Journal of Applied Microbiology ISSN 1364-5072

REVIEW ARTICLE

Prospects of photosensitization in control of pathogenic and harmful micro-organisms

Z. Luksienė and A. Zukauskas

Institute of Material Sciences and Applied Research, Vilnius University, Vilnius, Lithuania

Keywords
food pathogens, inactivation, photosensitization.

Correspondence
Zivile Luksienė, Institute of Materials Sciences and Applied Research, Vilnius University, Sauletekio 10, Vilnius, LT-10223, Lithuania.
E-mail: Zivile.Luksiene@mtmi.vu.lt

2009/0132: received 21 January 2009, revised 3 March 2009 and accepted 14 March 2009

doi:10.1111/j.1365-2672.2009.04341.x

Summary

Photosensitization is a treatment involving the interaction of the two nontoxic factors, photoactive compound and visible light, which in the presence of oxygen results in the selective destruction of the target cell. Different micro-organisms, such as multidrug-resistant bacteria, yeasts, microfungi and viruses, are susceptible to this treatment. Therefore, a photosensitization phenomenon might open a new avenue for the development of nonthermal, effective and ecologically friendly antimicrobial technology, which might be applied for food safety.

Introduction

> 'Photodynamic therapy is an entirely new treatment modality, and its development can be likened to that of the discovery of antibiotics. This is just the beginning, and its possible uses are only limited by the imagination.'
>
> J.S. McCaughan (1999) *Drugs and Aging* **15,** 49–68.

The discovery of antibiotics raised the belief that human beings found a powerful tool to control pathogens and infectious diseases. Unfortunately, after 70 years of hard work, we must state that the fight against microbes is still continuing and remains as one of the permanent challenges (Wainwright 1998, 2004; Durantini 2006).

Despite tremendous progress in food microbiology, the number of reported food-borne diseases continues to increase. Health experts estimate that every year food-borne illnesses in USA cost 5–6 billion US dollars in direct medical expenses and lost productivity. Infections with the bacteria *Salmonella* alone account for 2·5 billion dollars yearly. Thus, food-borne diseases are extremely costly (CDC 2003).

Today, *c.* 1 billion dollars each year are spent on 5000 antimicrobial products registered with the US Environmental Protection Agency. So far, antibacterials are ineffective and aid mostly in the development of resistant bacteria (US EPA 2004).

Traditional thermal technologies are effective antimicrobials, but usually they induce a plenty of uncontrolled chemical reactions in the food matrix and subsequently reduce the nutrition quality. The emerging food safety technologies being effective decontamination tools, most of them make different undesirable changes in the food matrix; for instance, they alter the structure of proteins and polysaccharides, cause changes in the texture, physical appearance, functionality of food and affect the organoleptic properties. Application of natural compounds, such as essential oils, chitosan, nisin or lysozime, is hampered because of the impact on organoleptic properties of foods (Leistner and Gould 2002; Senorans *et al.* 2003; Devlieghere *et al.* 2004; Manas and Pagan 2005).

Inevitably, a new approach to inactivate pathogenic and harmful micro-organisms in different fields, including medicine, food manufacturing and safety in a cost-effective and environmentally friendly means, is highly needed. To this end, modern biophotonic technology based on photosensitization, which is successfully used to cure cancer and infectious diseases (photodynamic therapy), might serve as a promising tool to decontaminate food and food-related surfaces from different pathogens. This inevitably poses a question: what is it and how does it work?

In general, photosensitization is a treatment involving the administration of a photoactive compound that selectively accumulates in the target cells and the following illumination. The interaction of two nontoxic elements, photoactive compound and visible light, in the presence of oxygen results in a plethora of cytotoxic reactions and consequently induces selective destruction of the target micro-organism (Sibata *et al.* 2001).

According to Dougherty *et al.* (1992), the era of photosensitization was initiated by Raab in 1900. He observed the death of *Paramecium caudatum* after light exposure in the presence of acridine orange (Kalka *et al.* 2000). Subsequently, Von Tappeiner and Jesionek described the use of topical eosin and visible light for the treatment of skin tumours (Wainwright 1998). The use of photodynamic cancer treatment is based on the pioneering work of Dougherty *et al.* (1992) who presented extensive data on the successful application of this novel technique.

The interest in photosensitization as an effective tool to eradicate pathogenic micro-organisms can be traced back to before the age of chemotherapy (Wilson 2004). Ehrlich was the first who introduced the idea of the 'magic bullet' and laid the foundations of modern chemotherapy: if living micro-organism accumulates vital stain and can be afterwards selectively detected, it should be possible to destroy the stained microbe after the illumination (Wainwright 1998).

The development of antimicrobial photosensitization-based treatment was stopped following the introduction of antibiotics. However, in recent years, attention has been drawn towards research into alternative antimicrobial approaches. Photosensitization can be a potential alternative to antimicrobials or antibiotics, because the mechanism of its action is different (Jori *et al.* 2006). This technique has been shown to be effective *in vitro* against resistant bacteria, yeasts, viruses and parasites (Durantini 2006). Currently, photosensitization is used for disinfection of blood and in treating local infections (Demidova and Hamblin 2004). The main advantages of antimicrobial action of photosensitization are as follows.

1. Treatment efficiency is independent of the antibiotic resistance pattern of the strain.

2. It inactivates pathogen population by up to six orders of magnitude without any harmful effect on the surrounding.

3. There is no mutagenicity or bacterial resistance to photosensitization.

4. It costs less, is easy to maintain and is an environmentally friendly and effective antimicrobial treatment (Jori *et al.* 2006).

Photophysical and photochemical processes: role of oxygen

Photosensitization is a result of the combined action of two indispensable and nontoxic components, photosensitizer and light, in the presence of oxygen. Thus, it is necessary to describe how they interact.

Absorption of light by a photosensitizer results in the excitation of molecules from the ground state (S_o) to the excited singlet state (S_1). The molecules can relax to the ground state by fluorescence or by internal conversion, whereby the energy is lost as heat to the surroundings. The third mode to relax is intersystem crossing, when the excitation transfers from the S_1 state to the lower excited triplet T_1 state with a longer lifetime (Fig. 1). Relaxation from the T_1 state results in either phosphorescence or induction of two types of photo-oxidative reactions. Type I pathway involves electron or hydrogen atom transfer, producing radical forms of the photosensitizer or the substrate. These intermediates may react with oxygen to form peroxides, superoxide ions and hydroxyl radicals, which initiate free radical chain reactions. Type II mechanism is mediated by an energy transfer process with ground state oxygen ($^3O_2$; Bissonette and Lui 1997). Both reactions occur simultaneously and in competition (Redmond and Gamlin 1999). It is worth

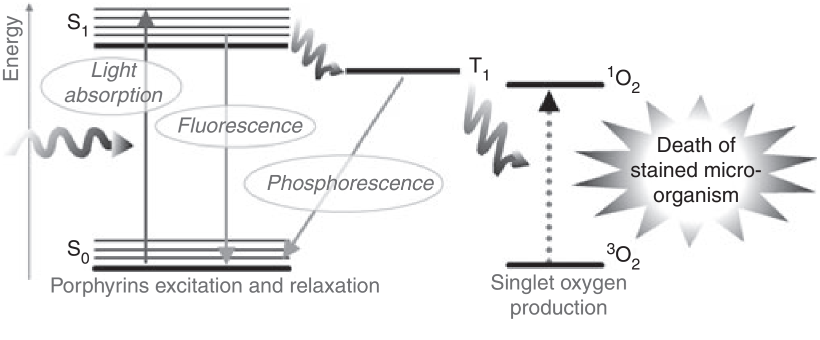

Figure 1 Scheme of photosensitization.

nothing that the destruction of a cell in this case is strictly localized because of a very short half-life of $^1O_2$ (ns) and consequently short diffusion distance (20 nm; Moan 1990). As a consequence, a plethora of cytotoxic reactions is triggered in the cells. These injuries induce cell damage and death (Luksiene 2003, 2005). Mostly, cell injuries induced by photosensitization include disruption of cell membrane, inactivation of different enzymes and damage of DNA (Demidova and Hamblin 2004).

Photosensitizers (photoactive dyes)

A great deal of work has been carried out to evaluate the correlation between antimicrobial efficiency and structure of the photosensitizer. As a rule, the photosensitizers are based usually on tetrapyrrole nucleus including porphyrins, chlorines, bacteriochlorins, phthalocyanines and texaphyrins. These molecules have low toxicity and can form long-lived triplet excited states (Demidova and Hamblin 2004). Several lines of evidence indicate that physico-chemical properties of a photosensitizer have potential impact on the efficacy of photosensitization. Lipophilicity (log P), ionization (pK_a), light absorption characteristics and the efficiency of singlet oxygen production (Φ_Δ) must be included in a putative photoantimicrobial profile (Reddi *et al.* 2002).

Some bacteria are known to produce significant amounts of endogenous porphyrins (Meffert *et al.* 1990). Thus, it seems reasonable to exploit cell metabolism for the production of endogenous photosensitizers using a well-known precursor, 5-aminolevulinic acid (ALA; Fig. 2).

ALA is a naturally occurring metabolite during heme synthesis in eukaryotic as well as prokaryotic cells, which induces the production of endogenous photosensitizer protoporphyrin IX (PpIX), uroporphyrin and coproporphyrin (Malik *et al.* 1992). Thus, photosensitization could be based on the activation of endogenous synthesis of porphyrin-type photosensitizer by δ-ALA, which is applied exogenously (Szocs *et al.* 1999; Nitzan *et al.* 2004). It was postulated that the presence of endogenous porphyrins within the cell with no need to penetrate any cell barriers would result in effective photodestruction of the strains that can produce high amounts of endogenous porphyrins. Hence, Gram-positive bacteria such as *Bacillus cereus* produce endogenous porphyrin ten times more than the Gram-negative *S. enterica* (Buchovec *et al.* in press). The outer membrane of Gram-negative bacteria forms a physical and functional barrier for different compounds. One of the approaches for the inactivation of Gram-positive bacteria can be the use of positively charged photosensitizers (Merchat *et al.* 1996).

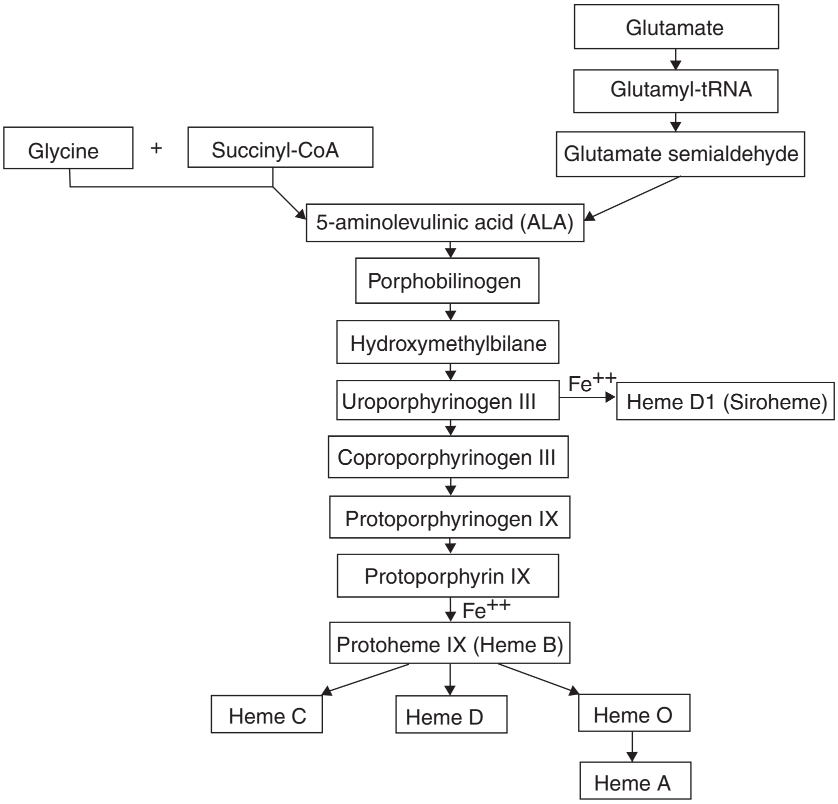

Figure 2 Synthesis of endogenous porphyrins from exogenously applied 5-aminolevulinic acid (adapted from Hamblin and Hasan 2004).

There are, however, many examples of natural photosensitizers. For instance, psoralen derivatives have been used in Asia for millennia for the treatment of various skin disorders. Similarly, traditional Chinese medicine used the extract of *Hypocrella bambusae* containing antiviral hypocrellin (Sibata *et al.* 2001). As a role, photosensitizers, derived from vital stains, are known to be nontoxic in much higher concentrations than those required for effective pathogen killing. The polycyclic phenanthroperylenedione hypericin (Hyp) is a natural pigment present in *Hypericum perforatum*, St John's wort. This natural pigment is described as 'one of the most powerful photodynamic agents in nature'. It has been convincingly shown that hypericin exerted high singlet oxygen generation, high fluorescence yield and strong absorption in the range of 600–700 nm. It is furthermore important to note that hypericin is devoid of toxic or genotoxic effects (Okpanyi *et al.* 1990; Luksiene and de Witte 2002).

To summarize, chemical purity, capability to accumulate in a micro-organism, strategically important localization inside the micro-organism, high killing efficiency and a lack of mutagenicity or genotoxicity are desirable features of an ideal photosensitizer (Kalka *et al.* 2000). In order to use it for food decontamination, additional features are essential. For instance, a desirable photosensitizer must be of low cost, a natural compound, may be a food additive or food component without strong colour, taste and flavour, working at very low concentrations with no negative effect on nutritional as well as organoleptic properties of the foods.

Light sources for photosensitization

The wavelength of light necessary for the induction of lethal photobiological reaction in a micro-organism depends on the structure and electron absorption spectrum of the photosensitizer (Nowis *et al.* 2005). Common porphyrin and chlorine derivatives have a characteristic absorption band between 400 and 430 nm (Soret band) and smaller absorption bands (Q-bands) above 600 nm (Redi and Jori 1988). Also, the wavelength determines the penetration depth of light into the tissue: 400–500 nm light penetrates by about 300–400 μm (surface treatment), whereas 600–700 nm does by about 50–200% more (deeper treatment).

Initially, photosensitization was performed by using conventional gas discharge and incandescent lamps equipped with colour glass filters for narrowing the spectrum. More recent applications involve incoherent sources of light, purposely designed for photosensitization needs: metal halide lamp, which emits in the range of 600–800 nm (Stables and Ash 1995), short-arc xenon lamp (400–1200 nm), as well as narrow-band ultraviolet (UV) lamp (407–420 nm; Nitzan *et al.* 2004). Alternative light sources for activation of photosensitizers are light-emitting diodes (LED), which are in between lasers and conventional lamps in view of the spectral properties and radiation pattern (Brancaleon and Moseley 2002). With the rapid development of LED, these devices achieved maturity sufficient for their application in life sciences (Zukauskas *et al.* 2002). The principle of operation of LED offers unsurpassed radiant efficiency. Besides, LED feature numerous advantages over conventional sources of light, such as low driving voltage, robustness, shock and vibration resistance, the absence of hazardous agents (mercury), compactness, light weightness, flexibility in assembling into arrays of various forms, narrow band emission and the absence of unwanted spectral components. This makes them attractive for use in photosensitizing luminaries that can be safe, portable, battery-driven, free of thermal side effect and low maintenance.

The present choice of the LED emission wavelengths covers a wide range from about 250 nm to about 7 μm. For surface treatment, deep blue and near-UV InGaN LED, which emit at 380–450 nm, can be used. UV LED have line widths in the range of 10–15 nm. The output power of LED ranges from *c.* 1 mW to 1 W. Different from many lamps, LED usually feature no abrupt failure. Instead, the output flux gradually decreases with time. Advanced manufacturers provide LED with the output maintaining 70% of the initial value after 50 000–100 000 h of exploitation.

Antimicrobial efficiency of photosensitization

ALA-based photosensitization was used to inactivate main food pathogens. According to the obtained data, fast and significant inactivation (6·5 log) of *B. cereus*, *Listeria monocytogenes* (4 log) and *Salmonella typhimurium* (6 log) was obtained after this treatment (Buchovec *et al.* 2009; Le Marc *et al.* 2009; Luksiene *et al.* in press).

It seems that photosensitization might help in overcoming the problem of bacterial multidrug resistance. For instance, gram-positive bacteria such as *Staphylococcus aureus*, *Deinococcus radiodurans* or the gram-negative *Acinetobacter baumannii*, which represent a significant problem in hospitals, are actually very sensitive to this treatment (Nitzan and Ahkenazi 1999).

The accumulation of photosensitizer in the cell strongly depends on the physiological state of the bacteria: in the exponential growth phase, bacteria exhibit better accumulation of the photosensitizer than the corresponding cells in the lag phase (Wainwright 1998). Moreover, spores usually more resistant to different treatments are susceptible to photosensitization (Buchovec *et al.* 2009). Other physiological conditions such as microbial biofilms are more resistant to any antibacterial treatment than the single cells (Maisch 2007). According to Soukos *et al.* (2003), *Streptococcus mutans*, *Streptococcus sobrinus* and *Streptococcus sanguis* biofilms can be destroyed by 95–99% after chlorine (e6)-based photosensitization. *Listeria monocytogenes* biofilms can be inactivated by 3·1 log after ALA-based photosensitization (Buchovec *et al.* in press).

So far, only few reports have been published on susceptibility of yeasts and microfungi to photosensitization (Carre *et al.* 1999).

Thus, we have focused on the possibility of inactivating series of harmful and pathogenic micro-organisms exploiting photosensitization. Our previous data (Luksiene *et al.* 1989, 2004a,b) indicate that yeast *Saccharomyces cerevisiae* as well as micromycetes *Ulocladium oudemansii*, *Trichotecium roseum* and *Aspergillus flavus* might be inactivated by photosensitization. Moreover, inhibition of spore germination was further observed in *Aureobasidium* sp., *Rhodotorula* sp., *Penicillium stoloniferum*, *Aspergillus fumigatus*, *Aureobasidium pullulans*, *Ulocladium chartarum*, *Alternaria alternata*, *Rhizopus oryzea*, *Fusarium avenaceum* and *Acremonium strictum* (Luksiene *et al.* 2005b). It seems that plethora of harmful micromycetes that destroys food might be inactivated by photosensitization, a method that is completely safe, reproducible, nonmutagenic, noncarcinogenic, environmental- and human-friendly.

It is accepted worldwide that the amount of accumulated photosensitizer plays a key role in the efficiency of treatment. According to our data, all selected fungi accumulate porphyrin in significant amounts, up to 30 mol - per μmol l^{-1} of protein. By no means, the accumulated amount of photosensitizer strictly depends on the dye concentration used in the medium. In all investigated cases, clear correlation between the accumulated amount of photosensitizer and its inhibiting activity was observed.

Mechanism of microbial inactivation

The successful implementation of a novel technology for food preservation relies on the progress in the understanding of the mechanism of microbial inactivation. As depicted in Fig. 3, accumulation of the photosensitizer in the bacteria is the main prerequisite for its photoinactivation.

As mentioned before, there was significant difference in susceptibility to photosensitization between gram-positive and -negative bacteria. It can depend on profound differences in their three-dimensional architecture. As a rule, neutral or anionic photoactive dyes might efficiently bind and subsequently, after the illumination, inactivate Gram-positive bacteria. This might be easily explained by the fact that gram-positive bacteria have a cytoplasmic membrane surrounded by a relatively porous layer of peptidoglycan (15–80 nm) with lipoteichoic acid traversing this wall and allowing the photosensitizer to cross it (Valduga *et al.* 1999). Macromolecules with a molecular weight of 30 000–60 000 (glycopeptides, polysaccharides) can readily diffuse into the inner plasma membrane. Taking into account that molecular weight of most photosensitizers does not exceed 1500–1800 Da, the outer wall of Gram-positive bacteria is not a permeability barrier. The cell envelope of Gram-negative bacteria consists of an inner cytoplasmic membrane and an outer membrane, which are separated by the peptidoglycan-containing periplasm (Hamblin and Hasan 2004). The outer wall has a highly heterogenous composition, including proteins with porin function, lipopolysaccharide (LPS) trimers and lipoproteins. It gives the outer surface a quasi-continuum of densely packed negative charges. Such a highly organized system inhibits the penetration of several compounds: even hydrophilic 600–700 Da molecules can diffuse through the porin channel (Nikaido 1994). It seems that the outer membrane of Gram-negative bacteria forms a physical and functional barrier to communicate with the surrounding.

As a rule, for Gram-positive bacteria and yeasts, the photosensitizer accumulates in the cell wall. After irradiation by visible light, reactive oxygen species (ROS) induce rapid disruption of the cell wall (Fig. 3). ROS interacts with unsaturated fatty acids, amino acid residues, such as cystein, histidine, tryptophan and nucleic acid bases of DNA, particularly guanine and thymidine (Girotti 2001; Milanesio *et al.* 2001).

Breaks in both single- and double-stranded DNA have been detected in both Gram-positive and -negative

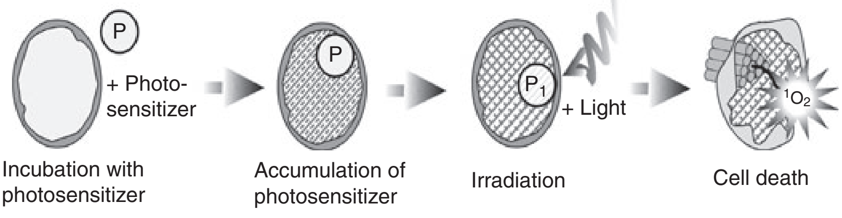

Figure 3 Mechanism of destructive action of photosensitization in the cell: P, photosensitizer; P_1, excited state of photosensitizer after absorption of light; $^1O_2$, singlet reactive oxygen.

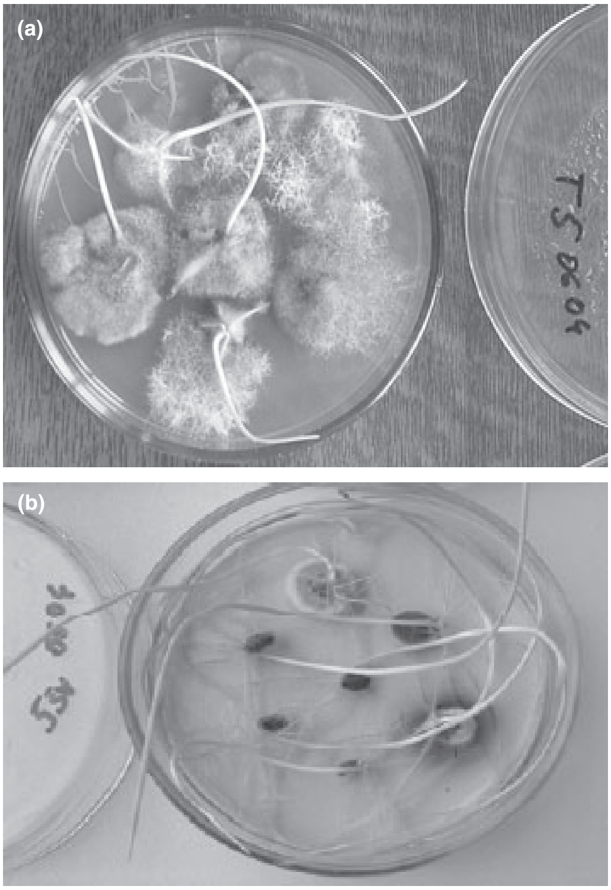

Figure 4 Contamination of organic wheat sprouts by microfungi in control (a) and photosensitization-treated (b) samples.

bacteria after photosensitization (Bertoloni *et al.* 2000). *Deinococcus radiodurans*, having very efficient DNA repair mechanism, can be easily killed by photosensitization as well (Wilson *et al.* 1996; Schafer *et al.* 1998).

A very attractive feature, peculiar to photosensitization as antimicrobial treatment, is the possibility of ROS to destroy several secreted virulence factors. For instance, Komerik *et al.* (2000) showed that LPS from *Escherichia coli* and proteases of *Pseudomonas aeruginosa* were inactivated after exposure to red light and toluidine blue O.

Photosensitization is able to inactivate the enveloped and nonenveloped viruses. Positively charged photosensitizers cause nucleic acid damage (oxidation of guanosine residues), whereas anionic photosensitizers act against the viral envelope. Aminolipids and peptides in the viral envelope are potential targets, leading to the inactivation of membrane enzymes and receptors (Wainwright 2004), whereas lipid peroxidation is detrimental to membrane integrity, leading to the loss of fluidity and increased membrane permeability (Fig. 4).

Decontamination of sprouts by photosensitization

The areas of organic production expand rapidly throughout the world as the demand of organic products is growing. Organic cereals of good quality are widely used both in product processing and in expansion of organic crop areas. Photosensitization being environment-friendly, effective and free from chemical substances can be used for decontamination of seeds.

Most prevailing genus of micromycetes, *Alternaria* (frequency of occurrence is 100%), was reduced from the surface of wheat grains by 75% after the photosensitzation treatment. Harmful micromycetes such as those of the *Penicillium* genus (frequency of occurrence is 62.5%) and *Mucor hiemalis* (frequency of occurrence is 37.5%) were effectively reduced from the surface of wheat grains as well (Luksiene *et al.* 2005a) (Tables 2 and 3).

Considering the application of ALA-based photosensitization to decontaminate food matrix, the question, 'how does ALA interact with food matrix?', arises. Our previous experiments on the decontamination of wheat sprouts by ALA indicated that ALA stimulates the growth of wheat seedlings and roots without impairing the vigour of germination and the viability of seeds. Moreover, 5-ALA increases the rate of photosynthesis and the activities of antioxidant enzymes, which can be associated with enhanced cellular capacity to detoxify ROS (Luksiene *et al.* 2007). Moreover, ALA is an essential precursor of tetrapyrrole compounds such as vitamin B12 and hemes, which serve as prosthetic groups of respiratory enzymes and chlorophyll in plants (Granick 1961). Suitable ALA concentrations have promotive effects on the growth rates and photosynthesis. Crop yields were enhanced by the application of ALA at the leaf stage for rice, barley, potato and garlic (Tanaka *et al.* 1992). Foliar application of ALA (100 mg ml^{-1}) on date palm has promoting effects on the fruit weight, volume and sugar content (Hotta *et al.* 1997).

Obviously, preliminary results obtained on the inactivation of pathogens on the surface of food matrix look promising. Meanwhile, more work must be performed on the examination of applicability of this treatment for different food matrixes, including evaluation of nutritional and organoleptic properties of treated foods.

Decontamination of packaging surface from pathogens by photosensitization

As a result of very high resistance of bacterial spores to UV (7–50 times more resistance than the vegetative cells; Nicholson *et al.* 2000), germicidal lamps are insufficient to ensure sterilization of surfaces, including packaging materials. More than 90% of the packaging contamination is composed of aerobic, spore-forming bacteria (Pirttijarvi *et al.* 1996). In some cases, hydrogen peroxide solution (35% v/v) is sprayed onto the surface of packaging and subsequently removed by a stream of hot sterile air (Holdsworth 1992). Hence, there is a recognized risk of chemical food contamination by residues of hydrogen peroxide, as relatively high concentrations of this compound are employed.

Decontamination of the packaging material from *B. cereus*, which adheres to the surface of the packaging material (polyolefine, a mixture of polyethylene/polypropylene), by photosensitization seems promising. More than 4 log inactivation was achieved after ALA-based photosensitization. Moreover, obtained data indicated that the bacillus spores are susceptible to this treatment as well. Even 3·1 log reduction in spore population was observed *in vitro* and 2·7 log on the surface of the packaging material after photosensitization (Luksiene *et al.* 2009a,b, in press; Paskeviciute *et al.* 2009).

In addition, the inactivation of *Listeria* cells attached on the surface of packaging after ALA-based photosensitization can reach 2·3–3·7 log. The inactivation of *Listeria* biofilms by 1·7–3·1 log indicates that this treatment has potential to combat biofilms as well (Buchovec *et al.* in press; Paskeviciute *et al.* 2009).

Photosensitization in connection with other emerging nonthermal technologies

Most of the currently employed preservation techniques act by inhibiting the growth of micro-organisms (Table 1) and preventing their multiplication (chilling, freezing, drying, curing, adding acids, preservatives), and just few of them act primarily by inactivating micro-organisms in the food. The major inactivation technique is heating.

Traditional thermal food preservation technologies provide safe foods, but there is a great loss of food quality, associated with the induction of thousands of uncontrolled chemical reactions that lead to undesirable protein denaturation, nonenzymatic browning, loss of vitamins and volatile flavour compounds (Gould 2001). Thus, the newly developed nonthermal technologies have complicated goals: the treatment must be effective against pathogenic and spoilage micro-organisms, and it must have no detrimental effects on nutrition and organoleptic food properties.

It is encouraging that most of the emerging nonthermal technologies can inactivate (not just inhibit, as existing technologies; Table 2) micro-organisms without application of heat and the following reduction of food quality (Gould 2001).

Meanwhile, most of the developing novel technologies such as high hydrostatic pressure (HHP), pulsed electric field (PEF), pulsed light, ultrasound, plasma technology and biopreservatives are mostly still in the developmental stage. HHP can be used for liquid and solid foods with or without packaging (100–1000 mol l^{-1} Pa, from milliseconds to 20 min duration) and can inactivate yeast, moulds and most vegetative bacteria, leaving flavour compounds, nutrients and vitamins intact. High-pressure products are on the market for over 20 years. The main disadvantage of this technique is that some types of spores (*Clostridium botulinum*) are resistant to this treatment. Moreover, the implementation of HHP is expensive, thus, its use is limited to high-value products (Senorans *et al.* 2003). High-intensity PEF processing involves the application of high-voltage pulses (20–80 kV cm^{-1}) for short period (less than a second) to fluid foods placed between two electrodes. PEF is an effective antimicrobial tool (but spores are resistant) reducing detrimental changes in sensory and physical properties of food. The main problems in the development of PEF technology deal with the generation of high electric field intensities and design of technique with optimal parameters (Devlieghere *et al.* 2004).

High-power pulsed light technique uses intense and short duration (μs) pulses of broad spectrum (from ultraviolet till infrared). This technology can be used to sterilize or reduce microbial population (vegetative cells and spores) on food surfaces, packaging materials or processing equipments. The main disadvantage of this technique is that light cannot penetrate deeper into the food matrix and can sterilize it just superficially (Gomez-Lopez *et al.* 2007).

Table 1 Major existing technologies for food preservation

Technique that slows or prevents the growth of micro-organisms
  - Reduction in temperature (chilling, freezing)
  - Reduction in water activity (drying, curing, conserving with added sugar)
  - Reduction in pH (acidification, fermentation)
  - Removal of oxygen (vacuum or modified – atmosphere packaging)
  - Addition of preservatives (inorganic – nitrite; organic – propionate, sorbate, benzoate, etc.)
  - Control of microstructure (in water-in-oil emulsion foods)

Technique that inactivates micro-organisms
  - Heating (pasteurization, sterilization)

Table 2 Micromycetes susceptible to photosensitization, *in vitro*

Object	References
Saccharomyces cerevisiae	Luksiene *et al.* (1989)
Ulocladium oudemansii	Luksiene *et al.* (2004a)
Aureobasidium sp.	Luksiene *et al.* (2004b)
Rhodotorula sp.	
Penicillium stoloniferum	
Aspergillus flavus	Luksiene *et al.* (2005b)
Fusarium avenaceum	
Trichothecium reseum	

Table 3 Inactivation of micromycetes on sprouts by photosensitization

Object	References
Aspergillus oryzae	Luksiene *et al.* (2005a)
Mucor hiemalis	
Ulocladium consortiale	
Alternaria alternata	
Rhizopus oryzae	
Acremonium strictum	
Verticillium sp.	Luksiene *et al.* (2006)
Mortierella sp.	
Mucor sp.	
Aspergillus sp.	
Alternaria sp.	
Penicillium sp.	
Rhizopus sp.	

High-intensity ultrasound (10–1000 W cm^{-2}, <0·1 MHz) can sterilize liquid and solid foods. Meanwhile, spores are resistant to ultrasound treatment.

Treatment of food with ionizing radiation is effective and legal in more than 40 countries. Nevertheless, this technology remains not approved in Europe mainly because of the suspicions of the consumers (Gould 2001).

A plethora of natural antimicrobial compounds derived from plants are known as antimicrobials (phytoalexins, phenolic compounds). The main disadvantages of these antimicrobials are strong flavour, high working concentration and relatively low antimicrobial efficiency.

In spite of the intensive research efforts and investments, very few of these new preservation methods are implemented by the food industry. Further research is required to use these new preservation techniques on industrial scale with total warranties for consumer's health.

Photosensitization, in comparison with the aforementioned emerging nonthermal techniques, is environment- and human-friendly, is of low cost, effective and is easy to maintain. It induces lethal effects in vegetative cells, spores and biofilms without any mutagenicity. No microbial resistance to this treatment was observed, whereas very high resistance to HHP and moderate resistance to ionizing radiation as well as PEF were identified (Manas and Pagan 2005). Moreover, photosensitization does not require high intensities (can be used as hurdle). Thus, the preservation of sensory, nutritional and functional properties of foods is higher. The main limitation of this technique is its superficial action.

Conclusions

Because of the wide variety of pathogens encountered, the field of antimicrobial fight must be emphasized as one of the permanent challenges. Multi-antibiotic resistance of pathogens is a rapidly growing and alarming phenomenon. Hence, the discovery of novel, cost-effective and human-friendly technologies to inactivate harmful and pathogenic micro-organisms becomes imperative. To this end, photosensitization as really an effective technique against a range of micro-organisms should encourage its use in a wider arena. Photosensitization of bacteria has repetitively been shown to be independent of the antibiotic resistance spectrum. It induces loss of viral infectivity, is effective against bacterial spores and biofilms and is not mutagenic or genotoxic. It is important to note that no bacterial resistance to this treatment was detected. In our opinion, this phenomenon opens a new and prospective avenue for the development of effective, human- and environment-friendly antimicrobial treatment. Its proper application for the treatment of food, packaging and processing equipments might be really useful in increasing microbial food control and subsequently decrease food-borne diseases. Nevertheless, this is just the beginning. Taking into account the complexity of the food matrixes, microbial ecology of food, interaction of food matrix with treatment, shelf-life and quality of foods after photosensitization treatment, further deep research is needed. While it is not suggested that photosensitization will solve all problems of antimicrobial issues, improvements may be obtained using this new approach in special cases or combining photosensitization with accepted thermal or nonthermal technologies for microbial control.

Acknowledgements

This work was partially supported by the European Commission (FP6, STREP project HighQRTE).

References

Bertoloni, G., Lauro, F.M., Cortella, G. and Merchat, M. (2000) Photosensitizing activity of hematoporphyrin on *Staphylococcus aureus* cells. *Biochim Biophys Acta* **1475**, 169–174.

Bissonnette, R. and Lui, H. (1997) Current status of photodynamic therapy in dermatology. *Dermatol Clin* **15**, 507–519.

Brancaleon, L. and Moseley, H. (2002) Laser and non-laser light sources for photodynamic therapy. *Lasers Med Sci* **17**, 173–186.

Buchovec, I., Vaitonis, Z. and Luksiene, Z. (2009) Novel approach to control *Salmonella enterica* by modern biophotonic technology: photosensitization. *J Appl Microbiol* **106**, 748–754.

Buchovec, I., Paskeviciute, E. and Luksiene, Z. Non-thermal photosensitization-based inactivation of food pathogen *Listeria monocytogenes*. *Int J Antimicrob Agents* (In press).

Carre, V., Gaud, O., Sylvain, I., Bourdon, O., Spiro, M., Blais, J., Granet, R., Krausz, P. *et al.* (1999) Fungicidal properties of meso-arylglycosylporphyrins: influence of sugar substituents on photoinduced damage in the yeast *Saccharomyces cerevisiae*. *J Photochem Photobiol* **48**, 57–62.

Center for Disease Control and Prevention (CDC)(2003). Division of Bacterial and Mycotic Diseases. Salmonellosis. Available at: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_t.htm (accessed 22 December 2008).

Demidova, T.N. and Hamblin, M.R. (2004) Photodynamic therapy targeted to pathogens. *Int J Immunopathol Pharmacol* **17**, 245–254.

Devlieghere, F., Vermeiren, L. and Debevere, J. (2004) New preservation technologies. Possibilities and limitations. *Int Diary J* **14**, 273–285.

Dougherty, T.J., Henderson, B.W., Schwartz, S., Winkelman, J.W. and Lipson, R.L. (1992) Historical perspectives. In *Photodynamic Therapy: Basic Principles and Clinical Applications* ed. Henderson, B.W. and Dougherty, T.J. pp. 1–19 New York: Dekker.

Durantini, E.N. (2006) Photodynamic inactivation of bacteria. *Curr Bioactive Compounds* **2**, 127–142.

Girotti, A.W. (2001) Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. *J Photochem Photobiol* **63**, 103–113.

Gomez-Lopez, V.M., Ragaert, P., Debevere, J. and Devlieghere, F. (2007) Pulsed light for food decontamination. *Trends Food Sci Technol* **18**, 464–473.

Gould, G.W. (2001) New processing technologies: an overview. *Proc Nutr Soc* **60**, 463–474.

Granick, S. (1961) Magnesium protoporphyrin monoester and protoporphyrin monomethyl ester in chlorophyll biosynthesis. *J Biol Chem* **236**, 1168–1172.

Hamblin, M.R. and Hasan, T. (2004) Photodynamic therapy: new antimicrobial approach to infectious disease. *Photochem Photobiol Sci* **3**, 436–450.

Holdsworth, S.D. (1992) Packaging operations. In *Aseptic Processing and Packaging of Food Products* ed. Holdsworth, S.D. pp. 253–306. Oxford, UK: Elsevier.

Hotta, Y., Tanaka, T., Tanaka, H., Takeuchi, Y., Konnai, M. and Al-Khateeb, H. (1997) Promotive effects of 5-aminolevulinic acid on the yield of several crops. *Plant Growth Regul* **22**, 109–114.

Jori, G., Fabris, C., Soncin, M., Ferro, S., Coppellotti, O., Dei, D., Fantetti, L., Chiti, G. *et al.* (2006) Photodynamic therapy in the treatment of microbial infections: basic principles and perspective applications. *Lasers Surg Med* **38**, 468–481.

Kalka, K., Merk, H. and Mukhtar, H. (2000) Photodynamic therapy in dermatology. *J Am Acad Dermatol* **42**, 389–413.

Komerik, N., Wilson, M. and Poole, S. (2000) The effect of photodynamic action on two virulence factors of gram-negative bacteria. *Photochem Photobiol* **72**, 676–680.

Le Marc, Y., Buchovec, I., Baranyi, J. and Luksiene, Z. (2009) Modeling the photosensitization-based inactivation of *Bacillus cereus*. *J Appl Microbiol* (In press).

Leistner, L. and Gould, G.W. (2002) *Hurdle Technologies: Combination Treatments for Food Stability, Safety and Quality*. New York: Kluwer Academic Premium Publishers.

Luksiene, Z. (2003) Photodynamic therapy: mechanism of action and ways to improve the efficiency of treatment. *Medicina* **39**, 1137–1150.

Luksiene, Z. (2005) New approach to inactivate harmful and pathogenic microorganisms: photosensitization. *Food Technol Biotechnol* **43**, 1–8.

Luksiene, Z. and de Witte, P. (2002) Hypericin-based photodynamic therapy: comparative antiproliferative activity and uptake studies in murine Ehrlich ascite carcinoma. *J Photosci* **9**, 524–526.

Luksiene, Z., Bagdoniene, I., Smilgevicius, V., Piskarskas, A. and Juodka, B. (1989) Action of sensitizers and light on biomolecules and cells: inactivation of *S. cerevisiae* growth after laser and porphyrin treatment. *Biology* **27**, 161–169.

Luksiene, Z., Peciulyte, D. and Lugauskas, A. (2004a) Photodynamic inactivation of harmful and pathogenic microorganisms. *Vet Med Zootech* **26**, 58–60.

Luksiene, Z., Peciulyte, D. and Lugauskas, A. (2004b) Inactivation of fungi *in vitro* by photosensitization: preliminary results. *Ann Agricult Environ Med* **11**, 215–220.

Luksiene, Z., Rutkoviene, V. and Sileikiene, D. (2005a) Biophotonic technology for decontamination of organic cereals from fungi. *Agricult Univ Plovdiv, Sci Works* **1**, 125–130.

Luksiene, Z., Peciulyte, D., Jurkoniene, S. and Puras, R. (2005b) Inactivation of possible fungal food contaminants by photosensitization. *Food Technol Biotechnol* **43**, 1–7.

Luksiene, Z., Danilcenko, H., Taraseviciene, Z. and Baliukoniene, V. (2006) Biophotonic technology for inactivation of micromycetes in germinated seeds. *Chem Technol* **4**, 64–68.

Luksiene, Z., Danilcenko, H., Taraseviciene, Z., Anusevicius, Z., Maroziene, A. and Nivinskas, H. (2007) New approach to the decontamination of germinated wheat from microfungi: effects of aminolevulinic acid. *Int J Food Microbiol* **116**, 153–158.

Luksiene, Z., Paskeviciute, E. and Buchovec, I. (2009a) Combined treatment of photosensitization and pulsed light to decontaminate fruits and vegetables. *Proceedings of Environmentally Friendly and Safe Technologies for Quality of Fruit and Vegetables*, Faro, Portugal.

Luksiene, Z., Buchovec, I. and Paskeviciute, E. (2009b) Decontamination of packaging by ALA-based photosensitization. *Proceedings of Environmentally Friendly and Safe Technologies for Quality of Fruits and Vegetables*, Faro, Portugal.

Luksiene, Z., Buchovec, I. and Paskeviciute, E. (in press) Inactivation of food pathogen *Bacillus cereus* by photosensitization *in vitro* and on the surface of packaging material. *J Appl Microbiol* (In press).

Maisch, T. (2007) Antimicrobial photodynamic therapy: useful in the future. *Lasers Med Sci* **22**, 83–91.

Malik, Z., Ladan, H. and Nitzan, Y. (1992) Photodynamic inactivation of gram-negative bacteria: problems and possible solutions *J Photochem Photobiol* **14**, 262–266.

Manas, P. and Pagan, R. (2005) Microbial inactivation by new technologies of food preservation. *J Appl Microbiol* **98**, 1387–1399.

Meffert, H., Gaunitz, K., Gutewort, T. and Amlong, U.J. (1990) Therapy of acne with visible light. *Dermatol Monatsschr* **176**, 597–600.

Merchat, M., Bertolini, G., Giacomini, P., Villanueva, A. and Jori, G. (1996) Meso-substituted cationic porphyrins as efficient photosensitizers of gram-positive and gram-negative bacteria. *J Photochem Photobiol* **32**, 153–157.

Milanesio, M.E., Alvarez, M.G., Yslas, E., Borsarelli, C.D., Silber, J.J., Rivarola, V. and Durantini, E.N. (2001) Photodynamic studies of metallo 5,10,15,20-tetrakis (4-methoxyphenyl) porphyrin: photochemical characterization and biological consequences in a human carcinoma cell line. *Photochem Photobiol* **74**, 14–21.

Moan, J. (1990) On the diffusion length of singlet oxygen in cells and tissues. *J Photochem Photobiol* **6**, 343–347.

Nicholson, W.L., Munakata, N., Horneck, G., Melosh, H.J. and Setlow, P. (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol Mol Biol Rev* **64**, 548–572.

Nikaido, H. (1994) Prevention of drug access to bacterial targets: permeability barrier and active efflux. *Science* **264**, 362–368.

Nitzan, Y. and Ahkenazi, H. (1999) Inactivation of *Deinococcus radiodurans*: an unusual gram-positive microorganism. *J Photochem Photobiol* **69**, 505–510.

Nitzan, Y., Salmon-Divon, M., Shporen, E. and Malik, Z. (2004) ALA induced photodynamic effects on gram positive and negative bacteria. *Photochem Photobiol Sci* **3**, 430–435.

Nowis, D., Makowski, M., Stoklosa, T., Legat, M., Issat, T. and Golab, J. (2005) Direct tumor damage mechanisms of photodynamic therapy. *Acta Biochim Pol* **52**, 339–352.

Okpanyi, S.N., Lidzba, H., Scholl, B.S. and Miltenburger, H.S. (1990) Genotoxicity of a standardized *Hypericum* extract. *Drug Res* **40**, 851–855.

Paskeviciute, E., Buchovec, I. and Luksiene, Z. (2009) Photosensitization as novel approach to decontaminate food or food-related surfaces. *Proceedings of Congress Environmentally Friendly and Safe Technologies for Quality of Fruit and Vegetables*, Faro, Portugal.

Pirttijarvi, T.S.M., Graeffe, T.H., Salkinoja-Salonen, M.S. and Pirttija, T.S.M. (1996) Bacterial contaminants in liquid packaging boards: assessment of potential for food spoilage. *J Appl Bacteriol* **81**, 445–458.

Reddi, E., Ceccon, M., Valduga, G., Jori, G., Bommer, J.C., Elisei, F., Latterini, L. and Mazzucato, U. (2002) Photophysical properties and antibacterial activity of meso-substituted cationic porphyrins. *Photochem Photobiol* **75**, 462–470.

Redi, E. and Jori, G. (1988) Steady-state and time-resolved spectroscopic studies of photodynamic sensitizers: porphyrins and pthalocyanines. *Rev Chem Intermed* **10**, 241–268.

Redmond, R.W. and Gamlin, J.N. (1999) A compilation of singlet oxygen yields from biologically relevant molecules. *Photochem Photobiol* **70**, 391–475.

Schafer, M., Schmitz, C. and Horneck, G. (1998) High sensitivity of *Deinococcus radiodurans* to photodynamically-produced singlet oxygen. *Int J Radiat Biol* **74**, 249–253.

Senorans, F.J., Ibanez, E. and Cifuentes, A. (2003) New trends in food processing. *Crit Rev Food Sci Nutr* **43**, 507–526.

Sibata, C.H., Colussi, V.C., Oleinick, N.L. and Kinsella, T.J. (2001) Photodynamic therapy in oncology *Expert Opin Pharmacother* **2**, 917–927.

Soukos, N., Mulholland, S., Socransky, S. and Doukas, A. (2003) Photodestruction of human dental plaque bacteria: enhancement of the photodynamic effect by photomechanical waves in an oral biofilm model. *Lasers Surg Med* **33**, 161–168.

Stables, G.I. and Ash, D.V. (1995) Photodynamic therapy. *Cancer Treat Rev* **21**, 311–323.

Szocs, K., Gabor, F., Csik, G. and Fidy, J. (1999) Delta-aminolevulinic acid-induced porphyrin synthesis and photodynamic inactivation of *E. coli*. *J Photochem Photobiol* **50**, 8–17.

Tanaka, Y., Tanaka, A. and Tsuji, H. (1992) Stabilization of apoproteins of light-harvesting chlorophyll-a/b protein complex by feeding 5-aminolevulinic acid under intermittent illumination. *Plant Physiol Biochem* **30**, 365–370.

US EPA, US Environmental Protection Agency. (2004) Antimicrobial Pesticide Products. Available at: http://www.epa.gov/opp00001/factsheets/antimic.htm (accessed 22 December 2008).

Valduga, G., Breda, B., Giacometti, G.M., Jori, G. and Reddi, E. (1999) Photosensitization of wild and mutant strains of *Escherichia coli* by meso-tetra (*N*-methyl-4pyridyl) porphine. *Biochem Biophys Res Commun* **256**, 84–88.

Wainwright, M. (1998) Photodynamic antimicrobial chemotherapy. *J Antimicrob Chemother* **42**, 13–28.

Wainwright, M. (2004) Photoinactivation of viruses. *Photochem Photobiol Sci* **3**, 406–411.

Wilson, M., Burns, T. and Pratten, J. (1996) Killing of *Streptococcus sanguis* in biofilms using a light-activated antimicrobial agent. *J Antimicrob Chemother* **37**, 377–381.

Wilson, M. (2004) Lethal photosensitization of oral bacteria and its potential application in the photodynamic therapy of oral infections. *Photochem Photobiol Sci* **3**, 412–418.

Zukauskas, A., Shur, M.S. and Gaska, R. (2002) *Introduction to Solid-State Lighting*. New York: Wiley.