Antimicrobial photodynamic therapy and its applicability in aquaculture systems and aquatic animal health management: An overview

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Abstract: Global aquaculture production in 2012 touched new high of 90.4 million tonnes including 66.6 million tonnes of fish food and 23.8 million tonnes of aquatic algae providing 19.2 kg per capita food fish supply. Aquaculture is reported to suffer heavy production and financial losses due to fish infections caused by microbial pathogens. Therefore in order to make aquaculture industry more sustainable, effective strategies to control fish infections are urgently needed. Antimicrobial Photodynamic Therapy (aPDT) is an emerging, low-cost anti-microbial approach to the treatment of locally occurring infections and also for the treatment of aquaculture water and waste waters. Already proven effective in various medical and clinical applications, it utilizes three vital components: a photosensitizing agent (PS), a light source of an appropriate wave length and oxygen. aPDT has got a potential of being a preferred choice over antibiotics in aquaculture systems because of its non-target specificity, few side effects, lack of the pathogenicity reversal and re-growth of the micro-organism after treatment and the lack of development of resistance mechanisms. The technique has been proved effective in vitro against bacteria (including drug-resistant strains), yeasts, fungi, viruses, parasites and even the stubborn biofilms. Although preliminary results indicate that this technology has a high potential to disinfect waters in aquaculture system and also in hatcheries and seed production units, but it clearly needs more deep knowledge and multi-dimensional approach.

Keywords: Antibiotic resistance, Antimicrobial photodynamic therapy, Fish farming, Photosensitizer

INTRODUCTION

Fish diseases affect the survival rate and growth of fish. Expensive drug treatments in aquaculture system contribute to higher cost as well as deterioration of environment. Control of infections is difficult in fish farming conditions (Defoirdt et al., 2007). Poor water quality, ubiquitous nature and rapid spreading of pathogens, environmental adverse conditions, high stocking densities, different stages of the fish life cycle, resistance in common pathogenic bacteria, low activity of chemotherapeutic agents against bacterial endospores and fungal zoospores, and few drugs licensed for fishery use are factors that make disease prevention difficult in aquaculture (Almeida et al., 2009). In the same context Antimicrobial Photodynamic Therapy (aPDT) also known as Photodynamic Chemotherapy (PACT) is a recent advanced non-antibiotic approach receiving considerable attention for its potentialities as a new form of antimicrobial treatment (Alves et al., 2011; Benov, 2014; Jui-Teng, 2014). At the very beginning of its history, photodynamic approach was applied in medicine (1903) by Albert Jesionek and Hermann von Tappeiner for the treatment of patients with malignant skin lesions as referred by Lin Jui-Teng, 2014. Although, since the arrival of “the Golden Age of antibiotics” after the discovery of penicillin and massive production of antibiotics in the middle of last century, antimicrobial photosensitizing reactions were largely forgotten (Alves et al., 2009). Moreover, in the last decade huge rise in antibiotic resistance encountered which has driven a need of research to the development of new potent anti-microbial strategies. In the same context, this new methodology has already proved to be effective in vitro against bacteria, viruses, fungi and protozoa (Merchat et al., 1996; Bonnett, 2000; Jemli et
Antimicrobial photodynamic therapy (aPDT) is based on the concept that a photosensitizer (PS) should be preferentially localized in the pathogens and not in the surrounding tissue, and subsequently activated by visible light of the appropriate wavelength to generate reactive oxygen radicals to destroy the microbes non-specifically. Recent studies, in particular, have demonstrated that aPDT can be effective in the selective inactivation of microorganisms and it can become a potential alternative for the treatment and eradication of microbial infections (Hamblin and Hasan, 2004; O’Riordan et al., 2005). Lin Jui-Teng in the year 2014 performed a comprehensive analysis for aPDT in aquaculture systems. To implement this technology in the environment, some additional aspects less relevant to aPDT in the clinic area needs to be considered, namely: 1) the removal of the sensitizer after photodynamic action to avoid the release of the PS to the water output; 2) the determination of the stability of the PS conjugates under sunlight irradiation conditions; 3) the assessment of the impact of this procedure on the natural non-photogenic microbial community structure, for instance when this technology is applied in extensive and semi-intensive fish farming systems; 4) the toxicity of the PS on aquatic organisms (e.g. fish and shellfish) at doses which induce marked mortality of microbial pathogens; 5) the effect of physical and chemical properties of environmental waters on aPDT; 6) the possibility of using natural sunlight as light source; 7) the efficacy of the porphyrin derivatives relatively to that of other PS in aPDT. Effectiveness of photodynamic inactivation has been evaluated for the destruction of faecal bacteria (Wolfsen and Wang, 1993, Konig et al., 2000, Smith et al., 2002, Chen-Collins et al., 2003; Akilov et al., 2007; viruses (Wilder-) and helminthes eggs (Ganz et al., 2005) in nature waters. Studies showed that cell cultures of gram-positive bacteria (e.g. meticillin-resistant S. aureus), gram-negative bacteria (e.g. E. coli), fungi (e.g. C. albicans) and fungal-like pathogens (e.g. Saprolegnia spp.) and parasitic protozoa (e.g. Acanthamoeba palestinensis) in the presence of micromolar PS doses (Schleier et al., 2004). Antimicrobial photodynamic therapy (aPDT) is based on the concept that a photosensitizer (PS) should be preferentially localized in the pathogens and not in the surrounding tissue, and subsequently activated by visible light of the appropriate wavelength to generate reactive oxygen species (ROS) in presence of oxygen that are cytotoxic to the pathogens. The significance of study lies in the fact that aPDT should be delivered to the various stakeholders in aquaculture industry as it is effective in the selective inactivation of microorganisms. In general, all organisms namely viruses, bacteria, protista, yeasts, algae, insects and cultured mammalian cells are sensitive to inactivation by the photodynamic effect (Wainwright, 1998; Makowski and Wardas, 2001; Lukšiene, 2005). The effects of aPDT on microorganisms depend on at least four factors: the concentration of the dye, the concentration of molecular oxygen and the appropriate wavelength and intensity of light (Capella and Capella, 2003). Increasing the concentration of a sensitizer at a fixed light dose leads to increased viral inactivation (Kasturi and Platz, 1992). Efficacy and the production rate of reactive oxygen species was found to be proportional to the PS (MB) concentration and the initial light intensity resulting into decreasing function of the light illumination time due to the depletion of PS concentration (Jui-Teng Lin, 2014). Dissolved oxygen concentration in water plays an important role in the production of the oxidative species required for the photodisinfection process (Aloui and Jenli, 2001). A very wide selection of light sources is available, ranging from basic tungsten-filament lamps to laser technology. However, aPDT uses low-power light rather than the lasers used in ablative therapy as microbial killing is attained with milliwatts rather than tens (or hundreds) of watts (Wainwright, 1998). In terms of molecular structure, molecular charge is important in determining antiviral activity. Thus, it is more likely that positively charged photosensitizers will be effective in causing nucleic acid damage than will neutral or anionic congeners (Wainwright, 2004). The positive charges on the photosensitizer molecule appear to promote a tight electrostatic interaction with negatively charged sites at the outer surface of bacterial cells. This behaviour also appears to apply to nonenveloped viruses such as hepatitis A virus and bacteriophage MS2, whose viral capsids and proteins are negatively charged at physiological pH (Casteel et al., 2004). This kind of association increases the efficiency of the photoinactivation process (Casteel et al., 2004; Lazzeri et al., 2004). Efficiency of photodynamic inactivation of V. fischeri in-situ were found unaffected by the variation of pH, temperature, salinity, or oxygen concentration within the characteristic ranges of aquaculture waters, although found to be affected by the content of the suspended solids in the medium, the concentration of PS and the light fluence rate (Alves et al., 2011). The objective of presenting this study is to collect and review information on Antimicrobial photodynamic therapy and present it before the academia, aquapreneurs, industry and other concerned users for its further study and application.

Photosensitizers (PS): A photosensitizer (PS) can be any natural or synthetic compound which undergoes excitation upon illumination to a certain wavelength of light, demonstrating antimicrobial efficiency against a broad spectrum of microorganisms. The photodynamic
treatment efficiency of a PS depends on several factors like the presence or absence of charge, charge distribution and the presence of peripheral substituents. The parameters in the make-up of the photosensitizer include lipophilicity (relative solubilities in water and lipids), degree of ionization, electric charge, non-specific protein binding (Maisch et al., 2004) and other factors, such as light absorption characteristics (the maximum wavelength of absorption and the intensity of the absorption) and the efficiency of formation of the triplet excited state or of singlet oxygen production and free radicals (Wainwright, 2000). Usually aromatic molecules have good light absorption capacity due to their ringed molecular structure and are efficient in the formation of long-lived triplet excited states, which in turn give rise to singlet oxygen (e.g., molecular oxygen) which are very reactive thus producing molecular consequences on important biological targets like plasma membrane, nucleic acids etc. For a compound to be considered as a PS for use in photodynamic treatment in an open system like a fish farm or hatchery system it must fulfill some properties which are application dependent viz. good absorption capacity at the wavelength of the spectral region, show good efficiency to generate singlet oxygen (De Rosa and Crutchley, 2002; Wainwright, 2007). Merch et al., 1996; Minnock et al., 1996; Reddi et al., 2002 shows killing efficacy against a broad spectrum of multi drug resistant Gram-positive and Gram-negative bacteria upon irradiation with visible light. Alves et al., in the year 2009 reviewed photosensitizing properties of various organic dyes (such as rose bengal, eosin, and methylene blue), fullerenez, porphyrins (of natural and synthetic origin), phthalocyanines and related tetrapyr rolic macrocycles. Synthetic non-porphyrin compounds, like the phenothiazine dyes: methylene blue and toluidine blue (Kem et al., 2001; Maclean et al., 2008) organic chemical dyes belonging to furanocou marians e.g. psoralen (Tanielian et al., 2001; Al-Rawahi et al., 2008) were also reported to have photosensitizing properties. Although no non-porphyrin sensitizer has been approved for PDT applications, a small number of anthraquinone, phenothiazine, xanthene, cyanine and curcuminoid sensitizers are under consideration and some are being evaluated in clinical trials (Ormond and Freeman, 2013).

**Preffered PS in aquaculture setup:** Porphyrins are a class of aromatic heterocyclic compounds that are largely ubiquitous in nature. Porphyrin derivatives which are used as photosensitizers can be natural por phyrins or chemically modified natural porphyrins. The second group is constituted only by synthetic por phyrins which can be neutral, cationic or anionic (Tang et al., 2007). Porphyrins and analogues have been the most promising compounds used in photochemotherapy in fish farming units (Milson et al., 1996), as they can utilize sun as light source since sunlight penetrates deeply into the water column resulting in a nearly uni- form illumination of large volumes of water, also it makes this approach more inexpensive. Moreover, the Soret absorption band of porphyrins in the 420–430nm spectral region allows a very efficient interaction with blue light wavelengths, resulting in maximum penetration into natural waters (Baker and Smith, 1982). Porphyrin derivatives show toxicity towards eukaryotic cells only at millimolar concentrations whereas microbial inactivation is effective at micromolar concentration (Jemli et al., 2002; Costa et al., 2008; Alves et al., 2009) suggesting their non-toxic property against higher organisms (as fishes) at photochemically active doses (namely, in the micromolar concentration range), also their excessive accumulation in the environment is unlikely because of their gradual photobleaching by solar light. The immobilization of the porphyrin is largely recommended as it allows PS recovery and reuse, avoiding the ingestion by fish and also the release into the open water.

**PS administration:** In clinical set-up photodynamic antimicrobial therapy for localized infections is carried out by local delivery of the PS into the infected area by several methods such as topical application, instillation, interstitial injection or aerosol delivery. While in case of internal or systemic infections, targeted photosensitizer delivery method is employed. Methods of targeting photosensitizers specifically to a certain type of microorganism include antibody conjugation (Tagmatischis and Shinohara, 2001) attachment of polycationic peptides (Menezes et al., 1990) and employing bacteriophages (Dogson and Wilson, 1992). There is intensive research on particulate delivery systems, e.g. nanoparticles, polymers or liposomes which can incorporate lipophilic photosensitizers and show selectivity against tumour cells. Nanoparticles can be ideal carriers of photosensitizer molecules for the photodynamic effect (Wang et al., 2004). Use of solid nanoparticles consists of polymers, metals, and ceramics. Polymeric nanoparticles are typically biodegradable, like poly lactide/polyglycolide copolymers (Konan et al., 2003). Gold nanoparticles having a high dye-loading capacity due to their small particle size (2–4 nm) showed very good results in animal studies (Hone et al., 2002; Cheng et al., 2008). Roy et al in the year 2003 and Ohulchansky et al., in the year 2007, individually prepared organically modified mesoporous silica particles containing a photosensitizer.

**PS localization:** The main targets are the external microbial structures, like cell walls, cell membranes, protein capsids, lipid envelopes and nucleic acids. Because of the limited migration of O₂ from the site of its formation (Moan and Berg, 1991) sites of the cell or tissue damage are closely related to the localization of the sensitizer (Peng et al., 1996). Selectivity with respect to space is noticed for many including lysyl chlor ine which is highly selective for lysosomes, the monocationic porphyrin are for membranes and the porphyrine monomer for mitochondria (Kessel et al., 1995).
Sensitizers that localize in mitochondria, like Photofrin, or are produced in mitochondria, like 5-aminolevulinic acid (ALA)-induced protoporphyrin IX, are likely to induce apoptosis, while sensitizers localized in the plasma membrane are likely to cause necrosis during light exposure. Aggregated as well as hydrophilic sensitizers are likely to be taken up by the cell and hence get localized onto the lysosomes or endosomes. Generation of reactive oxygen species (ROS) can follow two alternative pathways after illumination of a given photosensitizer. Upon absorption of a photon by the ground-state photosensitizer, the singlet excited state $1S^*$ is formed. Excited $1S^*$ state is short-lived and can undergo intersystem crossing to a long-lived triplet state, or alternatively can return to the ground state by fluorescence emission and/or heat. Generally the triplet state acts as a mediator of type-I / type-II photosensitization processes. Type-I: Generation of hydrogen peroxide ($H_2O_2$), hydroxyl radical (HO), and superoxide anion ($O_2^-$) by charge transfer from excited PS. Type-II: The triplet state of $3S^*$ can undergo energy exchange directly with triplet ground-state oxygen, leading to the formation of singlet oxygen, $O_2$. The generated ROS react rapidly with their environment depending on the localisation of the excited PS weather its bacteria cell wall, lipid membranes, peptides, or nucleic acid. Both reactions occur simultaneously and in competition.

**Photo damage:** Although antioxidant enzymes such as superoxide dismutase, catalase and peroxidase give protection against some ROS, they do not protect against singlet oxygen (Wainwright and Crossley, 2004) which, according to the literature, is the main ROS through which the PS exert their photodynamic action (Maclean et al., 2008). Moreover, singlet oxygen has been shown to inactivate these enzymes (Kim et al., 2001). The main targets of the bacterial and antiviral photodynamic activity are the external microbial structures (Hamblin and Hasan, 2004; Zupan et al., 2008). The damages to the external microbial structures can involve leakage of cellular contents or inactivation of membrane transport systems and enzymes (Mettath et al., 1999). Some damages produced in the nucleic acid chain can be repaired by the action of DNA repairing systems (Schafer et al., 1998). It has been concluded that although nucleic acids damage occurs, it cannot be the principal cause of microbial photodynamic inactivation (Hamblin and Hasan, 2004; Durantini, 2006). Fungi present much more complex targets than bacteria where photo-inactivation seems to be less dependent on cell-bound PS and needs PS to reach sub-cellular targets such as the mitochondria (Bertoloni et al., 1987) or the nucleus (Kassab et al., 2003). aPDT damage is manifested as swelling (Moan et al., 1979), bleb formation (Moan et al., 1979; Volden et al., 1981), shedding of vesicles containing plasma membrane marker enzymes, cytosolic and lysosomal enzymes (Volden et al., 1981), reduction of active transport (Moan et al., 1983), depolarization of the plasma membrane (Specht and Rodgers, 1990), increased uptake of a photosensitizer (Moan and Christensen, 1981), increased permeability to chromate (Moan et al., 1983) and even to cytosolic enzymes like lactate dehydrogenase (Christensen et al., 1982), inhibition of the activities of plasma membrane enzymes such as Na$^+$/K$^+$-adenosine triphosphatase (ATPase) and Mg$^{2+}$-ATPase (Gibson et al., 1988), a rise in Ca$^+$ (Joshi et al., 1994), up- and down-regulation of surface antigens (Davies et al., 1986), lipid peroxidation (Thomas and Girotti, 1989), that may lead to protein crosslinking (Reyffman et al., 1986) and damage to multidrug transporters (Kessel et al., 1995). Photo activity could rapidly induce apoptosis, both in vitro (Agarwal et al., 1991;) and in vivo (Zaidi et al., 1993; Webber et al., 1996) by release of cytochrome c and other mitochondrial factors into the cytoplasm (Kluck et al., 1997).

**APPLICATIONS OF ANTIMICROBIAL PHOTO-DYNAMIC THERAPY IN FISHERIES AND AQUACULTURE**

**Antibacterial photoactivation:** The major bacterial pathogens affecting various fishfin are Gram –ve including *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella tarda*, *Vibrio anguillarum*, *Pseudomonas* sp., *Yersinia ruckeri*, *Flexibacter columnaris*, *Flavobacterium* sp., *Photobacterium damselae piscicida* (formerly *Pasturella*), *P. damsela* (formerly *Vibrio damsela*) however, only a few Gram +ve species affect fishfin, such as *Renibacterium salmoninarum*, *Nocardia* spp., *Mycobacterium* sp., *Streptococcus* sp. (Shao, 2001; Toranzo et al., 2005; Meyer, 1991). The bacteria of public health significance that contaminate fish are classified into two broad groups the indigenous microflora (e.g., *Vibrio anguillarum*, *V. vulnificus*, *Photobacterium damselae*, *A. hydrophila*, *A. salmonicida*) and non-indigenous microflora (introduced through environmental contamination e.g. Enterobacteraceae such as *Salmonella* sp. and *Escherichia coli*) (Costa et al., 2008; Carvalho et al., 2009). Multiple antibiotic resistant (MAR) *V. harveyi* has been isolated from shrimp culture systems across Asia and Latin America. Tri- and tetracationic porphyrins, when irradiated with the appropriate light, can efficiently inactivate Gram (+) and Gram (–) faecal bacteria both when the PS are free or immobilized on solid matrixes (Alves et al., 2009). Ten bacterial species isolated from fish farming plants, namely *V. anguillarum*, *V. parahaemolyticus*, *P. damsela* subsp. *damsela*, *P. damsela* piscicida, *A. salmonicida*, *E. coli*, *Enterobacter* sp., *S. aureus*, *E. faecalis*, *Pseudomonas* sp. were successfully inactivated using aPDT methodology. In another study a cationic porphyrin Tri-Py+–Me–PF was found efficient against nine pathogenic bacteria isolated from a semi-intensive aquaculture system (Arrojado et al., 2011). Photodynamic therapy is found
equally efficient in controlling the growth of multiple antibiotic resistant V. harveyi strain under both in vitro and in vivo conditions using RB as the photosensitizer on Artemia nauplii model (shrimp larviculture systems) (Ashok et al., 2012). The authors reported that PACT was effective in killing the pathogen under both in vitro and in vivo conditions without deleterious effects on Artemia nauplii.

Water disinfection: Water disinfection using PACT is a relatively recent concept. Rose Bengal was found effective in killing 99.99% of E. coli in a contaminated water sample when immobilized on poly (styrene) beads (Bezman et al., 1978). aPDT methodology has been successfully employed to disinfect wastewater in a small scale, using cationic porphyrin 20a and sunlight (Jemli et al., 2002). The photocatalytic method can also be applied for the degradation of toxins secreted to water by bacteria (Makowski and Wardas, 2001). Savino and Angeli (1985) used methylene blue-PACT to disinfect water samples contaminated with E. coli to acceptable levels for drinking. Bonnett et al., (2006) used a phthalocyanine immobilized on a polymeric membrane of chitosan as a model reactor of water disinfection. Regenerated cellulose impregnated with 5, 10, 15, 20-tetrakis (1-methylpyridinium-4-yl) porphyrin tetra-p-tosylate showed photobactericidal activity against S. aureus, E. coli, Proteus vulgaris and Bacillus subtilis. Krouit et al. in 2006 showed efficient photoinactivation of Gram-positive and Gram-negative bacterial strains by cellulose films with immobilized porphyrin derivatives. It has got vast potential for application in aquaculture system, namely for use in water disinfection plants (Costa et al., 2008; Alves et al., 2009; Alves et al., 2008) and in fish-farming plants (Magaraggia et al., 2006; Arrojado et al., 2011). Costa et al. (2008) observed complete inactivation of viruses using a low light intensity concluding this methodology to be useful even on cloudy days and during winter, opening the possibility to develop new technologies for wastewater treatment.

Antiviral photoinactivation: Viral photoinactivation appears to be different for enveloped and nonenveloped viruses. Several viral components, including nucleic acids and lipid-rich envelopes, are potential targets for photodynamic attack. However, it has been shown that enveloped viruses are significantly more sensitive to photodynamic destruction than nonenveloped viruses (Wainwright, 2004; Egyeki et al., 2003; Demidova and Hamblin, 2005). It is supposed that the lipids and proteins in the envelope act as photosensitizer binding-sites and viruses can be inactivated due to damages caused in their protein molecules (Egyeki et al., 2003). Porphyrins are demonstrated to have effective virucidal effect in vitro, apparently causing photodamage to the viral envelope (Wainwright, 1998). It is more likely that positively charged photosensitizers cause nucleic acid damage (oxidation of guanosine residues), whereas anionic photosensitizers act against the viral envelope (Luksiene, 2005). Aminolipids and peptides in the viral envelope are potential targets, leading to the inactivation of membrane enzymes and receptors, whereas lipid peroxidation is detrimental to membrane integrity, leading to sudden loss of fluidity due to increased membrane permeability (Luksiene, 2005). For nonenveloped viruses, the photoinactivation effects depend mainly on damages in the protein capsid and/or loosening of protein-DNA interaction (Egyeki et al., 2003). So far, photodynamic inactivation has been proven to be a powerful method for inactivating enveloped viruses, such as murine retroviral vectors (Ben-Hur et al., 1992), human immunodeficiency viruses (HIV-1 and -2) (Schagen et al., 1999; Vzorov et al., 2002), herpes simplex viruses (Silva et al., 2005; Tome et al., 2007), hepatitis-B (Wagner et al., 2001) and vesicular stomatitis virus (Horowitz et al., 1991) and also for the inactivation of nonenveloped viruses, like the adenovirus (Schagen et al., 1999), hepatitis A virus (Casteel et al., 2004), human papilloma virus (Wainwright, 2004) and T7 (Egyeki et al., 2003), lambda (Kasturi and Plaz, 1992) and MS2 (Casteel et al., 2004).

Antifungal photoinactivation: Fungi are much more complex targets than bacteria. For example, yeasts, which constitute a large group of rather disparate eukaryotic organisms, are enveloped by a thick external wall composed of a mixture of glucan, mannan, chitin and lipoproteins and separated from the plasma membrane by a periplasmic space. The phenothiaziniums, such as TBO and MB are known to localize in the plasma membrane of yeasts. Although phenothiazin dyes have traditionally been used more in the aPDT of fungi (Donnelly et al., 2008; Gonzales et al., 2010) porphyrin compounds have been successfully tested in the inactivation of yeasts (Carre et al., 1999; Oriel and Nitzan, 2010), dermatophytes (Donnelly et al., 2005; Smjts et al., 2007), conidia- forming fungi (Friedberg et al., 2001; Luksiene et al., 2004) and fungal fish pathogens (Magaraggia et al., 2006). Micromolar concentration of a porphyrin analogue is reported to promote the cure of saprolegniosis in trout farming pools containing Saprolegnia infected fish without causing perilesional damage of the fish (Magaraggia et al., 2006).

Anti-parasitic photoinactivation: Clinically successful photodynamic inactivation of Leishmania sp. (Morgenstaller et al., 2008) is reported where ALA and analogues, porphyrins of natural and synthetic type and other porphyrin related compounds are employed as PS resulting in treatment of cutaneous leishmaniasis (Latorre-Esteves et al., 2010). ALA has also been reported to be used in vitro for the inactivation of Plasmodium falciparum (Smith and Kain, 2004). Extensive photodamage of Colpoda infata cysts, previously loaded with meso-tetrakis (1-methylpyridinium-4-yl) porphyrin tet- ratosylate (20a) and analogues was observed upon visible light irradiation (Kassab et al.,
2002). The same tetracationic porphyrin 20a, upon irradiation with white light, could successfully inactivate eggs of the helminths *Ascaris lumbricoides* and *Taenia sp.* (Alouini and Jemli, 2001).

**Biofilm photoinactivation:** Microbial biofilm cells are highly resistant to antibiotics and other antimicrobial treatments due to their distinct gene expression patterns, phenotypic variations in enzymic activity, cell wall composition and surface structure. It has been demonstrated that aPDT using MB as a PS is effective against *S. mutans* and *S. aureus* biofilms (Pereira et al., 2010).

**Conclusion**

Although there are only few studies done on aPDT in aquaculture systems, preliminary results indicate that this technology has a high potential to disinfect aquatic cultures. The effective inactivation of microorganisms, the improbable development of photo-resistant strains, and the possibility of irradiating fish-farming waters in the presence of immobilized PS using solar light, suggest that aPDT can be considered an alternative technology to disinfect aquatic waters. Low-cost visible light source is most preferred light source and free and immobilized PS represents a promising alternative to this kind of treatment since it allows the recovery and future re-utilization of the photosensitizer. This obviously turns it an easily applicable, less expensive and an environmentally safe technology. aPDT can be considered as a new approach to control fish infections in aquaculture systems, but the process is clearly more difficult to inactivate the complex natural bacterial communities of aquatic waters than pure cultures of bacteria isolated from aquaculture systems.

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