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Review

Applications of Antimicrobial Photodynamic Therapy in Aquaculture: Effect on Fish Pathogenic Bacteria

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Abstract: Increased infectious diseases and the reduced effectiveness of antibiotics due to antimicrobial resistance pose global challenges affecting the aquaculture industry. As bacteria increasingly develop antibiotic resistance, research scientists are shifting their focus to technologies such as antimicrobial photodynamic therapy (aPDT), which show potential for treating and controlling fish infections without promoting the development of resistant bacteria. Various photosensitizers (PSs), both natural and synthetic, are under investigation for their application in aPDT within the aquaculture industry. This shift is crucial for the sustainability of the aquaculture industry, which plays a significant role in achieving several of the United Nations (UN) Sustainable Development Goals (SDGs). This review highlights the application of aPDT against fish pathogens in the industry and the types of PSs utilized. It also explores the potential application of this technique for treating and controlling fish infections, along with the advantages and limitations of its use in aquaculture production systems. Finally, a conclusion and future perspectives are provided.

Keywords: antimicrobial photodynamic therapy; infections; aquaculture; pathogens; photosensitizer

Key Contribution: Antimicrobial photodynamic therapy (aPDT) using natural and synthetic photosensitizers (PSs) has shown antimicrobial activity against fish pathogenic bacteria, including multidrug-resistant ones. As a result, aPDT can be applied in the aquaculture industry to treat and prevent fish infections. Self-disinfecting surfaces utilizing aPDT can be designed to control bacterial pathogens on floors, pond surfaces, and utensils. Recirculating aquaculture systems can also incorporate PS-embedded membranes for aPDT disinfection in water. Thus, aPDT can play a vital role in advancing the sustainability of the aquaculture industries while supporting the United Nations Sustainable Development Goals (SDGs) 2, 3, and 6.

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1. Introduction

Global aquaculture production is on the rise, driven by the continual decline of natural fish populations in the oceans and the high demand for seafood, especially fish, due to rapid human population growth [1]. Despite this growth, the industry faces significant challenges due to substantial production and financial setbacks resulting from fish infections caused by various microbial pathogens, notably multidrug-resistant bacteria [2]. Several fish pathogenic bacterial infections, due to *Aeromonas*, *Edwardsiella*, *Flavobacterium*, *Pseudomonas*, *Yersinia*, *Vibrio*, *Stenotrophomonas* and *Streptococcus* species, have been reported. Additionally, significant increases in bacterial resistance to various antibiotics have been observed in fish farms attributed to the excessive use, incorrect prescription and/or over-prescription of antibiotics [3]. For instance, Pandey et al. reported the resistance of *Vibrio* species (*Vibrio proteolyticus*, *V. campbellii*, *V. nereis*, *V. cincinnatiensis*, and *V. harveyi*) to antibiotics ampicillin and cephalexin [4]. Adah et al. reported multiple antibiotic resistance index values between 0.20 and 0.80 from *Aeromonas* strains (*Aeromonas caviae*, *A. veronii*, *A. hydrophila*, *A. dhakensis* and *A. enteropelogenes*) isolated from *Clarias gariepinus* (40.9% isolated from diseased fish,

and 25% from healthy fish) acquired from farms that intensively utilized antibiotics [5]. Unfortunately, these resistance determinants can be transferred to other animal and human pathogenic bacteria [6], making antibiotic resistance a global challenge to aquaculture and human health.

Consequently, there is an urgent need for effective strategies to manage and control fish infections to enhance the sustainability of the aquaculture sector. Aquaculture strengthens food systems, improves livelihoods, and enriches nutrition [7], thereby supporting Sustainable Development Goal (SDG) 2 (Zero Hunger), which aims to achieve food security and improved nutrition.

For sustainable aquaculture production, there is a need for alternative technologies for controlling fish pathogenic infections. Antimicrobial photodynamic therapy (aPDT) has recently emerged as one of the technologies that can be successfully applied for the treatment of bacterial diseases and for the prevention of antibiotic resistance [8,9]. This review aims to give an overview of the use of aPDT against fish pathogenic bacteria. It accentuates both the natural and synthetic PSs reported against these fish pathogenic bacteria, together with the possible application of aPDT for both treatment and disease control in aquaculture, showing the potential of innovative designs towards the development of sustainable aquaculture.

1.1. Antimicrobial Photodynamic Therapy

Antimicrobial photodynamic therapy is a treatment that uses chemicals called photosensitizing agents, together with light, in an oxygen-rich environment to kill pathogenic bacteria [10,11]. aPDT involves the application of a photosensitizer (PS), which, after cellular uptake by bacteria, is irradiated with light (of appropriate wavelength), promoting the PS from the ground state to an excited state (which is unstable and can change spin), thus undergoing intersystem crossing (ISC) to a triplet state. The triplet state PS can react in type 1 or type 2 reactions [12,13]. In type 1 reactions, the PS can transfer electrons to the surrounding molecules inside the bacterial cells, producing free radicals that interact with molecular oxygen (O_2) in cells, giving rise to reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl (·OH) radicals [11–13] (as illustrated in Figure 1).

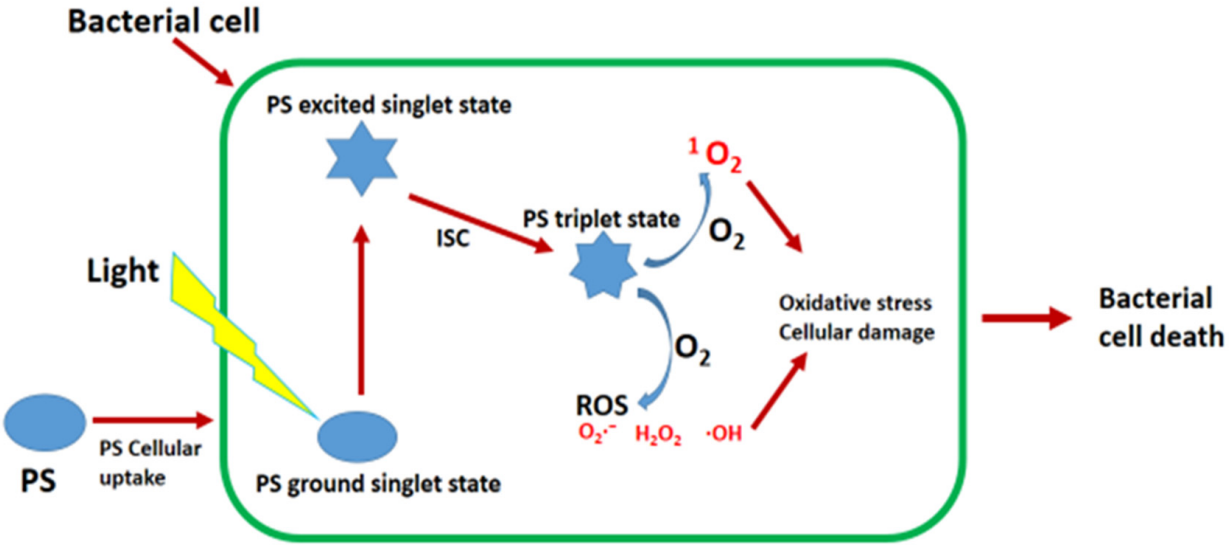


Figure 1. Illustration of the conversion of molecular oxygen to toxic singlet oxygen and ROS after excitation of PS to a triplet state on irradiation with light of specific wavelength. ISC—intersystem crossing.

These ROS react with bacterial biomolecules, causing damage and cell death. In type 2 reactions, the triplet state PS transfers energy to the ground-state molecular oxygen, producing singlet oxygen ($^1O_2$) that interacts with biomolecules in the cell, resulting in cell damage and death [10–13].

1.2. Fish Pathogenic Bacteria and the Effect of Their Membrane Structure on PS Uptake and aPDT Activity

Bacteria, due to their differences, especially in the membrane structure, can be grouped into Gram-negative (with fish pathogens from the *Acinetobacter*, *Aeromonas*, *Edwardsiella*, *Yersinia*, *Piscirickettsia*, *Flavobacterium*, *Photobacterium*, *Pseudomonas*, *Vibrio*, *Moritella*, *Tenacibaculum*, and *Serratia* species) and Gram-positive, (with fish pathogens from *Streptococcus*, *Mycobacterium*, and *Lactococcus* species). The cell wall structure of bacteria influences the aPDT of PSs. The cell wall of Gram-positive bacteria is characterized by a porous layer of peptidoglycan and lipoteichoic acid, which enables PSs to reach the cytoplasmic membrane. As a result, most PSs efficiently inactivate Gram-positive bacteria. For example, Yang et al. reported the aPDT inactivation of the drug-resistant *Mycobacterium marinum* using 5-aminolevulinic acid as a PS [14].

The cell wall of Gram-negative bacteria is characterized by negatively charged lipopolysaccharide, making the interaction with positively charged PSs effective, ultimately causing bacterial inactivation. However, these negatively charged bacterial surfaces impede the neutral or anionic PSs from permeating the cell. As a result, Gram-negative bacteria are resistant to neutral or anionic PS treatment. For example, Caprara et al. demonstrated the high potency of the positively charged PS, meso-tetra(4-N-methyl-pyridyl)porphyrin ($H_2TMePyP^+$) against Gram-negative *Acinetobacter baumannii* compared to the negatively charged PS, meso-tetra(4-sulfonate phenyl)porphyrin (H_2TPPS^-) [15]. Positively charged PSs from tetra(4-pyridyl)porphyrin) cisplatin derivatives have also been reported to be efficient against Gram-positive and Gram-negative bacteria [16]. This shows that the positive charge on the PS makes it effective against both Gram-positive (since the porous nature of the cell wall allows uptake) and Gram-negative bacteria (since the negative bacterial cell wall can interact with the positive charge on the PS).

For practical application of neutral or anionic PSs, the cell wall of Gram-negative bacteria is first destabilized to enhance permeability. For example, Zhao et al. linked protoporphyrin IX to an ethylenediamine derivative for enhanced permeability of *Pseudomonas aeruginosa's* outer membrane. They observed an effective inhibition of *P. aeruginosa* growth both in vitro and in vivo [17]. Strong chelating agents (such as ethylenediaminetetraacetic acid) destabilize membranes by sequestering divalent metals (mostly Mg^{2+} and Ca^{2+}, which are vital for the integrity of the bacteria's outer membrane) from lipopolysaccharides and membrane-associated proteins resulting in increased permeability [18].

Alternatively, cationic groups are attached to the anionic or neutral PS molecule for improved cell uptake. Zhou et al. utilized polymyxin B, a cationic peptide, for improved uptake of the PS curcumin by *P. aeruginosa*, which significantly enhanced its aPDT action [19]. PSs have also been incorporated onto nanoparticles (NPs) for improved uptake by bacteria. NPs, due to their tunable size, composition, and ability to be functionalized, can enhance the PS cellular uptake, thus increasing the therapeutic efficacy [20]. Rupel et al. demonstrated enhanced aPDT activity against *P. aeruginosa* when curcumin was loaded with amphiphilic spermine nanomicelles [21]. Table 1 shows the common fish pathogenic bacteria and the PSs utilized for aPDT.

Table 1. Common fish pathogenic bacteria, PSs used for aPDT against the specific fish pathogenic bacteria and the aPDT disinfected areas in aquaculture.

Pathogenic Bacteria	Bacteria Type	PSs Reported against Fish Pathogens	aPDT Disinfected Areas
Acinetobacter baumannii	Gn	Radachlorin [22], protoporphyrin IX [23] and Methylene blue [23,24], riboflavin, chlorophyllin [25,26], Toluidine blue O combined with TiO_2 and ZnO nanoparticles [27], red-carbon dots [28], aloe-emodin [29], fotoenticine [30], erythrosine [31], Chlorin e6 and Perfluorodecalin nanoemulsion [32], *meso*-tetra(4-*N*-*methyl*-pyridyl)porphyrin [15], 5, 10, 15, 20-trakis(4-((s)-2, 6-diaminohexanamido)-phenyl) porphyrin [33], hypericin nanoparticles combined with D-Tryptophan [34].	*
Aeromonas salmonicida	Gn	5,10,15-tris(1-methylpyridinium4-yl)-20-pentafluorophenylporphyrin tri-iodide (Tri-Py+-Me-PF) [35].	Aquaculture water [35]
Aeromonas hydrophila	Gn	Curcumin [36], 2,(3),9(10),16(17),23(24)-Tetrakis-[(2-pyridyloxy) hthalocyaninato] Palladium (II) and 2,(3),9(10),16(17),13(24)-Tetrakis-{[(2-(N-methyl)pyridyloxy]phthalocyaninato} Palladium (II) Sulphate [37], 1(4),8(11),15(18),22(25)-Tetrakis-[(2-pyridyloxy)phthalocyaninato]nickel (II) [38], tetrakis-(3-methylpyridyloxy-phthalocyanine Zn(II) [39].	*
Flavobacterium hydatis	Gn	Palladium phthalocyanines with methylpyridiloxy groups linked peripherally or non-peripherally [40]	*
Photobacterium damselae	Gn	5,10,15-tris(1-methylpyridinium4-yl)-20-pentafluorophenylporphyrin tri-iodide (Tri-Py+-Me-PF) [35]	Aquaculture water [35]
Pseudomonas aeruginosa	Gn	Toluidine blue-carbon nanotube conjugate [41], Radachlorin [42], methylene blue [43,44], methylene blue-polymyxin conjugate [45], Temoporfin [44], prodigiosin [46], curcumin [19,47], Rose Bengal alone and when combined with methicillin [48], metal phthalocyanines [M = Zn(II), Cu (II), Co(II), In(III), and Lu (III)] bearing chlorine and dipentylmalonyl groups on peripheral positions and hexyloxy groups on non-peripheral positions [49], tetrasulfonated hydroxyaluminum phthalocyanine [50], zinc phthalocyanine-colistin conjugate [51], meso-tetra(3-N-methylpyridyl)porphyrin and meso-tetra(4-N-methylpyridyl)porphyrin [52], isomeric meso-tetra (pyridyl)-substituted porphyrin (5,10,15,20-Tetra(4-pyridyl)porphyrin (TPyP)) derivatives of cisplatin (3-cis-Pt-TPyP and 4-cis-Pt-TPyP) [16], dimethyl-8,13-divinyl-3,7,12,17-tetramethyl-21H, 23H-porphyrin-2,18-bis[-N-2-(dimethylamine)ethyl] propenamide [53], 5,10,15,20-tetrakis(N-4-methylpyridyl)porphyrin and 5,10,15,20-tetrakis[(N-4-methylpyridyl)porphyrinate]zinc(II) [54], N, N′-bis (2-aminoethyl)-2,7,12,18-tetramethyl-3,8-divinyl-21H, 23H-porphyrin-13,17-bispropanamide porphyrin [17], 5,10,15,20-trakis(4-((S)-2,6-diaminohexanamido)-phenyl) porphyrin [55], sulfonated polystyrene nanoparticles with encapsulated 5,10,15,20-tetraphenylporphyrin [56], 7(12)-(1-methoxyethyl)-12(7)-(1-hydroxyethyl)-3, 8, 13, 17-tetramethyl-21H, 23H-porphyrin-2, 18-dipropionic acid [57], 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphyrin [58], [13,17-bis(1-carboxyethyl)carbamoyl(3-methylpyridine)-3-(1,3-dioxane-2-yl) methylidene-8-ethenyl-2-hydroxy-2,7,12,18-tetramethyl chlorin, diN-methy iodide [59], meso-tetrakis(1-undecyl-3-pyridyl)bacteriochlorin tetrabromide, meso-tetrakis [1-(4′-bromobutyl)-3-pyridyl]bacteriochlorin tetrabromide, meso-tetrakis[1-(4′-pyridiniobutyl)-3-pyridyl]bacteriochlorin octabromide, Meso-tetrakis(1-heptyl-3-pyridyl)-bacteriochlorin tetrabromide, meso-tetrakis[1-(2′-bromoethyl)-3-pyridyl]-bacteriochlorin tetrabromide, meso-tetrakis[1-(2′-pyridinioethyl)-3-pyridyl]bacteriochlorin octabromide [60], boron-dipyrromethenes (BODIPYs) [61].	*
Pseudomonas putida	Gn	trans-AB-porphyrin, trans-AB-porphyrin-gelatin nanoparticle conjugate [62], Rose bengal-gold nanoparticle conjugate, pyridyl porphyrin-gold nanoparticle conjugate [63], Rose Bengal-gelatin nanoparticle conjugate [64]	*
Vibrio aestuarianus	Gn	Curcumin, methylene blue and eosin Y [65]	Microalgae feed and tank seawater [65]
Vibrio alginolyticus	Gn	Curcumin [66]	*
Vibrio anguillarum	Gn	5,10,15-tris(1-methylpyridinium4-yl)-20-pentafluorophenylporphyrin tri-iodide (Tri-Py+-Me-PF) [35]	Aquaculture water [35]

Table 1. *Cont.*

Pathogenic Bacteria	Bacteria Type	PSs Reported against Fish Pathogens	aPDT Disinfected Areas
Vibrio campbellii	Gn	Tetra-cationic 5,10,15,20-tetrakis (1-methyl-4-pyridinio) porphyrin tetra (p-toluenesulfonate) [TMPyP] [67,68], curcumin [69]	Microalgae feed [67] water [68]
Vibrio cholerae	Gn	Curcumin [69]	Water [69]
Vibrio fischeri	Gn	5,10,15-tris(1-methylpyridinium-4-yl)-20-(pentafluorophenyl)porphyrin tri-iodide (Tri-Py+-Me-PF) [70]	aquaculture water [70]
Vibrio harveyi	Gn	Rose Bengal [71], curcumin [69]	larviculture systems [71], water [69]
Vibrio parahemolyticus	Gn	methylene blue [72], curcumin [36,73], 5,10,15-tris(1-methylpyridinium4-yl)-20-pentafluorophenylporphyrin tri-iodide (Tri-Py+-Me-PF) [35], 5,10,15,20-Tetrakis[*N*-methyl-4-pyridyl)porphyrin and 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin [74]	Aquaculture water [35,74]
Vibrio splendidus	Gn	Curcumin, methylene blue and eosin Y [65]	Microalgae feed and tank seawater [65]
Vibrio vulnificus	Gn	Toluidine blue O [75]	*
Serratia marcescens	Gn	Rose Bengal [76], toluidine blue [77], methylene blue [78], meso-tetra(3-N-methylpyridyl)porphyrin and meso-tetra(4-N-methylpyridyl)porphyrin [52], trans-AB-porphyrin, trans-AB-porphyrin- gelatin nanoparticle conjugate [62].	*
Stenotrophomonas maltophilia	Gn	Riboflavin and chlorophyllin [26],	*
Mycobacterium marinum	Gp	5-aminolevulinic acid [14,79]	*

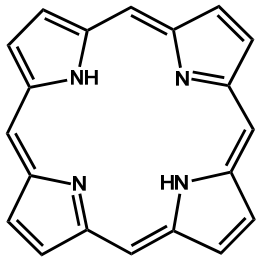
* Information not available Gn, Gram-negative; Gp, Gram-positive.

2. Photosensitizers Utilized for aPDT against Fish Pathogens

Different types of PSs (Table 1) have been reported against fish pathogenic bacteria. These include porphyrins, phthalocyanines, chlorins, methylene blue [23,24], riboflavin, chlorophyllin [25,26], toluidine blue O [27], red-carbon dots [28], aloe-emodin [29], fotoenticine [30], erythrosine [31], chlorin e6 (Ce6), perfluorodecalin (FDC), 5-aminolevulinic acid, Rose Bengal, Curcumin, and eosin Y.

2.1. Use of Porphyrins as PSs against Fish Bacterial Pathogens

Porphyrins (1) are a group of natural or synthetic aromatic heterocyclic compounds made of four pyrrole rings joined by methine bridges.



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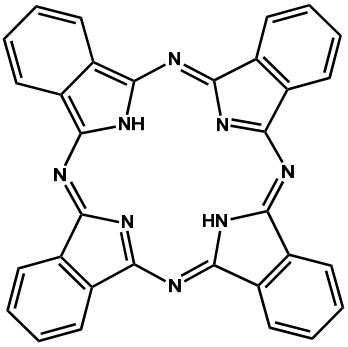
The aromatic macrocyclic ring can be substituted, and the molecules can be neutral, cationic or anionic. Due to their ability to interact with light and the high ROS production rate, these molecules can be photosensitizers in aPDT. Several fish pathogens have been inactivated using porphyrins as PSs in aPDT [15–17,35,52–58,62,67,68,70,74]. Cationic porphyrins are more active as PSs against Gram-positive and Gram-negative bacteria than anionic or non-ionic PSs. Work by Banfi et al. showed that positively charged groups of

porphyrins are important for PS antibacterial activity [80], as they promote PS permeation into the cell. Hence, cationic groups (including antimicrobial peptides) or cell-penetrating groups are now linked to porphyrins (especially anionic or non-ionic) to increase their efficiency. Karir et al. functionalized the porphyrins with gelatin nanoparticles (NPs) and observed efficient aPDT against both Gram-negative (*Serratia marcescens* and *Pseudomonas putida*) and Gram-positive bacteria (*Bacillus subtilis*) [62], which could be attributed to the increased permeability of the bacterial cells as a result of the linked NPs.

Interestingly, 5-Aminolevulinic acid, a precursor in the synthesis of porphyrins, has aPDT properties. This molecule demonstrated efficacy as a PS against the Gram-positive *Mycobacterium marinum* [14]. Wenlong et al. reported the treatment of *M. marinum* skin infections using 5-aminolevulinic acid-photodynamic therapy combined with fractional CO_2 laser ablation (where a 635 nm laser, with an output power of 80 mW/cm^2 was irradiated for 20 min) [79].

2.2. Use of Phthalocyanines As PSs against Fish Bacterial Pathogens

Phthalocyanines (Pcs (2)) are structurally related to porphyrins, being a benzoporphyrin consisting of nitrogen instead of carbon-bridging atoms.



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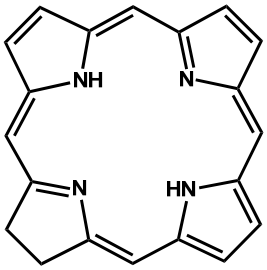
Pcs that are symmetrical and unsubstituted are mostly water-insoluble and aggregate (reducing the singlet oxygen generation), making their application for aPDT in aqueous media (such as in aquaculture) difficult [81]. Their structure can, however, be modified by introducing central metals and substituents on the axial, peripheral and non-peripheral positions [82]. This modification can also alter their photophysicochemical properties for enhanced singlet oxygen generation. Pcs have, as a result, shown aPDT against fish pathogenic bacteria [37–40,49–51]. For example, Mantareva et al. reported the inactivation of *Aeromonas hydrophila* and *Flavobacterium hydatis* using Pd(II) and Zn(II)-phthalocyanine complexes (activated using a 665 nm light source with an output power of 100 mW cm^{-2}, and at a light dose of 50 J·cm^{-2} for 20 min) [37,40].

Just like porphyrins, Pcs with cationic groups are more aPDT effective than neutral or anionic ones. Quartenisation of Pcs has been shown to introduce positive charges, increase water solubility and reduce aggregation, resulting in high singlet oxygen generation. For example, Günsel et al. showed that quaternization of 2(3), 9(10), 16(17), 23(24)-tetrakis (4-(dimethylamino)benzyloxy) metal-free, zinc (II) and cobalt (II) phthalocyanines imparted water solubility and enhanced the antibacterial activity [83], which could be attributed to enhanced singlet oxygen and ROS generation.

Linkage of PS to antibiotics, antibodies, peptides and nanocarriers has been shown to enhance the aPDT activity against bacterial pathogens. For instance, Bayat et al. (using laser light with the wavelength of 660 nm and an exposure time of 15 min), showed that a zinc phthalocyanine (ZnPc) linked to an antibiotic colistin has efficient aPDT activity against *P. aeruginosa* compared to the ZnPc alone [51].

2.3. Use of Chlorins as PSs against Fish Bacterial Pathogens

Chlorins (3), being derivatives of chlorophyll, are partially hydrogenated porphyrins consisting of three pyrrole rings and one pyrroline ring.



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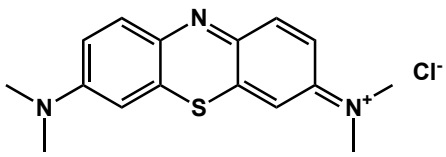
These molecules have displayed aPDT against fish pathogens [22,30,32,42,44,59,60]. aPDT using Radachlorin as a PS (and a 660 nm diode laser at an energy density of 5 J/cm^2 for about 13 min) was reported to significantly eliminate *A. baumannii* biofilm, with electron microscopy showing an abnormal structure due to the loss of the cell membrane [22]. Niu et al. made similar observations using Chlorin e6 and Perfluorodecalin nanoemulsion [32]. Since negatively charged bacterial surfaces impede the PS from permeating the cell, nanostructures such as the perfluorodecalin nanoemulsion enhance the PS cellular uptake due to their small size. Additionally, perfluorodecalin, due to its high affinity to oxygen, increases the oxygen in cells, ultimately increasing the levels of $^1O_2$ during aPDT [32].

2.4. Use of Methylene Blue and Toluidine Blue as PSs against Fish Bacterial Pathogens

Methylene blue (4) and toluidine blue (5) are cationic phenothiazine derivatives with a similar chemical structure together with photochemical properties.



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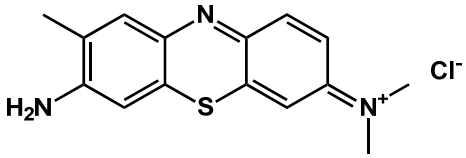

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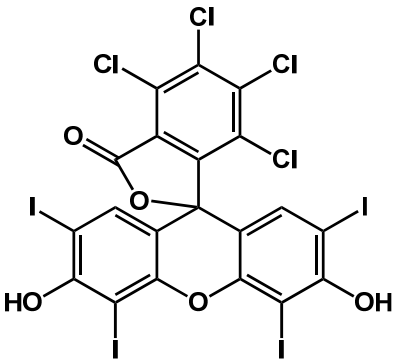
These molecules have, as a result, been utilized as PSs for aPDT [23,24,27,41,43,44,65,75,77,78]. Methylene blue mediated aPDT against *S. marcescens* has been shown to decrease the bacterial pathogen's quorum sensing-mediated virulence factor, together with the ability to form a biofilm [78]. A combination of these PSs and antibiotics has been shown to yield better results compared to the use of the PSs in aPDT alone or the use of antibiotics alone. For example, linkage of the PS, methylene blue with polymyxin has been reported to destroy 100% of the Gram-negative bacteria, especially *P. aeruginosa* (using a 630 nm light source with an output power of 40 mW cm^{-2} for 10 min) [45].

The uptake and internalization of the PS by the bacterial cells is paramount for effective aPDT. Linkage of toluidine blue to multiwalled carbon nanotube enhanced the PS uptake and delivery to the *P. aeruginosa* biofilm, enhancing the aPDT effect [41]. Boluki et al. also observed enhanced aPDT activity on *A. baumannii* attributed to the enhanced uptake of toluidine blue when linked to titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles [27].

2.5. Use of Rose Bengal and Eosin Y as PSs against Fish Bacterial Pathogens

Rose Bengal (6) and eosin Y (7) are xanthene molecules that have been utilized as PSs for aPDT against fish pathogenic bacteria [48,63–65,71,76].



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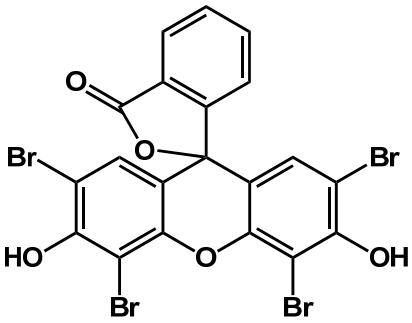

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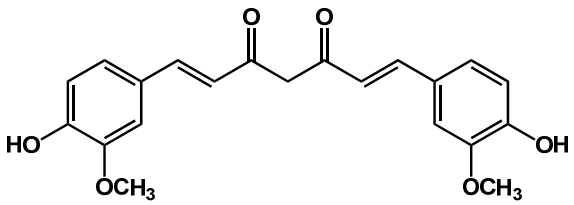
Rose Bengal and eosin Y are tetrachloro-tetraiodo and tetrabromo derivates of fluorescein, respectively, with three linearly arranged aromatic rings and an oxygen atom at the center of the ring. These molecules have displayed high singlet oxygen quantum yield; however, their hydrophilic and anionic nature limits them from crossing the hydrophobic cellular membrane, especially in Gram-negative bacteria, ultimately reducing cell uptake and application in aPDT [84]. For enhanced cellular uptake, Rose Bengal or eosin Y can be linked to NPs as nanocarriers. Due to their small size, NPs can easily cross cell membranes. For instance, when Rose Bengal is linked to gelatin NPs, it shows potent and broad-spectrum antimicrobial activity, even against fish pathogens such as *S. marcescens* and *P. putida* [64]. Notaro et al. demonstrated the capability of eosin Y for aPDT (in both fish feed and sea water) against *Vibrio* species when eosin Y was irradiated with a light-emitting diode (LED) (435–445 nm) for 5 min [65]. The aPDT of Rose Bengal and eosin Y demonstrates the potential of fluorescein derivatives for application in the disinfection and treatment against fish pathogenic bacteria.

2.6. Natural Occurring PSs Used against Fish Bacterial Pathogens

Curcumin (8), a naturally occurring molecule extracted from the roots of *Curcuma longa* L. (turmeric), in addition to medicinal properties, has shown great potential as a PS for aPDT [85].



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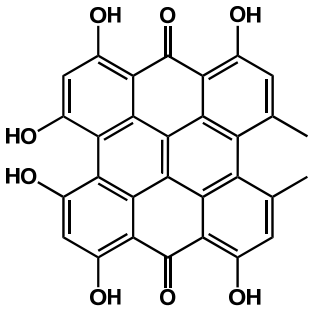
As shown in Table 1, this natural product has been greatly applied even against fish pathogenic bacteria [19,36,47,65,66,69,73]. For instance, Moideen et al. demonstrated the aPDT of curcumin against *V. cholera* and *V. harveyi* (on exposure to a 405 nm (10 mW cm^{-2}) LED for 60 min). However, *V. campbellii* was resistant to aPDT, which can be attributed to the low affinity of the *V. campbellii* cell wall for curcumin [69]. Nanomaterials, cell wall destabilizing molecules and cationic groups can be utilized to enhance permeability [17,19,21]. Due to the proven safety of curcumin for humans, it can be safely applied in aquaculture for aPDT treatment of diseases in fish caused by pathogenic bacteria.

Hypericin (9) is another naturally occurring molecule extracted from *Hypericum perforatum* L. (St. John's wort). Its photodynamic activities as a photosensitizer have drawn interest. However, as a naphthodianthrone, the poor solubility in water and aggregation makes its bioavailability in living organisms poor [86].



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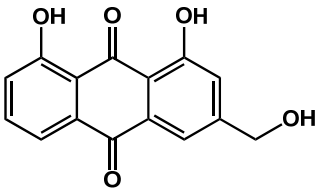
Nevertheless, just as with other PS, this can be improved by linking it with other compounds or groups for improved uptake by cells. Pourhajibagher et al. nanosized hypericin for enhanced uptake as a PS for antimicrobial photo-sonodynamic therapy (aPSDT). Upon combining hypericin nanoparticles-aPSDT with D-Tryptophan, a synergistic antibacterial effect against *A. baumannii* was observed (using a 450 nm blue light and an energy density of 25.56 J/cm^2 for 60 s) [34].

Aloe–emodin (10), a naturally occurring anthraquinone compound obtained from herbs like *Aloe vera*, *Cassia occidentalis* and *Rheum palmatum*, to mention a few, is reported to possess a wide range of medicinal properties, making it attractive for disease treatment.



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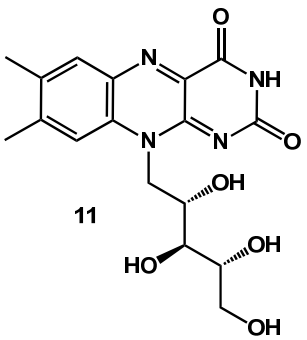
Due to its structural similarity to hypericin, together with its ability to absorb light in the UV-visible region, it has drawn attention as a PS for aPDT [87]. Work by Wang et al. showed that aloe–emodin-mediated aPDT is effective against infections caused by *A. baumannii* [29]. The effectiveness could be a synergistic effect of the antibacterial aloe–emodin and its aPDT effect.

Riboflavin (11), a naturally occurring, water-soluble vitamin, due to its photosensitizing properties, can be utilized for aPDT.



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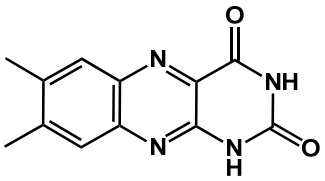
However, this compound photodegrades, reducing its effectiveness as a PS. It has been reported, however, that some of its photodegradation products, such as lumichrome (12), are photosensitizers [88], which could be advantageous for aPDT.



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Inactivation of *A. baumannii* has been reported using riboflavin–aPDT (on exposure to 440 nm blue LED light at 45 J/cm^2 for 60 min) [26], showing the potential of riboflavin to be applied as a PS in aquaculture.

Most natural photosensitizers are acquired from edible plants; hence, they are less toxic and environmentally benign [89]. Their application in aquaculture could reduce the environmental challenges caused by synthetic counterparts. However, the low solubility, aggregation, low triplet quantum yield, and singlet oxygen production limit the application of most natural photosensitizers in aPDT [89,90]. Nonetheless, natural PSs could be modified to yield better aPDT properties, making them lead compounds for developing new synthetic PSs [90].

3. Application of aPDT in Aquaculture

aPDT is a promising technology for application in the aquaculture industry in the treatment and prevention of fish infections caused by microbial pathogens, including the multidrug-resistant ones.

3.1. Treatment of Fish Infections

The application of aPDT for addressing deep tissue infections is constrained, with only a few documented attempts involving animal models like mice [91]. However, there is a noticeable lack of scientific research attention on the treatment of fish diseases through aPDT, which may be attributed to the localized delivery of visible light. Alves et al. proposed that fish with superficial infections, ulcers or lesions can be incubated in the dark and in water with dissolved PS. Subsequent irradiation with light should result in total recovery [92], as illustrated in Figure 2. However, this area of research still needs to be thoroughly investigated, considering the sensitivity of fish to changing environmental conditions, especially sudden changes in water conditions.

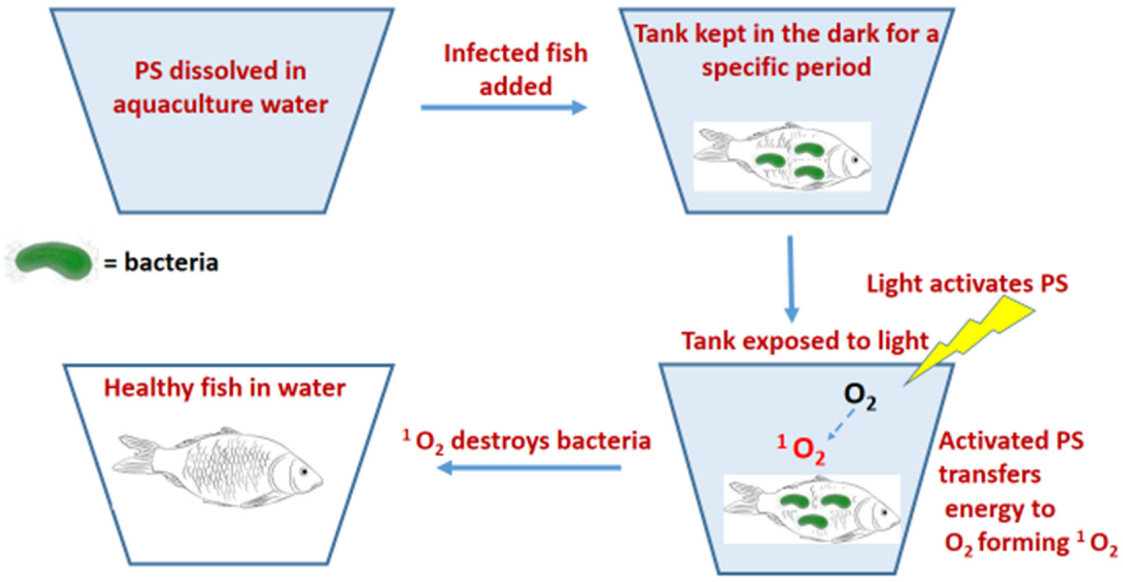

Figure 2. Illustration of the proposed treatment of localized fish infections using aPDT in aquaculture.

3.2. Prevention of Fish Infections

Effective health management of fish always includes prevention of disease instead of treatment. This involves measures that reduce pathogenic microbes in the environment, such as the continuous cleaning and disinfection of aquaculture water, good fish nutrition and good sanitation [93].

3.2.1. Disinfection of Aquaculture Water

Water quality is of importance in fish farms as it impacts the growth and health of fish. Fish not only live but feed and excrete waste in water. The residual feed, defecated waste and other water pollutants afford microorganisms favorable habitats. The water must be regularly cleaned and disinfected to reduce microbial infections. aPDT has been utilized for the disinfection of aquaculture water [35,65,68–70,74] by adding the PS to water or embedding the PS in a solid membrane [94] before irradiation with light of an appropriate wavelength. The irradiated light should be able to penetrate into the water (thus, water should be free of suspended matter) for the activation of the PS, and the water should be sufficiently oxygenated for increased singlet oxygen production [95]. Decontamination of water is done before adding it to fish tanks.

Magnetic nanoparticles have been linked to PSs for easy recovery of the PSs from the water matrix using a magnet, allowing for recycling and reuse [96,97]. Figure 3 illustrates the use of a magnetic PS in disinfecting aquaculture water, together with the recovery of the PS after disinfection using a magnet.

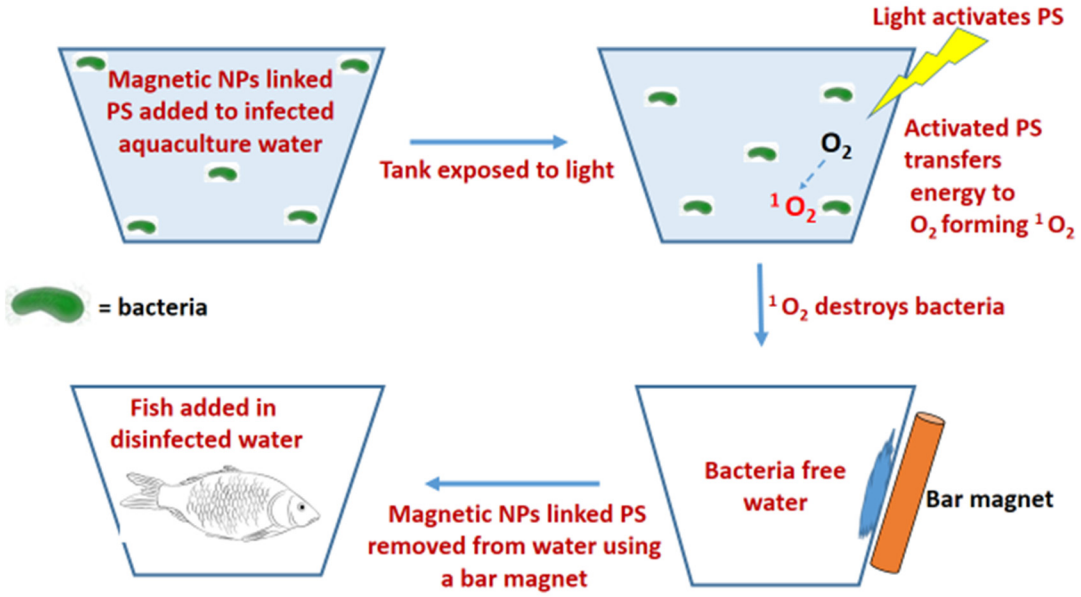

Figure 3. Illustration of the disinfection of aquaculture water using magnetic PS and recovery of the PS after disinfection using a magnet.

Dube et al. demonstrated the photoinactivation of *Staphylococcus aureus* and *Escherichia coli* using a magnetic PS (phthalocyanine linked to iron oxide nanoparticles), together with the recyclability of the PS [9]. PSs have also been immobilized on multi-walled carbon nanotubes for enhanced antibacterial activity (through photodynamic and photothermal therapy) while allowing for recovery and reuse in water and surface disinfection [98], making them cost-effective and environmentally friendly. For sustainability, natural light from the sun can be considered as it can penetrate deep and be utilized for large ponds [92]. This was demonstrated by Majiya et al. using 5, 10, 15, 20-tetrakis (1-methyl-4-pyridinio) porphyrin tetra *p*-toluene sulfonate embedded on a chitosan membrane [99].

3.2.2. Disinfection of Aquaculture Surfaces, Vessels, and Equipment

Disinfection of surfaces and equipment is a preventative measure to avoid opportunistic pathogens, including their transmission within the aquaculture facilities. After cleaning, surfaces, vessels, and equipment can be exposed to the PS and light for bacterial inactivation through aPDT [100]. Self-disinfecting surfaces that utilize aPDT have been reported [101]. For instance, Harada et al. utilized a phenoxy-substituted zinc phthalocyanine as a PS to fabricate cellulose acetate self-disinfecting films, which continuously generated $^1O_2$ for over 6 months under continuous exposure to room light [102]. Self-disinfecting aPDT coatings can, thus, be applied on aquaculture surfaces for room or natural light activation.

3.2.3. Disinfection of Aquaculture Feed

Aquafeed is formulated from plants and animal sources, including seaweed and microalgae. These can be contaminated with bacteria before harvesting, during drying, processing, packaging, storage and transportation [103]. To avoid the introduction of bacterial pathogens into the aquaculture production systems, the fish feed needs to be disinfected. aPDT has shown potential for the disinfection of fish feed. Malara et al., using the PS, 5, 10, 15, 20-Tetrakis (1-methyl-4-pyridinio) porphyrin tetra (p-toluenesulfonate), demonstrated the ability of aPDT to disinfect microalgal (*Tisochrysis lutea*, *Tetraselmis chui*, *Chaetoceros muelleri* and *Picochlorum atomus*) aquafeed contaminated with *Vibrio campbellii*. The microalgal aquafeed containing the PS was irradiated with a 465 nm LED light (1.179 mW cm^{-2}) for 6 h [67]. Notaro et al. also confirmed the ability of aPDT to destroy *V. splendidus* in both microalgal feed and tank water, as the levels of *V. splendidus* DNA and RNA in oysters fed with the aPDT-treated microalgae were reduced compared to those of oysters fed with an untreated diet [65].

4. Advantages and Limitations of Using aPDT in Aquaculture

4.1. Advantages of aPDT

aPDT, being a non-antibiotic strategy for the treatment and prevention of infectious diseases, has several advantages over the use of antibiotics, since antibiotics result in increased antibiotic-resistant bacteria together with the presence of residual antibiotics in food products.

aPDT has proven effective due to its broad spectrum of action since it can prevent infections caused by diverse organisms such as protozoa, viruses, fungi, parasites, and bacteria [104]. This is because aPDT generates highly reactive oxygen species (ROS) that oxidize cellular components, rapidly inactivating the cells of these infectious organisms [12]. aPDT can thus be used to control not only bacterial infection but a range of microbial infections.

aPDT has been shown to be effective against dangerous antibiotic-resistant bacteria [105]. Additionally, there are no reports of the development of resistance against the PS, even after multiple therapy sessions. This could probably be because, unlike antibiotic therapy, aPDT treatment is too short for resistance to develop. Additionally, the generated ROS species can oxidize numerous targets on the bacterial cell structure and components, unlike antibiotics, which work on a specific target [106].

For the treatment of infections in fish, aPDT can be designed to have minimal effects and damage to the host tissue through PS and light dosage control, as well as the targeting of infected areas, ensuring that only pathogens are destroyed [107]. Due to the short lifetime and high reactivity of singlet oxygen, photo-oxidative damage is restricted to the exposed infected parts [108].

Since the PS can also be embedded into polymers [109], aquaculture production systems that can kill microbes on their surfaces when activated by visible light can be designed. The self-disinfecting systems can reduce the spread of disease-causing microbes [110] during production and processing. Additionally, natural light can be utilized for PS activation, making aPDT technology cost-effective. Embedding the PS onto polymeric materials pre-

vents the release of the PS into the environment and promotes the reuse of the embedded PS [94,111].

aPDT can be combined with other technologies for enhanced efficiency. Combination therapy involving aPDT and antibiotics can be utilized, as aPDT therapy has been shown to induce damage to bacterial cell membranes, making the bacteria more susceptible to antibiotic treatment [95]. Combining aPDT with nanomaterials not only enhances uptake by bacteria but can also lead to the synergistic effect of aPDT and photothermal therapy (PTT), as specific nanomaterials generate localized high temperatures upon light absorption [112,113].

4.2. Limitations of aPDT

aPDT, though with several advantages, has limitations which reduce its applicability in aquaculture.

The application of aPDT for treating fish infections can only be restricted to infected fish parts where light can reach, such as the skin. It will not work for systemic infections due to poor light accessibility [114].

Light penetrability is important for aPDT; hence, in aquaculture, the technique can only work in water that can allow light through (water should be clear and free of debris) [92]. Considering that both faecal matter and uneaten food make the water in ponds murky, the material should be trapped and removed from the system for this technique to be effective. Thus, recirculating aquaculture systems might be required to continuously clean and disinfect aquaculture water.

aPDT is meant to target infectious microorganisms. However, it also damages unintended targets, such as fish tissue and beneficial microorganisms, due to its non-selectivity, which could manifest as side effects such as redness, swelling, and other allergic reactions [90].

Poor solubility and aggregation of some PSs limits their application in aPDT [90].

5. Conclusions and Future Perspectives

aPDT can inactivate fish pathogenic bacteria (especially Gram-negative bacteria). Although there are no reports of its application in the treatment of fish infections, this technology has the potential for use in aquaculture, especially for surfaces, equipment and aquaculture water disinfection, as it has shown advantages compared to traditional antibiotics. This technology can be effective in water if utilized under recirculating aquaculture systems. This will enable the continuous removal of sludge, allowing for aPDT treatment of water and, ultimately, water reuse.

Though not reported in aquaculture, self-disinfecting surfaces utilizing aPDT can go a long way in managing bacterial pathogens. PS can be embedded in surface coating materials for activation by light, including sunlight. The possibility of using sunlight and natural PSs could make the technology cheaper. Additionally, the technology can reduce PS release to the environment and allow for the re-use of the PS.

Though some limitations of aPDT, especially when using natural PS, have been reported, ways to overcome these limitations can be designed. For instance, the use of several PSs simultaneously can enhance antimicrobial effects [115]. The PSs combined may exhibit different photophysical and photochemical characters that complement each other. Additionally, aPDT has been combined with other antimicrobial therapies. The application of aPDT in the aquaculture industry still requires significant research and development efforts to ensure its efficacy, safety, and scalability.

This aPDT treatment not only supports aquaculture microbial treatment but also promotes the United Nations SDG2, 3 and 6 through promoting food security, human health and wellbeing, improving nutrition, and promoting sustainable aquaculture and infection-free water.

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