and immune clearance because the matrix restricts penetration and the biofilm contains slow-growing or physiologically heterogeneous subpopulations (Moormeier & Bayles, 2017; Peng *et al.*, 2023; Schilcher & Horswill, 2020; Sharma *et al.*, 2023). These characteristics make biofilm control an important preventive target in hatcheries, tanks, pipes, processing surfaces, and other aquaculture-related facilities.

Quorum sensing contributes to the regulation of *S. aureus* population behavior, especially through the accessory gene regulator (*agr*) system. *AgrD* is processed and exported as an autoinducing peptide (AIP), which activates the membrane sensor *AgrC* and the response regulator *AgrA*, leading to positive feedback through the P2 promoter and expression of *RNAIII* through the P3 promoter. This system strongly promotes many secreted virulence factors and is also connected to biofilm maturation and dispersal. Consequently, quorum-quenching strategies that interfere with AIP production, AIP-*AgrC* recognition, *AgrC* signaling, *AgrA* activity, or *RNAIII* have been proposed as antivirulence approaches (Chen *et al.*, 2026; Palit *et al.*, 2026; Tan *et al.*, 2018; Williams *et al.*, 2023). A critical point, however, is that *agr* inhibition may reduce acute virulence while simultaneously reducing biofilm dispersal, which can increase aggregation or persistent biofilm biomass in some strains (Jordan *et al.*, 2022; Wang *et al.*, 2026; Zhang *et al.*, 2022).

Natural bioactive compounds are therefore most attractive when they act on several stages of biofilm development rather than on *agr* alone. The supplied literature shows that cephalopod ink and octopus ink contain or are associated with alkaloids, melanin-related compounds, amino acids, phenolics, steroids, and bioactive peptides, and that octopus ink extract has demonstrated antioxidant, antimicrobial, growth-supporting, and immunomodulatory effects in aquaculture-related studies (Affandi *et al.*, 2019, 2023; Affandi, Azhar, *et al.*, 2025; Affandi, Scabra, *et al.*, 2025; Fadjar *et al.*, 2025; Sabathini *et al.*, 2026; Saputra *et al.*, 2025). Previous reviews have also proposed octopus ink as an anti-quorum-sensing or quorum-quenching material against *Aeromonas hydrophila*, *Edwardsiella tarda*, *Vibrio harveyi*, and *Streptococcus agalactiae* (Affandi, Rahmadani, *et al.*, 2026; Affandi, Setyono, *et al.*, 2026; Affandi & Setyono, 2025b, 2025a). Building on this evidence, the objective of this review was to evaluate the potential and mechanistic

plausibility of octopus ink extract as a multi-target quorum-quenching and antibiofilm candidate against *S. aureus* in aquaculture.

RESEARCH METHODS

Place and Time

This literature review was prepared in August 2026 in Mataram, West Nusa Tenggara, Indonesia. The review followed the structured literature-review approach used in previous aquaculture reviews while adding explicit eligibility criteria and a defined evidence set (Affandi & Diamahesa, 2023; Affandi & Diniariwisan, 2024; Affandi & Muahiddah, 2025).

Tools and materials

The tools used in the review consisted of a laptop computer, charger, mouse, reference-management software, spreadsheet software for article screening, and digital scientific articles. The literature set contained 67 articles were selected from international Scopus-indexed Q1-Q4 journals published within the last 10 years, with priority given to 2017-2026 evidence relevant to *S. aureus*, biofilms, quorum sensing, alkaloids, cephalopod ink, and aquaculture.

Research Procedures

The review used a structured systematic literature review. Searches were conducted through Scopus and were cross-checked using PubMed and publisher platforms including ScienceDirect, SpringerLink, Wiley Online Library, Taylor & Francis, Frontiers, MDPI, and ASM. Google Scholar was used only as a supplementary discovery tool. Search strings combined the terms "*Staphylococcus aureus*", "MRSA", "aquaculture", "fish", "seafood", "biofilm", "quorum sensing", "agr", "AIP", "quorum quenching", "alkaloid", "piperine", "berberine", "chelerythrine", "harmine", "sanguinarine", "octopus ink", and "cephalopod ink". Inclusion criteria were peer-reviewed research or review articles directly relevant to one or more review themes, international articles indexed in Scopus for the additional evidence set, publication mainly between 2017 and August 2026, availability of sufficient methodological or mechanistic information, and relevance to aquaculture, *S. aureus* virulence, biofilm biology, QS, natural antibiofilm agents, or cephalopod ink. Duplicate records, non-scholarly sources, articles without sufficient relevance, and retracted publications were excluded. The literature was then organized into six analytical themes: *S. aureus* pathogenesis, Agr quorum sensing, biofilm development, antibiofilm intervention, alkaloid mechanisms, and the proposed action of octopus ink extract. This process follows the objective, systematic, analytical, and critical workflow applied in the supplied review articles (Affandi, Rahmadani, *et al.*, 2026; Affandi, Setyono, *et al.*, 2026; Affandi & Setyono, 2023, 2024a, 2024b, 2025b, 2025a).

Data Analysis

Data were analyzed using qualitative content analysis. Each article was evaluated for relevance, study design, organism or strain, intervention, molecular target, biofilm stage, and major conclusion. Findings were compared across studies to identify consistent mechanisms and contradictions. Particular attention was given to distinguishing direct evidence from mechanistic inference. Because no article in the final evidence set directly demonstrated quorum quenching by octopus ink extract against *S. aureus*, the final octopus-ink mechanism is presented as an

evidence-based hypothesis rather than as a confirmed mechanism. This analytical approach is consistent with the content-analysis framework used in earlier aquaculture literature reviews (Affandi & Diamahesa, 2023; Affandi & Diniariwisan, 2024).

RESULTS AND DISCUSSION

Pathogenicity of *Staphylococcus aureus*

S. aureus establishes infection through a sequence of colonization, adhesion, immune evasion, tissue damage, and dissemination. Its cell-wall-anchored surface proteins enable attachment to host extracellular matrix components and abiotic materials. MSCRAMMs such as clumping factors and fibronectin-binding proteins strengthen adhesion, while protein A and capsule-associated traits can interfere with effective host recognition. Following attachment, the bacterium can produce extracellular enzymes and toxins that facilitate nutrient acquisition, tissue injury, and spread to new sites (Cheung *et al.*, 2021; Foster, 2019; Touaitia *et al.*, 2025). In aquaculture-related environments, the ability to adhere to organic residues, tank walls, pipes, nets, and processing surfaces can create a persistent reservoir even when overt infection of aquatic animals is not the primary ecological state.

The toxin repertoire of *S. aureus* includes alpha-toxin, other hemolysins, leukocidins, enterotoxins, exfoliative toxins, and superantigenic proteins. These molecules can damage cellular membranes, disrupt epithelial or immune barriers, provoke inflammation, and contribute to food poisoning or invasive infection. Production of many secreted virulence factors is coordinated by global regulators, including *agr*, which allows the bacterium to adjust its phenotype according to population density and environmental conditions (Ahmad-Mansour *et al.*, 2021; Cheung *et al.*, 2021). Antimicrobial resistance further increases the risk because MRSA isolates recovered from seafood and aquaculture settings can carry resistance determinants together with virulence genes, making contaminated products and environments relevant within a One Health framework (Lakhundi & Zhang, 2018; Okafor *et al.*, 2025; Sivaraman *et al.*, 2021).

Recent aquaculture and seafood studies demonstrate that *S. aureus* occurs at several points in the production chain. *S. aureus* and MRSA have been isolated from seafood, retail fish, shrimp farms, farm water, and people working in aquaculture. Genomic and epidemiological evidence also indicates exchange or shared lineages between humans and aquatic products, supporting the interpretation that handling, water, and production environments can act as transmission interfaces (Derke *et al.*, 2025; El-Ashker *et al.*, 2026; Matuszewska *et al.*, 2023; Murugadas *et al.*, 2017; Okafor *et al.*, 2025; Sivaraman *et al.*, 2022). Therefore, the aquaculture relevance of *S. aureus* includes both animal-health concerns and persistent contamination that can compromise product quality and food safety.

Biofilm formation links many of these pathogenic traits. Once attached, *S. aureus* can produce a matrix that protects the community and facilitates long-term persistence. Biofilm-associated cells frequently show lower susceptibility to antimicrobial exposure than planktonic cells, while the matrix can retain extracellular enzymes, DNA, and proteins that support community stability. This persistence may increase opportunities for recurrent contamination and horizontal exchange of resistance or virulence determinants. The combination of adhesion,

matrix formation, regulatory plasticity, and antimicrobial resistance makes biofilm control a rational target for preventive strategies in aquaculture facilities (L. A. D. S. De Silva & Heo, 2023; Peng *et al.*, 2023; Schilcher & Horswill, 2020).

Quorum Sensing Mechanism of *Staphylococcus aureus*

The principal quorum-sensing circuit in *S. aureus* is the accessory gene regulator system. The agr locus contains two divergent transcriptional units driven by the P2 and P3 promoters. RNAII contains agrB, agrD, agrC, and agrA. AgrD is synthesized as an AIP precursor and is processed by the membrane-associated machinery, including AgrB and MroQ, before mature AIP is released. As the population grows, extracellular AIP accumulates and binds AgrC, a membrane histidine kinase. AgrC autophosphorylation leads to phosphorylation of AgrA, which activates the P2 promoter to amplify the agr circuit and the P3 promoter to produce RNAIII (Chen *et al.*, 2026; Peng *et al.*, 2023; Tan *et al.*, 2018; Williams *et al.*, 2023).

RNAIII acts as a major regulatory effector that increases expression of numerous secreted virulence factors while reducing expression of several surface-associated colonization proteins. AgrA also directly regulates selected genes, including phenol-soluble modulins. This density-dependent switch supports a transition from an adhesive colonization phenotype toward a more invasive or dispersive phenotype at high cell density. Single-cell work has further shown that agr activation is heterogeneous across bacterial populations, meaning that community-level measurements can conceal subpopulations with different signaling states (Bär *et al.*, 2026). In addition, LuxS/AI-2-related signaling can influence stress tolerance and biofilm behavior, although its effects differ from the core Agr AIP system (Wang *et al.*, 2026).

Quorum quenching can theoretically interrupt the Agr circuit at several points. Candidate targets include AIP maturation and export, extracellular AIP stability, AIP binding to AgrC, AgrC kinase activity, AgrA phosphorylation or DNA binding, and RNAIII-mediated regulation. Reviews of *S. aureus* QS inhibitors show that peptides, natural products, antibodies, and small molecules can attenuate Agr-controlled virulence without necessarily inhibiting bacterial growth, which may reduce selective pressure compared with conventional bactericidal therapy (Chen *et al.*, 2026; Palit *et al.*, 2026). These characteristics make the Agr pathway attractive for natural-product screening.

Nevertheless, the relationship between Agr and biofilm formation is not linear. Agr activity can promote protease and phenol-soluble modulin production that supports biofilm structuring and dispersal. Consequently, Agr-defective or Agr-inhibited populations may show reduced toxin expression but stronger attachment, clumping, or persistent biofilm under some conditions. Jordan *et al.* (2022) demonstrated that agr status does not predict biofilm behavior identically in vitro and in vivo, while Zhang *et al.* (2022) showed that sub-inhibitory berberine reduced AgrA and inhibited biofilm dispersal while increasing PIA-associated clumping. Current reviews therefore emphasize a therapeutic trade-off between suppression of acute virulence and possible promotion of persistent biofilm (Chen *et al.*, 2026). Any octopus-ink quorum-quenching strategy must be evaluated against both outcomes. The quorum sensing mechanism of *Staphylococcus aureus* is presented in Figure 1.

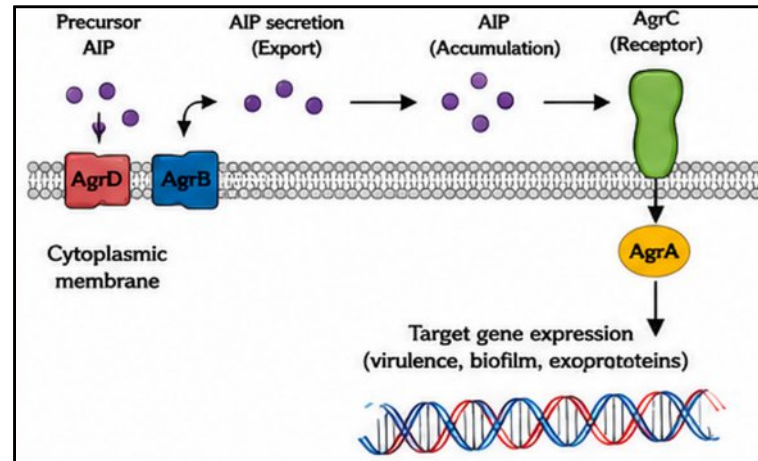


Figure 1. The Agr Quorum-Sensing System in *Staphylococcus aureus* (Kim *et al.*, 2017; Le & Otto, 2015)

Biofilm Formation Mechanism of *Staphylococcus aureus*

S. aureus biofilm development can be described as a sequence of attachment, irreversible adhesion, accumulation, maturation, and dispersal. Initial attachment depends on physicochemical interactions and bacterial surface proteins, including MSCRAMMs that bind host or environmental substrates. Clumping factors, fibronectin-binding proteins, and other cell-wall-anchored proteins promote stable contact with surfaces. If attachment persists, cell-cell interactions and matrix production convert the early community into an organized biofilm (Foster, 2019; Moormeier & Bayles, 2017; Schilcher & Horswill, 2020). This early stage is particularly important for aquaculture biosecurity because preventing attachment can stop downstream maturation before a resistant community becomes established.

During accumulation, *S. aureus* can use both polysaccharide-dependent and polysaccharide-independent mechanisms. The *icaADBC* operon directs synthesis and modification of polysaccharide intercellular adhesin, also called poly-N-acetylglucosamine. PIA/PNAG contributes to intercellular adhesion and matrix integrity. Proteinaceous adhesins and extracellular DNA can provide alternative or complementary scaffolds, which explains why some strains form strong biofilms even with limited PIA dependence. SarA, SigB, Agr, and other regulators coordinate expression of adhesins, enzymes, and matrix-associated genes in response to nutrient and stress conditions (Nasser *et al.*, 2022; Peng *et al.*, 2023).

Mature biofilms develop spatial and physiological heterogeneity. Microcolonies contain cells with different metabolic states, including slow-growing or persister-like subpopulations that survive antimicrobial exposure more effectively than actively dividing planktonic cells. Matrix components restrict diffusion, bind antimicrobial molecules, retain enzymes, and buffer changes in the local environment. These features contribute to antibiotic tolerance that can occur even without a new heritable resistance mutation (Moormeier & Bayles, 2017; Schilcher & Horswill, 2020; Sharma *et al.*, 2023). In production environments, mature biofilms can therefore act as recurring contamination sources after apparently successful cleaning.

Dispersal releases cells or cell clusters from the mature community and allows colonization of new surfaces. In *S. aureus*, proteases, nucleases, and phenol-soluble modulins can destabilize matrix components, while Agr signaling promotes a high-density phenotype associated with secreted factors and dispersal. The developmental cycle is therefore dynamic rather than a one-way progression toward greater biomass. An antibiofilm intervention should ideally reduce early attachment and matrix accumulation while avoiding unintended inhibition of dispersal that leaves a highly persistent sessile population. This principle is central when evaluating any Agr-targeting natural compound, including octopus ink-derived candidates (Jordan *et al.*, 2022; Peng *et al.*, 2023; Schilcher & Horswill, 2020). The mechanism of biofilm formation by *Staphylococcus aureus* is presented in Figure 2.

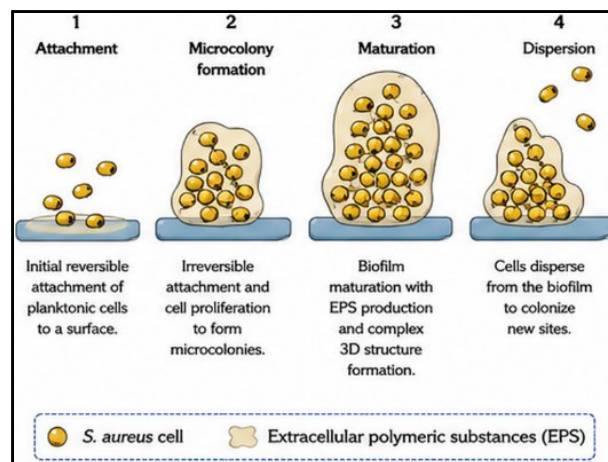


Figure 2. Developmental Stages of *Staphylococcus aureus* Biofilm Formation (Moormeier & Bayles, 2017; Schilcher & Horswill, 2020)

Intervention Strategies Against *Staphylococcus aureus* Biofilm

Strategies against *S. aureus* biofilm can be organized according to the stage or structural target of the biofilm. Prevention of initial attachment can involve surface modification, antifouling materials, interference with MSCRAMM-mediated adhesion, or changes in surface physicochemistry. After attachment, interventions can target PIA/PNAG synthesis, extracellular DNA, matrix proteins, or cell-cell aggregation. Once a mature biofilm is established, stronger strategies are usually required because the matrix and physiological heterogeneity reduce susceptibility to conventional antimicrobials (Ghosh *et al.*, 2020; Kang *et al.*, 2023; Nasser *et al.*, 2022).

Chemical and biological approaches include antibiotics in rational combinations, antimicrobial peptides, matrix-degrading enzymes, bacteriophages, postbiotics, and natural products. Enzymes such as nucleases can degrade extracellular DNA, while proteases or glycoside-targeting enzymes can weaken other matrix components. Bacteriophages may kill susceptible cells and can sometimes be engineered or selected for matrix-degrading activity. Natural products offer additional mechanisms, including membrane effects, inhibition of adhesion, QS modulation, and suppression of matrix-associated gene expression (Mishra *et al.*, 2020; Sharma *et al.*, 2023; Tuon *et al.*, 2023).

Modern materials-based approaches use nanoparticles, photodynamic or photothermal treatment, catalytic generation of reactive oxygen species, and

responsive drug-delivery systems to penetrate or destabilize biofilms. These approaches can be effective but may be difficult to translate directly to aquaculture because cost, environmental release, non-target toxicity, residue control, and large water volumes must be considered. In aquaculture, preventive strategies that reduce surface colonization and pathogen virulence while remaining biodegradable and safe for cultured organisms may offer greater practical value than highly complex biomedical platforms (Kang *et al.*, 2023).

Natural compounds are especially relevant for this objective because one molecule or extract may act on several biofilm targets simultaneously. Reviews of *S. aureus* indicate that natural compounds can reduce attachment, MSCRAMM expression, icaADBC-associated matrix production, QS signaling, or established biofilm biomass, and some can sensitize biofilms to conventional antimicrobials (Kashi *et al.*, 2024). The supplied aquaculture literature similarly emphasizes natural immunostimulants and bioactive feed additives as alternatives that may reduce dependence on synthetic chemicals (Affandi & Diamahesa, 2023; Affandi & Diniariwisan, 2024; Affandi & Muahiddah, 2025; Affandi & Setyono, 2023, 2024a, 2024b). For octopus ink, the most realistic development pathway is therefore a multi-target natural intervention rather than a single-target Agr inhibitor. Intervention strategies against *Staphylococcus aureus* biofilms are presented in Figure 3.

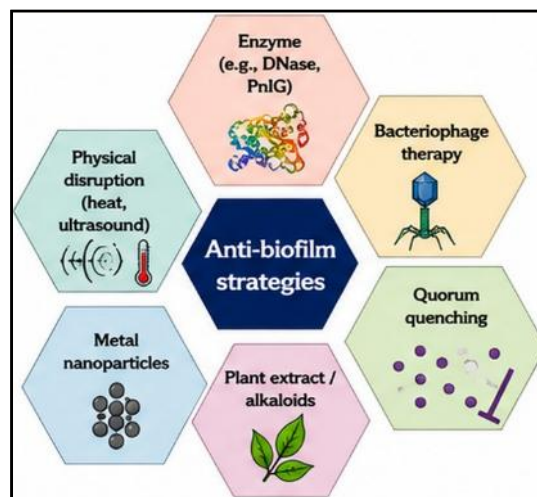


Figure 3. Major Intervention Strategies for Combating *Staphylococcus aureus* Biofilms (Fleitas Martínez *et al.*, 2019; Paluch *et al.*, 2020; Panáček *et al.*, 2018; Schilcher & Horswill, 2020)

To clarify the strength and limitations of the available evidence, Table 1 summarizes the main findings supporting the potential of octopus ink extract as an antibiofilm and quorum-quenching candidate against *Staphylococcus aureus*, while explicitly distinguishing experimentally demonstrated effects from indirect supporting evidence and mechanisms that remain hypothetical.

Table 1. Evidence Synthesis Supporting the Potential of Octopus Ink Extract as an Antibiofilm and Quorum-Quenching Candidate Against *Staphylococcus aureus*

Evidence Domain	Main Evidence From the Literature	Evidence Type	Relevance to Octopus Ink Extract	Current Evidence Status	Representative References
<i>S. aureus</i> occurrence and pathogenic relevance in aquaculture and seafood	<i>S. aureus</i> and MRSA have been detected in seafood, retail fish, aquaculture environments, farm water, and fish handlers. Several isolates carry both antimicrobial-resistance and virulence determinants.	Direct evidence in aquaculture/seafood settings	Confirms that <i>S. aureus</i> is a relevant microbial target for aquaculture hygiene, food safety, and disease-management strategies.	Demonstrated	(Derke et al., 2025; El-Ashker et al., 2026; Murugadas et al., 2017; Okafor et al., 2025; Sivaraman et al., 2021, 2022)
Agr quorum sensing in <i>S. aureus</i>	The AgrD-AgrB-AIP-AgrC-AgrA system regulates density-dependent expression of virulence-associated genes through RNAPIII and other downstream targets.	Direct mechanistic evidence in <i>S. aureus</i>	Establishes a biologically valid quorum-sensing pathway that could potentially be targeted by bioactive compounds.	Demonstrated	(Chen et al., 2026; Kim et al., 2017; Peng et al., 2023; Tan et al., 2018; Williams et al., 2023)
Role of Agr in biofilm regulation	Agr influences biofilm architecture, secretion of proteases and phenol-soluble modulins, and biofilm dispersal. However, Agr suppression may increase attachment or aggregation in some strains and conditions.	Direct but context-dependent evidence in <i>S. aureus</i>	Indicates that successful quorum quenching should be evaluated together with attachment, maturation, biomass, and dispersal rather than Agr inhibition alone.	Demonstrated, but context dependent	(Chen et al., 2026; Jordan et al., 2022; Peng et al., 2023; Zhang et al., 2022)
Biofilm development in <i>S. aureus</i>	Biofilm formation involves initial attachment, accumulation, maturation, and dispersal. MSCRAMMs, <i>icaADBC</i> -dependent PIA/PNAG, extracellular DNA, proteins, and regulatory networks contribute to	Direct mechanistic evidence in <i>S. aureus</i>	Defines measurable targets for evaluating any future antibiofilm effect of octopus ink extract.	Demonstrated	(Foster, 2019; Moormeier & Bayles, 2017; Nasser et al., 2022; Peng et al., 2023; Schilcher & Horswill, 2020)

Evidence Domain	Main Evidence From the Literature	Evidence Type	Relevance to Octopus Ink Extract	Current Evidence Status	Representative References
	biofilm stability.				
Alkaloids as antibiofilm compounds against <i>S. aureus</i>	Piperine, chelerythrine, sanguinarine, berberine, and solasodine have demonstrated antibiofilm activity through mechanisms involving reduced adhesion, matrix inhibition, oxidative stress, membrane disruption, metabolic interference, and modulation of virulence pathways.	Direct evidence for isolated alkaloids against <i>S. aureus</i>	Provides the strongest compound-level mechanistic support for proposing alkaloid-containing octopus ink as an antibiofilm candidate.	Demonstrated for specific alkaloids, not for octopus ink extract	(A. R. P. da Silva et al., 2025; Das et al., 2022, 2024; Lu et al., 2026; Qian et al., 2020; Qu et al., 2025)
Alkaloid-mediated interference with Agr signaling	Berberine has been reported to suppress the Agr system and reduce virulence-related gene expression and biofilm formation in MRSA. At sub-inhibitory concentrations, however, berberine may also inhibit dispersal and increase PIA-associated aggregation.	Direct mechanistic evidence for a specific alkaloid in <i>S. aureus</i>	Supports the plausibility that alkaloids may interfere with Agr-mediated signaling, while also showing that the biological outcome depends on dose and biofilm stage.	Demonstrated for berberine, not for octopus ink alkaloids	(Lu et al., 2026; Zhang et al., 2022; Zhou et al., 2026)
Bioactive composition of octopus ink extract	Octopus ink extract contains alkaloids, saponins, phenols, steroids, melanin-associated constituents, proteins, peptides, amino acids, and other small bioactive molecules. Strong antioxidant activity has also been reported.	Direct chemical evidence for octopus ink	Confirms the presence of compound classes that could potentially contribute to antimicrobial, immunomodulatory, or antibiofilm effects.	Demonstrated for composition, not for anti- <i>S. aureus</i> quorum quenching	(Affandi et al., 2023; Affandi, Azhar, et al., 2025; Hamdi et al., 2024; Hernández-Zazueta et al., 2021; Imran et al., 2023)
Biological activity of octopus ink in aquaculture	Octopus ink extract has been evaluated as a feed additive in catfish and Mozambique tilapia challenged with <i>Aeromonas hydrophila</i> , with improvements in	Direct evidence in aquaculture, but involving other pathogens	Demonstrates biological compatibility and practical potential of octopus ink-derived products as functional	Demonstrated in other aquaculture models	(Affandi, Scabra, et al., 2025; Sabathini et al., 2026; Saputra et al., 2025)

Evidence Domain	Main Evidence From the Literature	Evidence Type	Relevance to Octopus Ink Extract	Current Evidence Status	Representative References
	growth, survival-related performance, and hematological or immune parameters at selected doses.		aquaculture inputs.		
Biological activity of other cephalopod ink products	Squid ink contains several bioactive compounds and has shown immunostimulatory effects in vannamei shrimp, including enhancement of non-specific immune responses.	Direct evidence for cephalopod ink, but not octopus ink against <i>S. aureus</i>	Supports the broader biological plausibility of cephalopod ink-derived materials as functional aquaculture ingredients.	Indirect supporting evidence	(Affandi et al., 2019; Fadjar et al., 2025)
Octopus ink as a quorum-quenching material against other aquatic pathogens	Previous reviews proposed octopus ink extract as a quorum-quenching or anti-quorum-sensing candidate against <i>A. hydrophila</i> , <i>E. tarda</i> , <i>V. harveyi</i> , and <i>S. agalactiae</i> .	Review-based mechanistic inference	Supports conceptual transfer of the quorum-quenching hypothesis to <i>S. aureus</i> , but the relevant signaling systems differ among bacterial species.	Proposed, not experimentally confirmed	(Affandi, Rahmadani, et al., 2026; Affandi, Setyono, et al., 2026; Affandi & Setyono, 2025b, 2025a)
Proposed interference of octopus ink with the <i>S. aureus</i> Agr system	Alkaloids or other constituents of octopus ink could theoretically interfere with AIP maturation, AIP stability, AIP-AgrC recognition, AgrC kinase activity, AgrA/RNAIII signaling, or downstream virulence expression.	Mechanistic inference derived from separate evidence streams	Represents the central quorum-quenching hypothesis of this review.	Hypothesis, not directly demonstrated	Synthesized from (Affandi, Azhar, et al., 2025; Chen et al., 2026; Palit et al., 2026; Zhou et al., 2026)
Proposed multi-target antibiofilm action of octopus ink extract	A biologically plausible mechanism may combine Agr modulation with inhibition of adhesion, <i>ica</i> -associated matrix formation, membrane integrity, redox balance,	Evidence-based synthesis	Provides a broader and more defensible model than describing octopus ink as a specific Agr inhibitor.	Evidence-based hypothesis requiring validation	(Affandi, Azhar, et al., 2025; Das et al., 2024; Kashi et al., 2024; Mishra et al., 2020; Zhou et al.,

Evidence Domain	Main Evidence From the Literature	Evidence Type	Relevance to Octopus Ink Extract	Current Evidence Status	Representative References
	and biofilm maturation.				2026)
Direct octopus ink extract activity against <i>S. aureus</i> biofilm and AIP - AgrC signaling	No study in the reviewed evidence set directly tested octopus ink extract against <i>S. aureus</i> using Agr-specific or biofilm-specific molecular endpoints.	Direct evidence specifically required for confirmation	This represents the principal evidence gap of the review. Future studies should test sub-MIC activity, Agr signaling, <i>agrA/agrC/RNAlII</i> , <i>icaADBC</i> , PIA/PNAG, eDNA, biomass, viable cells, virulence, and dispersal.	Not yet demonstrated	No direct study identified among the 67 included articles

As summarized in Table 1, the evidence supporting octopus ink extract as a potential antibiofilm and quorum-quenching candidate against *S. aureus* is derived from several complementary but distinct evidence streams. Direct evidence confirms the importance of the Agr system and biofilm-related pathways in *S. aureus*, while compound-level studies demonstrate that several alkaloids can interfere with these processes. In parallel, studies on octopus ink confirm the presence of alkaloids and other bioactive constituents and demonstrate biological activity in aquaculture models. However, these evidence streams have not yet converged in a direct experiment showing that octopus ink extract disrupts AIP-AgrC signaling or inhibits *S. aureus* biofilm formation. Therefore, the proposed mechanism should be interpreted as an evidence-based hypothesis that requires direct experimental validation.

Mechanism of Alkaloids in Inhibiting *Staphylococcus aureus* Biofilm

Alkaloids are nitrogen-containing natural compounds with chemically diverse structures and equally diverse antibiofilm mechanisms. In *S. aureus*, piperine has been reported to reduce biofilm formation at sub-inhibitory concentrations, with evidence of reactive oxygen species accumulation and reduced biofilm-associated functions. A later MRSA study found that piperine reduced extracellular polysaccharide production and downregulated *icaA*, supporting a matrix-directed mechanism in addition to oxidative stress (Das *et al.*, 2022, 2024). Chelerythrine has also shown activity against staphylococcal biofilms, reducing adhesion and matrix-associated biomass in dual-species *S. aureus* and *S. lugdunensis* biofilms (Qian *et al.*, 2020).

Other alkaloids act through membrane and metabolic targets. Sanguinarine can disrupt membrane integrity, dissipate the proton motive force, and interfere with imidazoleglycerol-phosphate dehydratase and histidine biosynthesis, producing both antibacterial and antibiofilm effects against *S. aureus* (Qu *et al.*, 2025). Berberine, an isoquinoline alkaloid, has been shown to suppress the Agr

quorum-sensing system in methicillin-resistant *S. aureus*, resulting in decreased expression of virulence-associated genes and reduced biofilm formation (Zhou *et al.*, 2026). Solasodine, a steroidal alkaloid sapogenin, has also reduced *S. aureus* biofilm formation and biomass, demonstrating that antibiofilm activity is not restricted to one alkaloid subclass (A. R. P. da Silva *et al.*, 2025).

Berberine illustrates why alkaloid action must be interpreted by concentration and biofilm stage. Recent work reports direct antibiofilm activity of berberine, including reductions in biomass and metabolic activity at effective concentrations (Lu *et al.*, 2026). In contrast, sub-inhibitory berberine can suppress AgrA, inhibit biofilm dispersal, stimulate clumping, and increase PIA-related features without strongly suppressing growth (Zhang *et al.*, 2022). Thus, an apparent quorum-sensing inhibitory effect is not automatically equivalent to reduced biofilm. The desired profile is a coordinated decrease in attachment or matrix development together with reduced virulence, not simply suppression of agr.

The available alkaloid literature supports a multi-target model that includes inhibition of initial adhesion, modulation of ica-dependent matrix production, interference with Agr-related virulence signaling, membrane stress, metabolic inhibition, oxidative imbalance, and disruption of established biofilm architecture. This model is consistent with broader reviews of natural antibiofilm agents (Kashi *et al.*, 2024; Mishra *et al.*, 2020). It also provides a mechanistic basis for evaluating octopus ink because alkaloids have repeatedly been detected in cephalopod ink preparations, including the supplied squid-ink and octopus-ink studies (Affandi *et al.*, 2019, 2023; Affandi, Azhar, *et al.*, 2025). The mechanism of alkaloids in inhibiting *Staphylococcus aureus* biofilm is presented in Figure 4.

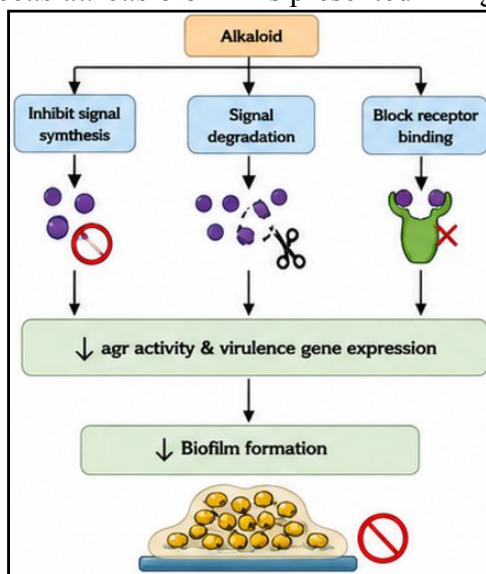


Figure 4. Proposed Mechanisms of Alkaloids in Interfering with Quorum Sensing and Biofilm Formation (Arya & Usha, 2024; Kalia, 2013)

Mechanism of Octopus Ink Extract as Quorum Quenching in *Staphylococcus aureus* Biofilm Formation

Octopus ink is a chemically complex marine by-product rather than a single-compound preparation. Studies on *O. vulgaris* and other octopus species identify or indicate proteins, peptides, melanin-associated constituents, amino acids, and

multiple small bioactive molecules. The supplied aquaculture study by Affandi, Azhar, *et al.* (2025) detected alkaloids, saponins, phenols, and steroids in octopus ink extract and reported strong antioxidant activity with an IC_{50} of 94.4661 ppm. Earlier work on squid ink identified betaine, choline, cinnamic acid, and other bioactive constituents, supporting the broader concept that cephalopod ink contains compounds with antimicrobial and immunomodulatory potential (Affandi *et al.*, 2019). External studies further show antimicrobial and bioactive properties of *O. vulgaris* ink extracts and reveal a rich ink proteome containing candidate bioactive peptides (Hamdi *et al.*, 2024; Hernández-Zazueta *et al.*, 2021; Imran *et al.*, 2023). Kalor *et al.* (2019) also demonstrated biologically active extracts from *O. cyanea* ink, although its study focused on cytotoxicity rather than quorum sensing.

The supplied aquaculture evidence strengthens the case for biological compatibility and application potential. Octopus ink extract has been evaluated as a feed supplement in catfish and Mozambique tilapia challenged with *A. hydrophila*, with reported improvements in growth, survival-related performance, or hematological immune indicators at selected doses (Affandi, Scabra, *et al.*, 2025; Sabathini *et al.*, 2026; Saputra *et al.*, 2025). Squid ink powder has also enhanced non-specific immune parameters in vannamei shrimp challenged with infectious myonecrosis virus, showing that cephalopod ink-derived materials can function as biologically active feed ingredients (Fadjar *et al.*, 2025). These studies do not demonstrate anti-*S. aureus* quorum quenching, but they support the feasibility of developing cephalopod ink as a functional aquaculture input.

Previous reviews from the supplied literature have proposed octopus ink as an anti-quorum-sensing or quorum-quenching material against *A. hydrophila*, *E. tarda*, *V. harveyi*, and *S. agalactiae* (Affandi, Rahmadani, *et al.*, 2026; Affandi, Setyono, *et al.*, 2026; Affandi & Setyono, 2025b, 2025a). For Gram-negative bacteria, such models often focus on interference with acyl-homoserine lactone or related autoinducer pathways. *S. aureus* requires a different mechanistic model because its dominant Agr system uses peptide signals. A plausible *S. aureus* mechanism would therefore involve one or more octopus-ink constituents interfering with AIP maturation, AIP-AgrC recognition, AgrC kinase activity, AgrA/RNAIII signaling, or downstream virulence, while other constituents simultaneously reduce adhesion, ica-associated matrix formation, membrane integrity, or redox balance. This multi-target requirement is consistent with the broader alkaloid evidence but remains a hypothesis.

No article among the 67 sources directly demonstrated that octopus ink extract blocks AIP-AgrC signaling or suppresses *S. aureus* biofilm formation. The most defensible conclusion is therefore that octopus ink extract is a candidate for experimental validation, not a confirmed quorum-quenching agent against *S. aureus*. Future studies should separate antibacterial activity from true quorum-quenching activity by using sub-MIC concentrations and should measure agrA, agrC, RNAIII, P3 activity, toxin or protease outputs, initial adhesion, icaADBC expression, PIA/PNAG, eDNA, total biomass, viable biofilm cells, and dispersal. A successful formulation should reduce virulence and total biofilm burden without producing the undesirable phenotype of strong persistent aggregation caused by isolated Agr suppression. The mechanism of octopus ink extract as a quorum-quenching agent in *Staphylococcus aureus* biofilm formation is presented in Figure 5.

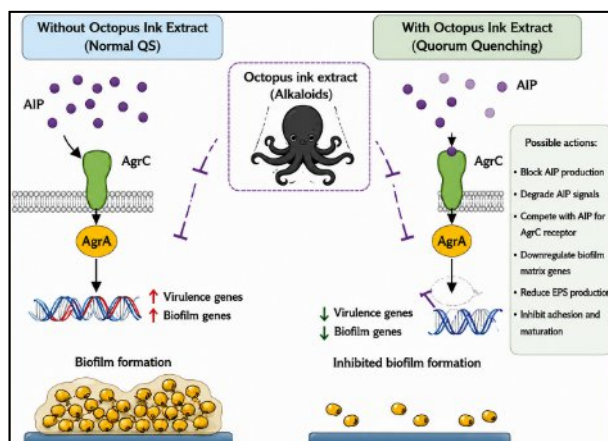


Figure 5. Proposed Mechanism of Octopus Ink Extract as a Quorum-Quenching Agent Against *Staphylococcus aureus* Biofilm Formation. The Proposed Pathway was Synthesized from Affandi *et al.* (2019, 2023); Affandi, Azhar, *et al.* (2025); Affandi, Scabra, *et al.* (2025); Arya & Usha (2024).

CLOSING

Conclusion

Octopus ink extract has strong potential as a multi-target natural candidate for controlling *Staphylococcus aureus* biofilm in aquaculture because it contains several bioactive groups, including alkaloids, and cephalopod ink preparations have shown antimicrobial, antioxidant, and immunomodulatory activities. Evidence from *S. aureus* alkaloid studies supports possible effects on adhesion, matrix production, membrane function, metabolism, oxidative stress, and Agr-related signaling, but direct quorum-quenching activity of octopus ink against *S. aureus* has not yet been demonstrated. Therefore, octopus ink extract should currently be positioned as a promising research candidate whose antibiofilm effect must be confirmed experimentally, especially because inhibition of Agr alone can reduce virulence while increasing biofilm persistence in some conditions.

Suggestion

Further studies should identify and quantify the specific octopus-ink constituents responsible for activity against *S. aureus* and test purified compounds together with standardized whole extracts. Mechanistic experiments should use sub-MIC concentrations and simultaneously measure Agr/AIP signaling, virulence expression, adhesion, ica-associated matrix production, mature biofilm viability, and dispersal to distinguish true quorum quenching from nonspecific antibacterial effects. After in vitro validation, safety, effective dose, delivery method, residue risk, effects on beneficial microbiota, cultured-animal performance, and efficacy under hatchery or farm conditions should be evaluated before aquaculture-scale application.

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