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Review

Current Advances in the Concept of Quorum Sensing-Based Prevention of Spoilage of Fish Products by Pseudomonads

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Abstract: Microbial spoilage of fish is attributed to quorum sensing (QS)-based activities. QS is a communication process between the cells in which microorganisms secrete and sense the specific chemicals (autoinductors, AIs) that regulate proteolysis, lipolysis, and biofilm formation. These activities change the organoleptic characteristics and reduce the safety of the products. Although the microbial community of fish is diverse and may consist of a range of bacterial strains, the deterioration of fish-based products is attributed to the growth and activity of *Pseudomonas* spp. This work summarizes recent advancements to assess the influence of QS mechanisms on seafood spoilage by *Pseudomonas* spp. The quorum sensing inhibition (QSI) in the context of fish preservation has also been discussed. Detailed recognition of this phenomenon is crucial in establishing effective strategies to prevent the premature deterioration of fish-based products.

Keywords: *Pseudomonas* spp.; quorum sensing (QS); autoinductors (AIs); quorum sensing inhibition; spoilage of fish-based products; fish preservation

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1. Introduction

The nutritional and health value of fish, as well as their relatively low price and availability, affect global production, which is projected to reach 200 Mt by 2029, increasing by 25 Mt from the base period (average of 2017–2019) [1]. As reported by FAO [1], 44% of total fish production is utilized in the fresh/chilled form, which are usually more perishable than most other foodstuffs [2]. The high content of free amino acids, high post-mortem pH, high water content, and presence of trimethylamine oxide (TMAO) promote the growth of microorganisms in fish-based products. Psychrotolerant species of the genera *Pseudomonas* mainly contribute to seafood deterioration; the bacteria may decompose nitrogenous/lipid substances [3], which can result in greater weight loss, a reduction in water-holding capacity, textural changes, and off-odor effects of products [4,5]. *Pseudomonas* spp. have successfully evolved genotypic and phenotypic traits enabling growth and defeating the negative effects of conventional seafood antimicrobials and temperature stress [4,6–8] (Figure 1). Despite implementing quality control programs in the seafood industry, about one-fourth of the world's supply and 30% of landed fish are lost due to microbial activity [8]. Therefore, it is necessary to develop new solutions to prevent spoilage of fish-based products caused by *Pseudomonas* spp.

The metabolic activities of *Pseudomonas* spp. are regulated via a quorum sensing (QS) system, which was perfectly described in the work of Venturi [9]. In that system, small-signal molecules (autoinductors, AIs), followed by interaction with cognate receptor proteins, lead to a coordinated regulation of specific gene expression [9]. Two acyl-homoserine lactone (AHL) QS systems, LasI-LasR and RhlI-RhlR, were identified in *P. aeruginosa*; these two systems also interact with a 2-heptyl-3-hydroxy-4-quinolone signal (PQS) and 2-2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS) systems [10].

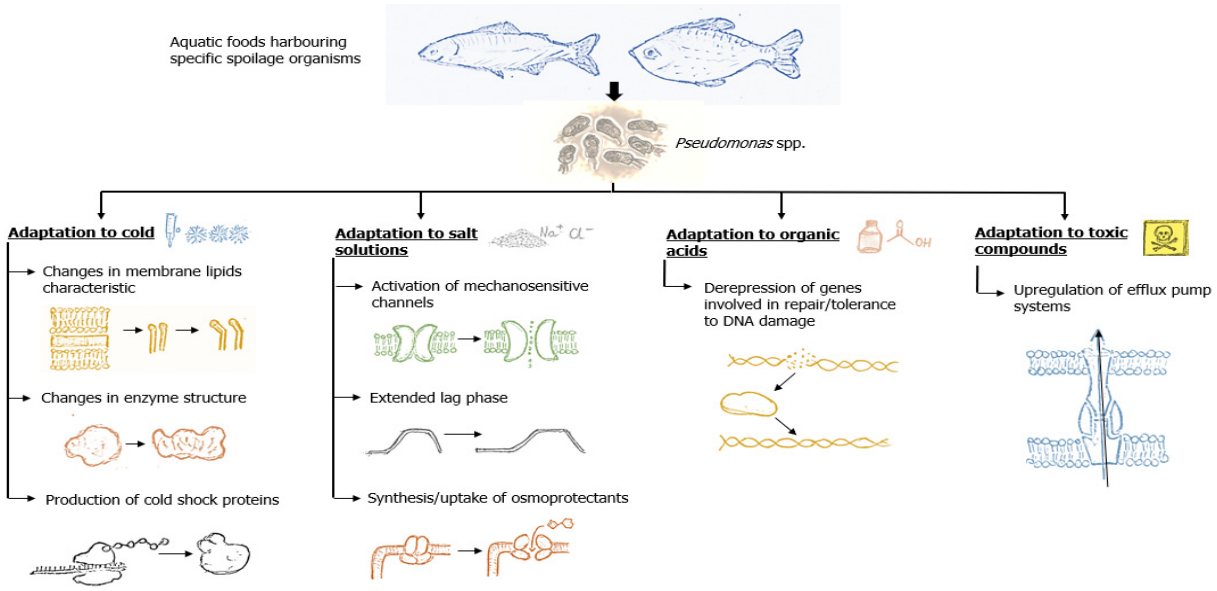

Figure 1. Schematic explanation of *Pseudomonas* spp. adaptation strategies to environmental conditions.

Numerous articles are available in which the background of food-associated stresses and their impact on the cellular response of bacteria are expertly described [9,11]. However, few of them address the effect of inhibition of QS mechanisms on seafood quality. Due to the resistance of *Pseudomonas* spp. to conventional food preservation systems, QS-based control methods are often considered [12]. Their application to the food industry would greatly aid efforts to eradicate undesirable microflora from food processing environments and, ultimately, from food products. These approaches, in contrast to bactericidal treatments, exert less selective pressure, which in turn reduces the likelihood of resistance development. QS inhibitors would help design approaches for reducing or preventing spoilage reactions or even controlling the expression of virulence factors of *P. aeruginosa* [13].

This review summarizes recent research reports on the QS phenomenon in *Pseudomonas* spp. We present the role of QS in regulating the metabolic activity of *Pseudomonas* spp. (i) and review of the current applications of QS inhibitors in preventing bacterial food spoilage (ii). Our goal is to provide a new perspective for the search for more effective food antimicrobials through the use of QS inhibitory agents.

2. QS System in Pseudomonads

The AHLs-depended QS networks studied are the best so far and the focus of this review will be on the structural basis of that systems in pseudomonads [9]. Moreover, transcriptomic studies have revealed that the AHL-regulated genes and operons constitute over 6% of the genome and are scattered through the chromosome [14,15]. That supports the view that the AHL system in bacteria constitutes a global regulatory system [16].

AHL signals differ in the chemical structure; the differences are in the length of the acyl chain and the substituents (usually 3-oxo or 3-hydroxy groups) at the 3 position of the acyl chain [17,18].

In *P. aeruginosa*, two AI synthase genes, *lasI and rhlI*, are involved in the synthesis of AHLs; they share significant sequence homologies to *luxI* of *Vibrio fisheri* [10]. AHLs accumulate both in the cells and in the environment. As the bacteria grow, the concentration of secreted AHL molecules increases [19]. When the population density reaches the "quorum", AIs exceed the critical threshold and are recognized by specific receptors that belong to a large class of DNA-binding transcription factors named "R-proteins" [18]. In *P. aeruginosa*, the LasR, upon binding to the specific AHL, directly regulates the transcription of target genes by binding to or dissociating from corresponding promoters [10,18].

In *P. aeruginosa*, there are also two other types of AHL-mediated systems, the *pqs* system and the *iqs* system. These networks work in a manner similar to the *las* and *rhl* systems, though their AIs are PQS and IQS [20,21].

PQS is synthesized via a "head to head" condensation of anthranilate and β-keto dodecanoate and requires the products of the *pqsA*, *pqsB*, *pqsC*, and *pqsD* genes, which generate over 50 other 2-alkyl-4-quinolones including 2-heptyl-4-quinolone (HHQ) [22]. Many of these signals are produced at low levels; thus, their biological function is not yet clear [16]. In addition to their regulatory role in the pathogenicity of *P. aeruginosa*, it is believed that PQS can also induce outer membrane vesicle formation, activate the oxidative stress response, act as a stress warning signal, and modulate the host immune response [16,23,24].

The role of the last gene of the *pqs* operon (*pqsE*) is not known, but while *pqsE* mutants produce parental levels of PQSs, the strains do not exhibit any PQS-related phenotypes; consequently, PqsE is considered to facilitate the response to PQS [25]. The immediate precursors of PQS are HHQ, and its conversion to PQS depends on the activation of PqsH, a putative mono-oxygenase [22] that is LasR regulated, so linking AHL and PQS regulatory networks.

IQS belongs to a new class of AIs of the QS system. The genes that are involved in IQS synthesis are a non-ribosomal peptide synthase gene cluster *ambBCDE*. When inhibited, it caused a decrease in the production of PQS and C4-HSL AIs, as well as QS-regulated activities of bacteria [21]. IQS has been shown to contribute to the full virulence of *P. aeruginosa* in different animal models, highlighting the function of this new QS system in the modulation of bacterial pathogenesis. Importantly, upon phosphate depletion in the culture medium, IQS was demonstrated to be able to partially take over the function of the central *las* system in pseudomonads [26].

There are interconnections between the aforementioned QS systems in pseudomonads. The *las* system is activated by 3-oxo-C12-HSL. Next, the LasR-3-oxo-C12-HSL complex multimerizes and stimulates the transcription of *rhlR*, *rhlI*, *lasI* (hence a positive feedback loop), and other genes that are part of this regulon [27]. The RhlR-C4-HSL also multimerizes and activates its own regulon; *rhlI* forms the second positive feedback loop [10,28]. LasR-3-oxo-C12-HSL positively controls PqsR, a transcriptional regulator of operon *pqsABCD,* and the expression of *pqsH*, the gene encoding the final converting enzyme of PQS from precursor HHQ [10]. Interestingly, *pqsR* and *pqsABCDE* expression can be inhibited by RhlR-C4-HSL, suggesting that the ratio of the concentration of 3-oxo-C12-HSL and C4-HSL plays a crucial role in the dominance of the *pqs* signaling system [29]. As stated above, LasR-3-oxo-C12-HSL regulates the onset and activation of both the *pqs* and *rhl* systems in *P. aeruginosa*. These networks represent a step-wise activation cascade that is triggered by the attainment of the "quorum" in pseudomonads [10]. Recently detected IQS is also controlled by LasRI; disruption of either *lasR* or *lasI* limits the expression of *ambBCDE* and production of IQS [26].

3. Examples of QS-Based Activities of *Pseudomonas* spp. Affecting Spoilage of Fish

Extracellular enzyme biosynthesis and biofilm development are regulated by the QS system. The above microbial activities play a major role in the fish spoilage process [13,30–32]. AIs of the QS system have been detected in spoiled fish filets, cold-smoked, and minced fish products [13]. The degree of fish spoilage was correlated with the concentration of AIs [30]. Examples of the effects of AIs on fish spoilage are presented in Table 1.

Table 1. Examples of *Pseudomonas* spp. phenotypes regulated by AHLs-mediated system.

Microorganism	AHL	Phenotypes Regulated by QS	Reference
P. aeruginosa	3-oxo-C12-HSL	Pyoverdine production	[33]
P. psychrophila	C4-HSL	Exoenzyme production	[34]
P. fluorescens, *P. putida*	3-oxo-C6-HSL, C6-HSL, C8-HSL, C12-HSL	Proteolytic activity	[34]
Pseudomonas spp.	C4-HSL, 3-oxo-C6-HSL, C6-HSL, C8-HSL, C12-HSL	Slime formation	[13]
P. fluorescens, *P. putida*	3-oxo-C6-HSL, C6-HSL, C8-HSL, C12-HSL	Proteolytic activity	[13]
P. fluorescens	C4-HSL	Biofilm formation, EPS production	[35]
P. fluorescens	C4-HSL	Biofilm formation	[19]
P. fluorescens	3-oxo-C14-HSL, 3-oxo-C6-HSL, C4-HSL	Lipolytic activity	[5]

3.1. QS and Proteolysis

Pseudomonas spp. isolated from fishery products displayed proteolytic activities accompanied by an increase in amino acids and volatile sulfur compounds such as mercaptans and H_2S [32]. The proteases of fish-borne *Pseudomonas* spp. are serine, thiol, or metalloproteases stabilized by Ca^{2+}. They exhibit low activation energies compared to the classical trypsin protease [36]. Proteolytic enzymes degrade the fish muscle and connective tissue, facilitating bacterial penetration to deeper structures and causing textural changes in fish [37].

AHLs have been identified in proteinaceous foods and were correlated with the proteolytic activity of microflora [34]. AHLs were found in rainbow trout fillets contaminated by *P. fluorescens* and *P. putida* [13]. Moreover, *P. fluorescens* exhibited significantly higher proteolytic activity when exogenous C4-HSL and C6-HSL were added to the culture [30]. A significant increase in total volatile basic nitrogen (TVB-N) (from 5.21 to approximately 60 mg N/100 g) during storage was observed with the addition of C4-HSL and C14-HSL. These AIs had a stimulatory effect on the *aprX* metalloprotease gene expression and proteolytic activity of *P. fluorescens* (by 63 and 52%, respectively) [38]. In *P. psychrophila*, *P. orientalis*, and *P. fluorescens*, changes in QS systems involving downregulation of AHL and the PQS molecules resulted in inhibition of proteolysis (approximately 15 to 30%) along with downregulation of genes encoding metalloproteases [39]. A significant inhibition by about 40% of extracellular protease activity was finally confirmed by Tang et al. [35] in ΔluxI and ΔluxR mutants of *P. fluorescens*.

3.2. QS and Lipolysis

Fish muscle is characterized by a relatively higher content of lipids that accounts for 16% [4]. *Pseudomonas* spp. produce lipolytic enzymes that catalyze the hydrolysis of triglycerides to glycerol and free fatty acids, resulting in unpleasant odors related to the development of aldehydes [2]. The lipolytic activity of fish-borne spoilers *Pseudomonas* spp. was described by Myszka et al. [5] and Sterniša et al. [37]. It has been established that QS-regulated *lipA* and *lipB* genes are responsible for fish spoilage [40]. The lipase encoded by *lipB* is solely responsible for "lipolytic phenotype" of *P. fluorescens*, which leads to rancidity, a soapy off-flavor, and other quality defects of fish [41]. *lipA* is located at the end of a polycistronic operon in the *apr* gene cluster; its downregulation results in the loss or relatively low lipolytic activity of bacteria [42]. Moreover, studies performed by Riedel et al. [43] demonstrated that Lip exporter is regulated by the QS system; its function is essential for lipase secretion. Supplementation of the culture medium with AHLs increased transcription of *lipA* and *lipB* of bacteria [44]; the bacteria lost the ability to synthesize lipase since they contained a nonfunctional AHL of the QS system [45].

3.3. QS and Biofilm Formation

The formation of biofilms is a stepwise process involving the initial attachment of bacteria to surfaces, microcolonies growth and maturation into expanding structures, and further detachment of aged microorganisms [46]. It has been proposed that AHL-mediated QS is involved in all stages of biofilm formation. The influence of the *las* AI, 3-oxo-C12-HSL on biofilm maturation in *P. aeruginosa* has been described by Davies et al. [47]. Strain deficient in the production of 3-oxo-C12-HSL formed very thin biofilms that lacked the three-dimensional architecture observed with the parent. In addition, while the wild-type biofilm was resistant to sodium dodecyl sulfate (SDS), the biofilm formed by the *lasI* mutant was easily dispersed upon exposure to SDS [47].

The relationship between QS and biofilm formation was also described indirectly by evaluating the effects of AIs on twitching and swarming motilities, rhamnolipids, and exopolysaccharides (EPS) production [33]. Swarming motility, which is an organized form of structure translocation, is useful in the early stages of biofilm development and is regulated by the *rhl* system similarly to twitching motility—a flagella-independent way of translocation necessary for microcolony development [48]. EPS holds all cells of biofilm in the near vicinity to enable QS interactions [49]. Rhamnolipid production is involved in several aspects of biofilm formation, such as the formation of microcolonies, maintaining the open channel structure, facilitating mushroom-shaped structures, and aiding cell dispersion [50].

In general, the addition of AHLs to the culture medium was shown to affect EPS (alginate)/rhamnolipid production, twitching and swarming motilities, and biofilm maturity by *Pseudomonas* spp. [30,51]. Inhibiting OS would be, therefore, an alternative to combat the biofilm problems [51,52]. Adhered *Pseudomonas* spp. are found in many locations on seafood processing lines, despite the fact that thorough cleaning and disinfection are carried out regularly. Moreover, biofilms of *Pseudomonas* spp. enhanced the colonization of *Listeria monocytogenes* on food contact materials which promoted food contamination [53].

4. QS Inhibition in the Context of Fish Preservation

As researchers correlated metabolic activities with the QS mechanism [13,54], the search for QS inhibiting agents as an alternative approach for fish preservation has been extensively studied [55]. Disrupting the bacterial QS network is a reverse bactericidal strategy that will not exert selection pressure, leading, as with conventional preservatives, to the development of resistance [56]. Moreover, blocking QS renders the bacteria less virulent [9]. In general, QS inhibitors (QSIs) and quorum quenching enzymes (QQ) can be successfully used for food safety control. QS inhibiting agents can target AIs, and QS receptors and interfere with signaling cascades [54]. All of these mechanisms are present in *Pseudomonas* spp.; a detailed description is given below.

4.1. QS Inhibiting Agents

The agents that target pseudomonads AIs include mainly lactonases, acylases (also known as amidases or aminohydrolases), and oxidoreductases. Enzymes can lead to one of the following effects: (i) AI-degradation for fine-tuning the endogenous QS system, (ii) AI-degradation for modulating the QS system, and (iii) AI-degradation as a mechanism to use AIs for nutrient sources. Inactivation of AI synthases and modification/degradation of AIs affect the QS system of pseudomonads [57].

Among lactone-degrading enzymes, metallo-β-lactamase-like lactonases (MLL) and phosphotriesterase-like lactonases (PLL) are the main studied families [57,58]. They share a common catalytic mechanism and their differences in AHL substrate preference lie in how the acyl chain can be accommodated into the catalytic site [59]. PLL favors long aliphatic lactones as substrates, whereas MLL exhibits board AHL specificity. The work of Rémy et al. [60] confirms that levels of C4-HSL and 3-oxo-C12-HSL and low expression of *las* and *rhl* genes in *P. aeruginosa* were due to PLL and MLL activity.

Acylases hydrolase the amide bond between the acyl chain and the homoserine lactone ring [57]. Four AHL-acylases are present in *P. aeruginosa*: namely *pvdQ*, *quiP*, *hacB*,

and PA1893 [61]. When *P. aeruginosa* is grown in a rich medium, the constitutive expression of these acylases shows a decreased level of 3-oxo-C12-HSL. Vice versa, disruption of the acylase genes resulted in a higher concentration of AIs. In addition, a Δ*hacB* single mutant and a Δ*pvdQ*, Δ*hacB*, and Δ*quiP* triple mutant secrete more efficiently 3-oxo-C12-HSL in comparison to Δ*pvdQ*, Δ*quiP* double mutant that produced AHLs in the same level as wild-type pseudomonads. This observation indicates that HacB might be working as the main acylase in controlling 3-oxo-C12-HSL accumulation in pseudomonads [62].

Oxidoreductases modify AIs by oxidizing or reducing the acyl chain at the third or distal carbon without degrading the AHLs [58]. Such modification may also affect the specificity and recognition of the AIs, thus disturbing the activation of the QS-mediated genes [63]. BpiB09 oxidoreductase derived from a metagenomics library was found to be capable of inactivating 3-oxo-C12-HSL in *P. aeruginosa* [64]. Its expression in pseudomonads resulted in significantly reduced QS-controlled bacteria phenotypes.

QS inhibiting agents also target QS receptors that inactivate the receptor or compete with the receptor. Terpenes and flavonoids of plant essential oils (EOs), as well as halogenated furanones derived from algae, can bind to QS receptors [21]. Examples of natural QS inhibiting agents against *Pseudomonas* spp. are presented in Table 2. For instance, a plant flavonoid naringenin competes with the 3-oxo-C12-HSL by binding to the LasR receptor leading to inhibition of QS-regulated virulence factors in *Pseudomonas* spp. [65]. Methyl eugenol and β-phellandrene of tarragon EO [39] and quercetin [54] show similar anti-QS activities [66]. Methyl eugenol, β-phellandrene, and quercetin were successfully docked into the LasR of pseudomonads [54,66]. High docking score values of the examined agents-LasR receptor were due to the range of H-bones created with negatively charged residues of the proteins [66]. According to Klebe [67], hydrogen bonds provide stability to the complex ligand-receptor and play a key role in molecular recognition. Moreover, the effective binding of compounds of EOs results in conformational changes in the proteins [68].

Table 2. Impact of natural QS inhibiting agents on fish-associated *Pseudomonas* spp.

QS Inhibiting Agent	Target Microorganism	Impact on Bacterial QS-Controlled Processes	Reference
Piper nigrum L. EO	*P. psychrophila*	reduction of proteolytic and lipolytic activities	[66]
Ferula asafoetida EO	*P. aeruginosa*	Reduction of pyocyanin and elastase production; prevention of biofilm formation	[69]
Myrtus communis L. EO	*P. fluorescens*, *P. orientalis*	Reduction in the EPS production	[52]
Origanum majorana EO	*P. putida*	Prevention of biofilm formation	[70]
Juniperus phoenicea EO	*P. fluorescens*	Reduction of proteolytic and lipolytic activities	[5]
Cinnamaldehyde	*P. fluorescens*	Reduction of proteolytic activity; prevention of biofilm formation	[71]
Quercetin	*P. aeruginosa*	Reduction the EPS production and bacterial motility, prevention of biofilm formation	[54]
Garlic extract	*P. aeruginosa*	Reduction of rhamnolipid production	[72]

Selective metabolites produced by lactic acid bacteria can also bind simultaneously to different QS receptors. The affinity of 3-benzene lactic acids from *Lactobacillus* spp. for RhlR and PqsR receptors is higher compared to C4-HSL and PQS ligands in *P. aeruginosa* [73]. In addition, flavonoids can also non-competitively bind to the LasR receptor and prevent the protein from binding to DNA. The agents also cause the repression of QS-mediated activities [74].

The third mechanism of inactivation of the QS system in *Pseudomonas* spp. blocks the signaling cascade by deactivating the downstream response regulators or other regulatory factors [21]. For instance, an efflux pump inhibitor PAβN reduces the accumulation of AIs in supernatants and significantly decreases the relative expression of the QS cascade (*pqaA*, *pqsR*, *lasI*, *lasR*, *rhlI*, and *RhlR*) in *P. aeruginosa*. Limonene and β-caryophyllene from

black pepper EO also exhibited the anti-QS and anti-efflux pumps of pseudomonads [66]. The mRNA transcript levels of autoinductor synthases, membrane fusion, outer membrane proteins, and transcription of repressor regulators MarR and TetR were observed in *P. psychrophila*. In general, limonene and β-caryophyllene affected the functioning of the QS system in *P. psychrophila* and consequently reduced the spoilage potential [66].

4.2. Examples of Use Anti-QS Agents in Fish Preservation

To delay spoilage and extend the shelf life of fish, alternative preservation methods involving the addition of compounds with known anti-QS activity were proposed [75]. However, new preservative techniques require extensive research to be effectively applied to fish products due to the different sensitivity of microorganisms to QS inhibiting agents introduced directly into the food matrix compared to the sensitivity of cells to the above agents recorded in vitro [37,67].

The most convenient method of using anti-QS agents is to add them directly to marinades or brine. For instance, fresh salmon samples were immersed in marinade composed of 95% olive oil and 5% vinegar and enriched with pepper EO. After 72 h of incubation, the protease and elastase activities of *P. aeruginosa* were suppressed by 30–50% and 60–70%, respectively [76]. Moreover, Van Haute et al. [77] and Eskandari et al. [78] reported that the shelf life of salmon and silver carp could be extended when marinated with anti-QS agents. Both the addition of cinnamon EO and black cumin extract inhibited microbial proliferation. After 15 days of storage, total psychotropic pseudomonads content was low, lipid oxidation was delayed, and sensory quality (texture, color, and odor) was high compared to control samples [78]. In the work of Sterniša et al. [37], rosemary extract, buffered vinegar, and their combinations were used as dip treatments against *P. fragi*, *P. psychrophila*, *Shewanella putrefaciens*, and *Shewanella xiamenesis* in common carp meat. The results showed that *Pseudomonas* strains were more resistant to applied antimicrobials, and only a concentration of 3.13 mg/mL rosemary extract in vinegar resulted in growth inhibition and lowered lipolytic and proteolytic activity.

Although the direct application of anti-QS EOs to fish-based products is the most common application method, the technique has some disadvantages. Indeed higher concentrations of EOs are needed to achieve the same effect in food compared to in vitro approaches. In addition, even at low doses, some EOs could have a negative impact on the sensory attributes. Therefore, an alternative solution is to use edible coating/films enriched with anti-QS EOs.

The advantages of edible films are stabilizing volatiles entrapped into their structure, increasing the oxygen barrier, and maintaining sensory and texture attributes [79]. The application of the anti-QS myrtle EO to the chitosan-based nanomatrix of salmon-based products enhanced the activity of the agent against *Pseudomonas* spp. [52]. *Pseudomonas* population was maintained at approximately under 10^4 CFU/g relatives to the control, reaching 10^8 CFU after 5 days of storage. Chitosan coatings in combination with whey protein and tarragon EO were also tested for their preservative effects on *Scomberoides commersonnianus* fillets under refrigerated conditions. The applied treatment inhibited the growth of psychrotrophic bacteria, delayed the increase in TVB-N content and pH value while significantly reducing lipid oxidation [80]. Xu et al. [81] evaluated the preservative effect of two gelatin coatings, the first combined with ginger and the second with garlic EO for turbot fillets stored for 20 days at 4 °C. The results showed the ability of garlic EO coating to prolong the shelf life of fresh fish. After incubation, the total viable counts of bacteria did not exceed 10^6 CFU/g, the TVB-N concentration was approximately 20 mg/100 g of product, and the hardness decreased by approximately 53% compared to the control samples [81].

To the best of our knowledge, the use of an AHL-degrading enzyme for fish preservation has only been evaluated in the work of Gui et al. [82]. A combination of bacteriocin nisin, AiiAAI96 AHL-lactonase, and vacuum packaging was used to preserve chilled stur-

geon fillets. The treatments acted synergistically in inhibiting the growth of psychrotrophs and delaying food spoilage; they extended the shelf life of the fish samples by 5 days [82].

5. Future Work and Conclusions

QS plays a prime function in regulating fish spoilage by *Pseudomonas* spp. Thus a proper and in-depth understanding of the QS system in those bacteria is very much essential. Of particular interest are studies that will clarify the following issues related to QS inhibition: (i) what are the best conditions for QS inhibiting agents in controlling bacterial phenotypes? and (ii) whether QS inhibiting agents impact beneficial microflora in food ecosystems. In addition, more than one AHL-mediated QS system occurs in a particular strain, which are all involved in metabolic activity regulation. Some investigated QS inhibiting agents show high target specificity. These agents present a challenge in developing approaches that influence a broad range of AIs of *Pseudomonas* spp. Further investigations should focus on studying the "universal" anti-QS agent that targets a broad range of AIs to inhibit QS activity efficiently. A combination of the anti-QS approach with other biocontrol treatments to obtain a synergistic effect is a promising strategy that could increase the susceptibility of pseudomonads to the conventional preservative treatment of seafood.

In this minireview, a summary of the results related to the contribution of QS in the metabolic activities of *Pseudomonas* spp. is provided. QS inhibiting agents with examples of their application in seafood products were also described. With the increasing amount of information available, QS-based strategies can be used more effectively to extend the shelf life of perishable fish products and as innovative strategies for controlling foodborne *P. aeruginosa*.

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