Journal of Photochemistry & Photobiology, B: Biology 197 (2019) 111554

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Determining and unravelling origins of reduced photoinactivation efficacy of bacteria in milk

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A B S T R A C T

Bovine mastitis is an endemic disease of dairy cattle that is considered to be one of the most frequent and costly diseases in veterinary medicine. An increase in the incidence of disease results in the increased use of antibiotics, which in turn increases the potential of bacterial resistance. This study aimed to investigate the effectiveness of antimicrobial photodynamic therapy (aPDT) in the treatment of bovine mastitis, as an alternative to systemic antibiotics. To identify the key factors affecting photoinactivation efficacy, realistic experiments in view of the end-use were conducted in milk samples using two different photosensitizers: methylene blue (MB) and silicon (IV) phthalocyanine derivative (SiPc). We explored the effects of divalent ions and fat content on the aPDT outcome and determined influence of different proteins on aPDT efficacy. Levels of bacterial sensitivity to PSs varied depending on the type of bacteria (Gram-positive vs. Gram-negative) and light exposure time. Critical interrelated factors affecting aPDT in milk were identified and an efficient combination of treatment conditions that can lead to a full photodynamic inactivation of bacteria was determined.

1. Introduction

Antimicrobial resistance has increased to a dramatic extent in recent years and it is the use of antibiotics in animals that have contributed to the escalation of this global challenge [1]. Efforts to tackle antimicrobial resistance dissemination thus require the adoption of a "One-Health" approach that promotes the integration of public health and veterinary disease, food, and environmental surveillance [2]. Mastitis is one of the most common and detrimental diseases in veterinary medicine and is the largest health cost in most farms. It is ranked second after infertility as the main reason for culling cows. Mastitis is a multifactorial disease, which results from injury, chemical irritation and infection caused by different bacterial species. In Europe and USA approximately 20–50% of dairy cows receive antibiotic treatment for infections. The wide use of antibiotics can affect animal welfare, milk quality and, most importantly, public health due to the increased risk for the spread of the antibiotic-resistant bacteria [3,4]. Thus, novel approaches to identify, eliminate and prevent bacterial infections are urgently required.

Antimicrobial photodynamic therapy (aPDT) is emerging as a new and very effective treatment option of local infections in veterinary medicine [5]. This modality is based on a dynamic interaction of light, oxygen and photoactive drug to induce oxidative damage to the bacterial cells [6]. The basic principle of aPDT is the following: absorption of a photon of light promotes light-activated compound called photosensitiser (PS) into a long-lived excited triplet state, which further reacts with molecules from its direct environment by electron transfer (type I mechanism) or energy transfer to ground state molecular oxygen (type II mechanism), leading to the production of highly reactive oxygen species (ROS). The cytotoxic effects arise from the potential of ROS to react with nucleic acids, proteins, or cell membranes, thus destroying bacterial cells in the shortest possible time-frame. A clear benefit of this approach is that in contrast to standard antibiotic treatments, aPDT does not lead to the selection of resistant mutants [7]. Although aPDT has been suggested as a good option to kill bacteria [8,9], studies where aPDT was used to treat bovine mastitis are very scarce [10,11]. The microenvironment of PS is essential for the efficacy of photo-induced therapies and in order to transfer this promising strategy to practice, PSs that are active in milk samples are required. An in vitro study performed by Sellera and coworkers indicates that MB at a concentration of 50 μM and red light with irradiance 100 mW/cm^2 was effective for treatment of mastitis in a dose-dependant way, regardless of their antibiotic resistance phenotype. Moreira and colleagues showed that aPDT using 2.5% toluidine blue and LED irradiation at 635 nm with 200 J/cm^2 fluency was efficient when applied in vivo for the treatment of subclinical mastitis and can induce a significant reduction of the total number of bacteria. For their studies an acrylic light guide that was coupled to the LED equipment allowed irradiation to reach the infected mammary tissue. However, whereas under these conditions a 2-log reduction for *Streptococcus dysgalactiae* and a 5-log

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https://doi.org/10.1016/j.jphotobiol.2019.111554
Received 15 May 2019; Received in revised form 30 June 2019; Accepted 10 July 2019
Available online 12 July 2019
1011-1344/

reduction for coagulase-negative *Staphylococcus* were found in the first 24 h after irradiation, treatment was not effective against *Escherichia coli* (*E. coli)*. This is not surprising since photoinactivation of Gram-negative bacteria is rather challenging due to the more complex structure of their cellular envelope. Gram-negative bacteria possess a complex outer membrane with lipopolysaccharides and tightly packed phospholipids that present a very strong barrier between the cell and its environment, hindering binding and uptake of PS [12,13]. For this reason commonly high concentration of PS and longer exposure times are required for inactivation. The gram-negative bacterium *E. coli* is a part of the normal intestinal flora of humans and animals. It is the most common facultative anaerobic bacterial species in the gut and constantly excreted in the faeces into the environment. The source of mastitis-causing *E. coli* stains can be found in the intestinal flora of the affected cow. The efficacy of the treatment of *E.coli* mastitis with known antimicrobials is very limited [14]. Although fluoroquinolones and cephalosporins show some beneficial effects, they are last resort antibiotics that should be reserved for use in humans and used with caution.

The challenge for aPDT of bovine mastitis is to define the PS, which is more suitable for the inactivation of both Gram-positive and Gram-negative bacteria in milk samples than previously used phenothiazine-based dyes. To address this issue two different PSs were used: methylene blue (MB), a 'first in class' clinical entry phenothiazine-based PS and tetrapyrrole-based 1,(4)-[Tetra-(3-pyridyloxy phthalocyaninato)] dihydroxy silicon(IV) (SiPc) (Fig. 1).

Besides well-studied characteristics, such as the nature of bacterial cells and photoactive drugs, light exposure time etc., the physiological environment has a big impact on photoinactivation efficacy and may complicate aPDT treatment. Although encouraging results have been reported for in vitro studies in aqueous media, significant differences in inactivation largely occur in vivo. To address this shortcoming and provide a solid translational basis, critical factors governing the efficacy of inactivation of bacteria were investigated, with the view to maximize the potential of using aPDT for treatment of bovine mastitis. When studying aspects affecting photoinactivation efficacy, the first problem encountered centers around polydispersity of the milk. Light scattering from the milk components, notably fat globules and casein micelles, can reduce absorbance of light by PSs. Second, milk components may interact with PSs decreasing their bioavailability or influencing their photophysical characteristics that may diminish the efficacy of PSs. Third, milk components, particularly divalent cations, can stabilize the outer membrane barrier of Gram-negative bacteria and hinder binding and uptake of PSs.

The purpose of this in vitro study is to compare the efficacy of aPDT to reduce the viability of bacteria in milk samples employing MB and SiPc as photoactive agents. For this reason commercially available milk samples were experimentally contaminated with six different Gram-positive and Gram-negative bacterial strains and subsequently irradiated with red light. We have determined the antibacterial effect of these PSs in different formulations and identified the components that may hamper the activity of the PSs.

2. Experimental

2.1. Chemicals and Reagents

The 1,(4)-[Tetra-(3-pyridyloxy phthalocyaninato)] dihydroxy silicon(IV) (SiPc) was synthesized according to the previously published method yielding a purity of 98% [15]. Methylene blue, purity > 99%, was purchased in powder form from Sigma-Aldrich and used as it is. Stock solutions were prepared in Millipore water and diluted to the final concentrations using milk with 0.3% or 3.8% fat content. Milk used in our experiments was commercially available, homogenized and was kept refrigerated until use. Casein, α-lactalbumin, β-lactoglobulin were purchased from Sigma-Aldrich. Protein solutions were prepared in Millipore water immediately before measurement. Casein enriched solution was prepared at pH 8.

2.2. Bacterial Strains and Culture Conditions

Gram-positive model organisms *Staphylococcus aureus* 3150/12, *Staphylococcus hominis* 3934/16 and *Staphylococcus warneri* 3930/16, but also Gram-negative *Escherichia coli* bovine mastitis isolates 1303, ECC-147 and 131/07 were used to investigate the potential of MB and SiPc as aPDT agent for the treatment of bovine mastitis. The *bacterial* strains were maintained on lysogeny broth (LB) agar and were stored at 4 °C. A single colony was picked from plate, transferred into 3 ml LB broth and incubated aerobically at 37 °C overnight in a shaker incubator at 180 rotations per minute (rpm). On the next day, the bacteria were suspended in 20 ml of fresh LB medium to an optical density (OD_{600}) of 0.1 and grown in a flask to an attenuance of ca. OD_{600} = 0.4. The bacterial suspensions were then centrifuged at 4000 rpm for 5 min, resuspended in milk samples to the final bacterial concentration of ca. 1×10^9 cells per mL and subsequently used for the experiments.

2.3. Photoinactivation of Bacteria

To induce $^1O_2$ generation the 1 ml PS stained bacteria (15 min, 37 °C) were placed in 24-well plate and were irradiated red light at a

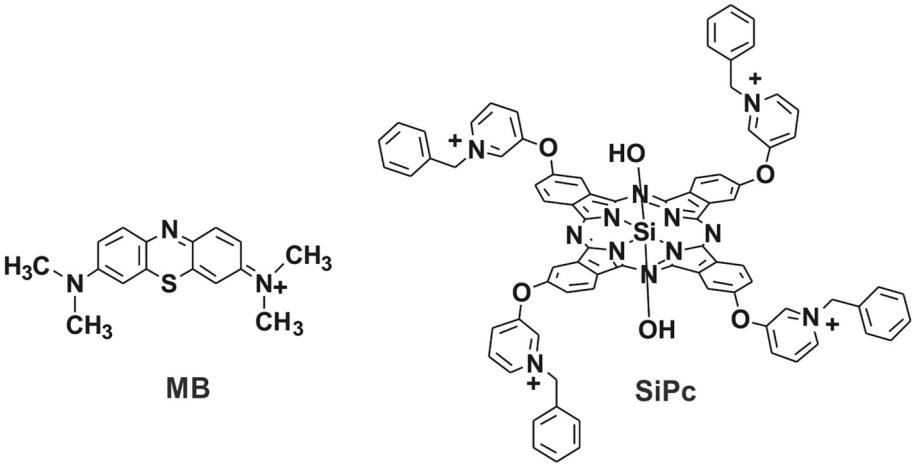

Fig. 1. Chemical structures of methylene blue (MB) and silicon(IV)phthalocyanine (SiPc) used in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

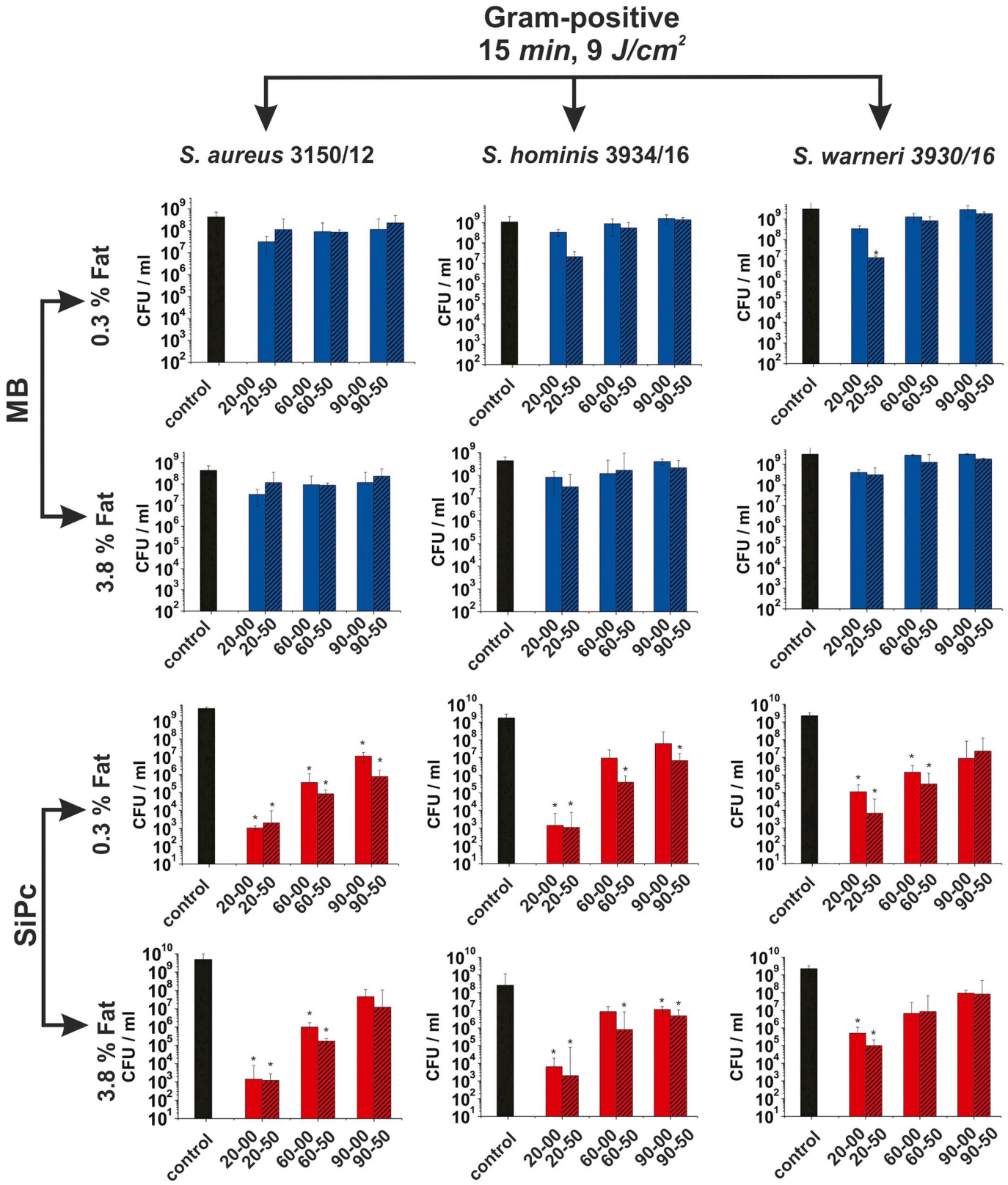

Fig. 2. Efficacies of killing of Gram-positive *S. aureus* 3150/12, S. hominis 3934/16, S. warnei 3930/16 ($\lambda > 610$ nm, 10 mW/cm^2, 9 J/cm^2) by MB and SiPc after 15 min of incubation. Data show formulations with 20%, 60% and 90% milk without EDTA marked as 20–00, 60–00, 90–00, correspondingly (blue and red columns) and formulations containing 50 μM EDTA, marked as 20–50, 60–50, 90–50, correspondingly (dashed columns). Data are presented as mean ± SD; (*$p < .05$ statistical difference vs control). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fluence rate 10 mW cm^{-2} from the top of the plate using a projector lamp equipped xenon lamp. Cut-off filter at > 610 nm was installed to coincide emitting energy with the absorption maxima of both PSs. Fluence rates were routinely measured using power meter (Solar Meter from Solartech). After irradiation, aliquots of bacteria were serially diluted and the living bacterial cells were determined by plating on LB agar plates. The plates were incubated overnight at 37 °C and the number of CFU/mL was counted using automated colony counter ProtoCOL from Synbiosis.

2.4. Spectroscopic Instrumentation

Absorption spectra were measured on Agilent 8453 spectrophotometer using 1-cm optical path-length quartz cells and baseline corrected. Steady-state emission spectra were recorded on a HORIBA Jobin-Yvon IBH FL-322 Fluorolog 3 spectrometer equipped with a 450 W xenon-arc lamp, double-grating excitation and emission monochromators (2.1 nm/mm dispersion; 1200 grooves/mm). All experiments were performed at room temperature.

2.5. Photobleaching

Two-ml samples containing 10 μM PS with or without 1 mM cysteine were placed in an open quartz cuvette and irradiated for certain time period from a projector lamp passing through a cut-off filter at 610 nm, 10 mW cm^{-2}. The absorption spectra of the irradiated samples were recorded and the Q_{max} was plotted against time.

2.6. Singlet Oxygen Production

Singlet oxygen production of PS and PS-cysteine mixtures were determined by the relative method. Polychromatic irradiation from a projector lamp passing through a cut-off filter at 610 nm, 10 mW cm^{-2} was used to carry out the experiments. Freshly prepared dye solution in a dark flask was mixed with the PS/PS-cysteine only immediately before taking the samples at time point "0". $^1O_2$ photogeneration rates in water were derived using 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABMDMA) as a fluorescent monitor (λ_{exc} = 370 nm) for photosensitized bleaching rates monitored between 0 s to 100 s.

3. Results and Discussion

3.1. Bacterial Inactivation by aPDT, Influence of Dilution, Fat Content and Cations

Both PSs used in this study have absorption bands in the visible region of the electromagnetic spectrum with maxima at 664 nm (logε = 4.89) for MB and 678 nm (logε = 5.10) for SiPc. Singlet oxygen quantum yields for both compounds are comparable in organic solvents (Φ_Δ = 0.57 for MB and Φ_Δ = 0.59 for SiPc), but differ in aqueous media (Φ_Δ = 0.56 for MB and Φ_Δ = 0.22 for SiPc), probably due to the formation of the higher order structures in the case of SiPc. These aggregates are, however, loosely bound since the formation of a face-to-face arrangement is excluded by the presence of hydroxyl groups on the axial positions of the macrocycle and only edge-to-edge contacts are possible. Upon binding to bacteria, SiPc aggregates commonly break up and singlet oxygen quantum yield can be recovered [15].

For the purpose of direct comparison between their antibacterial efficacies, the same end concentration of 10 μM for both PSs was used in our studies. Additionally, formulations containing 50 μM ethylenediaminetetraacetic acid (EDTA) and different milk content (20°%, 60% and 90%) were used to find out whether binding or uptake of PS by bacterial cells plays a role. EDTA and other ion chelators are known to bind divalent cations such as Ca^{2+} and Mg^{2+} and can thereby weaken the bacterial cell envelope and consequently potentiate the killing efficacy of PSs [16]. We show that, however, EDTA alone do not cause any toxicity (Fig. S1, Supporting Information). The results of the determination of antibacterial effect are summarized in Figs. 2, 3 and S2 of Supporting Information. When suspensions of *S. aureus* 3150/12, *S. hominis* 3934/16 and *S. warnei* 3930/16 were exposed to 36 J cm^{-2} light passing through 610 nm cutoff filter, a significant decrease of viability and complete loss of viability were achieved with MB and SiPc, respectively. Thus, to obtain more details of the aPDT effect of PSs used, inactivation of the tested Gram-positive bacteria was determined after 15 min irradiation (9 J cm^{-2}, Fig. 2). Shorter periods of irradiation revealed significant differences between SiPc and MB in inactivation efficacy. SiPc was much more effective than MB under the same conditions applied. Dilution of the samples and addition of EDTA resulted in a shift in survival when SiPc was used. At this concentration eradication of Gram-positive bacteria resulted in a disinfecting effect (> 5 $\log_{10}$ steps) when the milk content was 20% and 60% for SiPc and only ca. 1 $\log_{10}$ reduction with MB when the milk content was 20%. In the case of Gram-negative bovine mastitis isolates *E.coli* 1303, *E.coli* ECC-147 and *E.coli* 131/07 only formulations containing EDTA and 20% milk led to a reduction of the bacterial load. In the case of SiPc, a 5–7 $\log_{10}$ reduction of the bacterial count was obtained for milk samples with 0.3% and 3.8% fat content, while for MB we saw about 2–4 $\log_{10}$ of killing only in the milk samples containing 0.3% fat. SiPc was also active against *E.coli* 1303 and *E.coli* 131/07 in formulations containing EDTA and 60% milk (Fig. 3). Among many factors that affect the toxicity of PS to bacteria, the ability to bind to the bacterial cell and enter it is one of the most important factors. Previous studies showed that Gram-positive bacterial pathogens are usually highly sensitive to photosensitizing agents in contrast to Gram-negatives [17]. The outer membrane and the thin peptidoglycan layer in the periplasmic space of Gram-negative bacteria limit the access and thus the effective PS concentration reaching the sensitive cytoplasmic membrane [18]. The integrity of the outer leaflet of the outer cell wall of Gram-negative bacteria is maintained by lipopolysaccharides and divalent cations are essential for stabilizing the negative charges of the oligosaccharide chains [19]. For this reason, in Ca^{2+} and Mg^{2+}-rich media the efficacy of PS-mediated killing of Gram-negative bacteria is significantly reduced [20]. This could explain why both PSs were unable to kill *E. coli* strains in suspensions containing 90% milk, while a considerable decrease in bacterial survival was observed in the presence of EDTA, when divalent cations are chelated. In the case of Gram-positive bacteria influence of EDTA had no effect when MB was used as PS (Fig. S2, Supporting Information).

3.2. Influence of Proteins on the Stability of PS

The rate of bacterial inactivation in milk is not only reduced due to the presence of divalent ions. Other milk components can also contribute to the reduction of the antibacterial PS activity. Milk composition has a dynamic nature; generally, bovine milk contains approximately 3.5% protein of which 80% are caseins and 20% whey proteins. Whey contains β-lactoglobulin, α-lactalbumin and several minor proteins with different biological activities such as enzymes, mineral-binding proteins, and immunoglobulins. Although a number of published results show that proteins can help to solubilize hydrophobic and highly aggregated PSs, significantly contributing to the improvement of their photophysical properties [21,22], in some cases, a reduced activity of PSs was found in protein-rich media. For instance, in their recent study Rodriguez-Amigo and co-workers showed that β-lactoglobulin can serve as a carrier for the natural photosensitizer hypericin and improve singlet oxygen quantum yield. However, the photo-inactivation effectivity of the hypericin-β-lactoglobulin complex against *Staphylococcus aureus* was not much different from free Hypericin [23] or even reduced when a small amount of dimethyl sulfoxide was present in the media [24]. In another study Chen et al. showed that the antibacterial efficacy of MB was reduced in blood plasma compared to protein-free media. This cannot be attributed to the decrease in the light

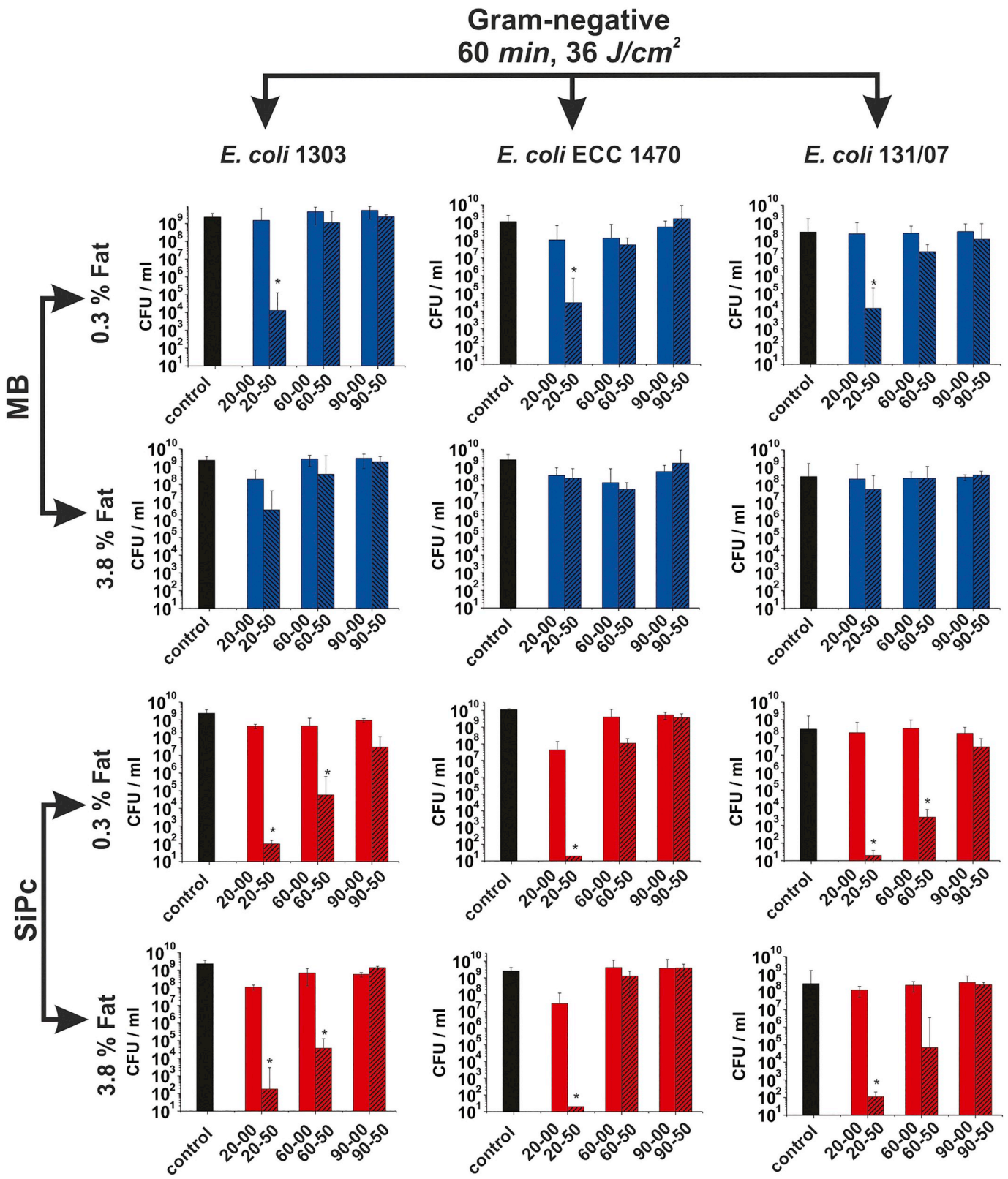

Fig. 3. Efficacies of killing of bovine mastitis isolates Gram-negative E.coli 1303, E.coli ECC 1470, E.coli 131/07 ($\lambda > 610$ nm, 10 mW/cm^2, 36 J/cm^2) by MB and SiPc after 15 min of incubation. Data show formulations with 20%, 60% and 90% milk without EDTA marked as 20–00, 60–00, 90–00, correspondingly (blue and red columns) and formulations containing 50 μM EDTA, marked as 20–50, 60–50, 90–50, correspondingly (dashed columns). Data are presented as mean ± SD; (*$p < .05$ statistical difference vs control). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

intensity due to absorption or scattering, because fresh human plasma is clear. The authors showed that attachment of a hydrogen atom from the S—H group of cysteine to the central ring nitrogen of MB destroys the ring conjugation and forms Leuco-MB, which does not absorb in the 660 nm region and does not generate singlet oxygen [25]. Later the same authors showed that the addition of ~ 10^{-4} M acetic acid to the

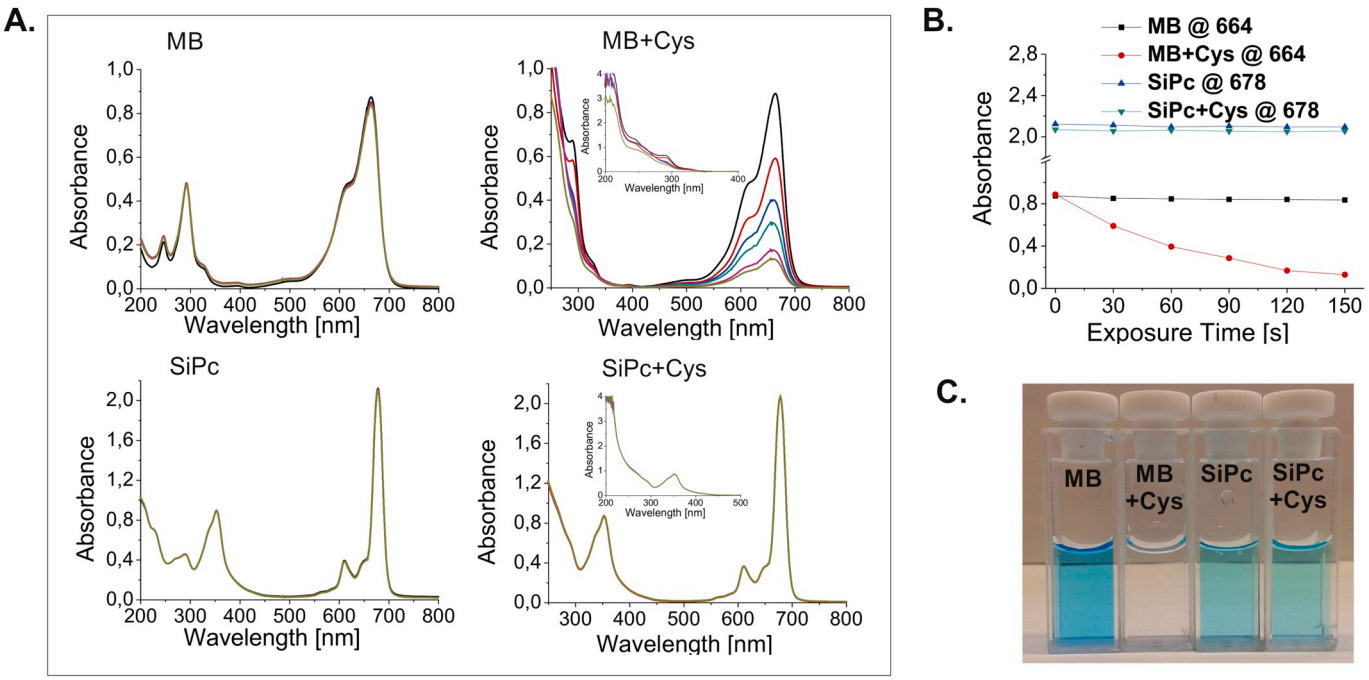

Fig. 4. (A) Time-resolved (0–160 s) UV–vis spectra of MB and SiPc (10 μM) upon irradiation in the presence and absence of cysteine (1 mM), (B) decrease of the λ_{max} as a function of the time, (C) digital photographs of solutions after irradiation.

human plasma prevents H-atom attachment to MB and formation of non-active Leuco-MB. The mechanism proposed is based on the oxidation of cysteine to cystine, thus the elimination of the thiol hydrogen atom [26].

In milk, whey proteins have proportionally more sulfur-containing amino acids (1.7°%) than casein (0.8°%). The sulfur in casein is mainly contained in methioine, while whey proteins are relatively rich in cysteine [22]. In our study we intended to find out whether cysteine can also influence photostability of SiPc, therefore, UV–vis analysis of both PSs were studied under the same irradiation conditions, both in the presence and absence of cysteine. Changes in the absorption spectra were recorded for predetermined periods of time. As expected, 663 nm absorption band of MB in water hardly changes upon irradiation, whereas MB bleaches within few minutes in the solution containing cysteine. Analysis of the absorption spectra of SiPc shows that under a similar condition almost no changes in the Q band intensity occurs with and without cysteine (Fig. 4). As proposed by Chen et al., the key step of the proposed mechanism of MB bleaching is the formation of Leuco-MB, which inhibits generation of MB triplets and consequently generation of reactive oxygen species (ROS).

3.3. Influence of Proteins on the Generation of ROS

Generation of ROS is a crucial indicator when evaluating aPDT efficacy. Singlet oxygen produced in biological systems is commonly very short-lived, since it can react readily with surrounding molecules. Reactivity of thiols makes them uniquely susceptible to oxidation by ROS and indeed they play an important role in the protection against ROS-induced damage to the biomolecules. Together with glutathione and homocysteine, cysteine plays a vital role in maintaining the biological redox homeostasis [27]. To assess the quenching ability of thiols, exemplified by cysteine, solutions containing PS, 9,10-anthracenediyl-bis (methylene) dimalonic acid (ABMDMA), with or without cysteine, were irradiated. The reaction of ABMDMA with singlet oxygen leads to the formation of non-emissive endoperoxide, which could be used for the direct measure of the amount of singlet oxygen.

As expected, ABMDMA conversion was slower when cysteine was present in the solution for both PSs. The slope of the photodegradation kinetics, which is proportional to the rate of singlet oxygen generation, indicated that for both PSs ca. 60°% reduction occurs, when the concentration of cysteine was 10 μM (Fig. 5). In contrast to the effect on MB, cysteine does not contribute to the photobleaching of SiPc, however, it can still reduce its activity by scavenging ROS. In biological media, many antioxidants can scavenge different types of ROS. For instance, intracellular antioxidant enzymes are produced in the cell and provide an important defense against free radicals. Such a defense mechanism can be activated also during aPDT. However, the effect of quenching agents such as superoxide dismutase (SOD) or catalase (CAT) on different types of PSs can be different. Recently Faraj Tabrizi et al. showed that *E.coli* strain, which is not able to produce SOD A and SOD B, resulted in a disinfectant effect (≥ 5 $\log_{10}$steps). On the contrary only an antimicrobial effect (≥ 3 $\log_{10}$ steps) was detected with wild type strain. This was not the case when tetrapyrollic porphyrin-based PS was used; the survival levels were almost the same in the mutant strain and in the wild type background [28]. Together, these findings show that defense mechanisms against ROS might influence bacterial susceptibility against Type I aPDT, while this is not the case when PS that mostly acts via the Type II pathway.

3.4. Influence of Proteins on the Aggregation of PS

Furthermore, aggregation of PSs can also have a tremendous effect on their photodynamic efficacy [29]. Different proteins present in the milk can shift the dynamic equilibrium between aggregated and disaggregated species. The degree of aggregation can be easily determined by an analysis of the absorption spectra, providing additional insights on the influence of the biological medium on aPDT efficacy. When MB is aggregated, the absorption spectrum features a blue-shifted band at 610 nm with respect to that of the monomer at 664 nm [30]. Generally, for phthalocyanines the presence of a blue-shifted band corresponds to the formation of face-to-face H-aggregates, whereas red-shifted Q-band results from the formation of slipped-cofacial J-aggregates [31]. Because of their random coil structure and lack of ordered secondary structure elements, caseins are expected to have more pronounced effect on the aggregation of PSs as compared with globular whey proteins, which are much more rigid in their tertiary structures.

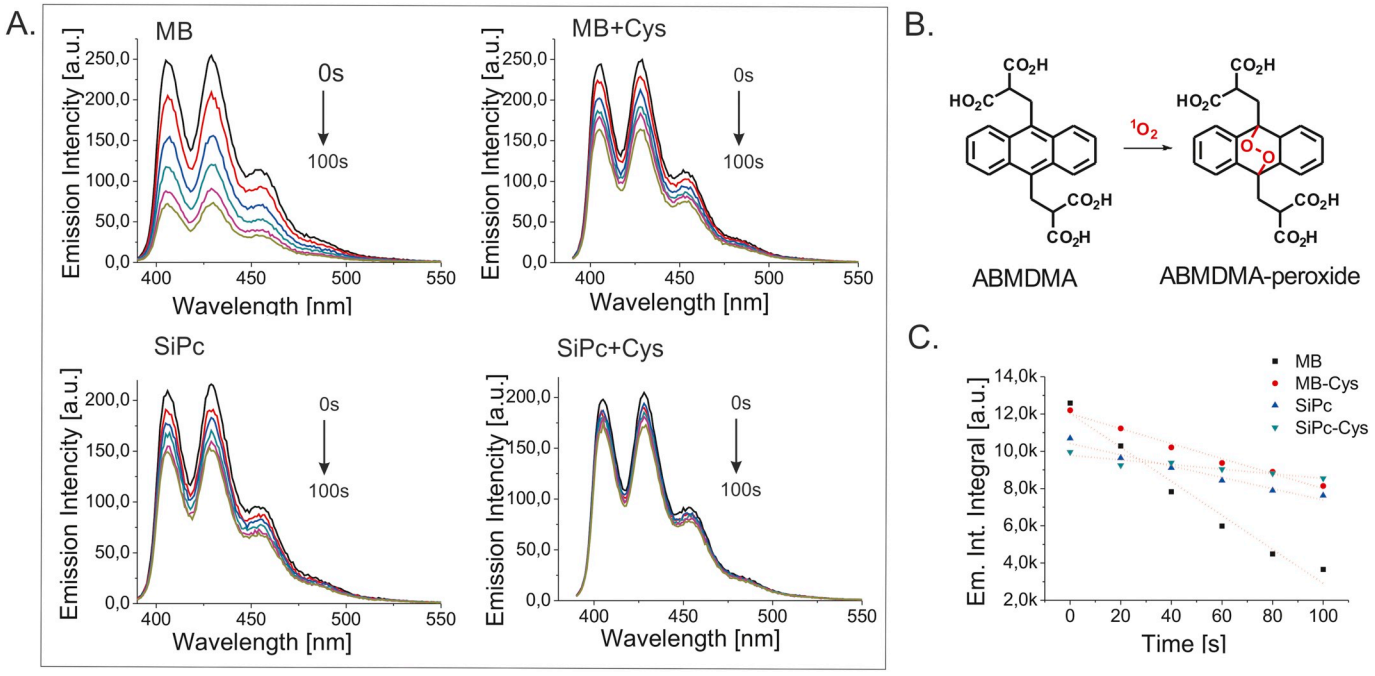

Fig. 5. Impact of cysteine in ROS formation by MB and SiPc. (A) Time-resolved emission spectra of ABMDMA upon irradiation, (B) ABMDMA's reactivity in the presence of $^1O_2$, (C) corresponding decays.

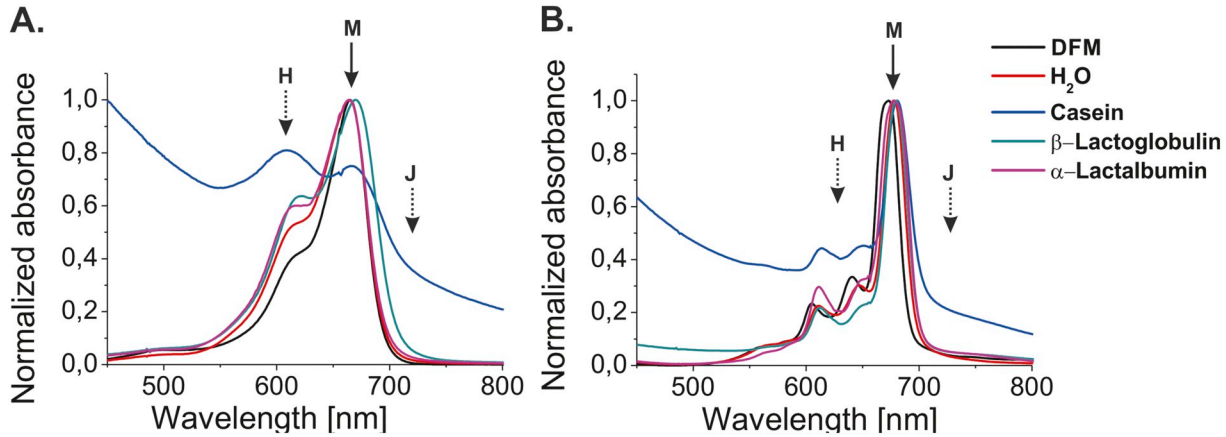

Fig. 6. Impact of different milk proteins on PS aggregation. Displayed are normalized absorption spectra of MB and SiPc in different solutions as indicated in the legend (M = monomers, H = H-aggregates, J = J-aggregates).

Fig. 6 shows normalized absorption spectra of PSs dissolved in DMF, H_2O or together with one of the three main milk proteins casein, β-lactoglobulin and α-lactalbumin (at 10 mg/ml concentrations). In the casein solution, the MB absorption band at 664 nm, assigned to the monomers, was reduced upon aggregation and a relatively large fraction of the PS absorbed light at lower wavelengths (< 620 nm). β-Lactoglobulin and α-lactalbumin also induced some aggregation of MB. Most likely, axial and peripheral ligands in SiPc can effectively isolate chromophoric phthalocyanine rings and substantially decrease aggregate formation. Contrary to MB, only relatively small perturbations of the Q-band were observed in case of SiPc.

4. Conclusions

This study demonstrates a systematic approach to assess the effect of different milk components on the photodynamic efficacy of MB and SiPc. It was found that fat content and dilution of the milk samples can influence aPDT outcome. Even though divalent cations such as Ca^{2+} and Mg^{2+} did not affect the photophysical characteristics of the PSs, they severely impaire the binding of the PSs to the Gram-negative bacteria. Our findings show that when a PS is administered in tandem with EDTA its activity against *E.coli* was improved significantly, as the cell envelope no longer represents a significant barrier for the PS. To provide insights into the effect of different proteins on aPDT efficacy, photobleaching and singlet oxygen quantum yields were measured in the presence of the proteinogenic amino acid cysteine. Our results show that whereas MB bleached within minutes in a cysteine containing solution, SiPc remained largely unaffected. Physical quenching of singlet molecular oxygen by cysteine in water solution was measured by the inhibition of the rate of singlet oxygen oxidation of ABMDMA. We found that cysteine was able to quench the singlet oxygen of both PSs and might influence the final aPDT outcome. We also showed that the interactions between different milk proteins and PSs can affect the aggregation equilibrium and alter PSs photophysical and photochemical pathways. Casein and whey proteins were found to contribute to the aggregation of MB, while SiPc remained mostly in its monomeric form. Overall, our results show that the phthalocyanine-based PSs have a number of advantages for the treatment of bovine mastitis in

comparison to the phenylthiazolium-based PSs. Thus, the SiPc scaffold can be used for the further development of new and more effective PSs.

Declaration of Competing Interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (grant GA 2362/2-1 to AG and SFB1009/B05 to UD). Financial support of AG by the Fonds der Chemischen Industrie, the WWU Graduate Center and Santander Universities greatly acknowledged. We thank O. Mantel (Münster) for technical assistance.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphotobiol.2019.111554.

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