SAARC J. Agric., 21(2): 239-250 (2023) *DOI: https://doi.org/10.3329/sja.v21i2.68226*

PHOTODYNAMIC ANTIMICROBIAL CHEMOTHERAPY REDUCES BACTERIAL LOAD IN LIVE FEEDS IN AQUACULTURE

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ABSTRACT

Live feeds are one of the route of entry of pathogenic bacteria in larval rearing systems in aquaculture. Current study discusses the disinfection of bacteria associated with live feed (*Artemia* nauplii) using photodynamic antimicrobial chemotherapy (PACT). More than 90 % of *Aeromonas hydrophila* in solution and total bacteria associated with live feed were killed on exposure to 5 µM of curcumin under photoexcitation for 30 minutes. The cell wall of *A. hydrophila* was disrupted by the reactive oxygen species generated by photoexcited curcumin. Up to 50 µM curcumin and their photoexcitation products were not toxic to *Artemia* nauplii. The findings of the current study propose photodynamic antimicrobial chemotherapy as a propitious tool for One Health approach and restricting the use of antibiotics in aquaculture settings.

Keywords: Aquaculture, Live feeds, One Health, Photodynamic antimicrobial chemotherapy, Reactive oxygen species.

INTRODUCTION

Variety of organisms such as microalgae, copepods, rotifers, cladocerans, ostracods, protozoans and planktonic forms of crustaceans are used as live feed in shrimp and fish hatcheries across the globe (Das et al., 2012). Live feeds are rich sources of proteins, vitamins, minerals and lipids and enhance the growth and overall health of larval fish and shrimps (Ansari et al., 2021; Samat et al., 2020). Live feeds also carry multiple antibiotic resistant pathogens which causes disease incidents and mass mortalities in hatcheries and some of them are pathogenic to humans too (Hurtado et al., 2020; Levican and Avendaño-Herrera, 2015; McIntosh et al., 2008). Therefore, it is important to identify alternative strategies to control their proliferation in

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Received: 16.08.2023 *Accepted: 20.12.2023*

aquaculture settings. Current study explored the possibilities of utilizing photodynamic antimicrobial chemotherapy (PACT) for preparing bacteria free live feed with the aim of reducing the use of antibiotics in aquaculture.

PACT uses a combination of photosensitizer and light source, in which the former undergoes a series of electron transfer reactions on photoexcitation to generate reactive oxygen species (ROS). The photoexcitation induces the shifting of electrons from its ground state (S0) to short lived singlet (S1) state and to a highly unstable triplet state species (T1). In an attempt to return to its stable state, triplet state species releases extra energy to other molecules in the proximity through type I or type II reactions, leading to the generation of an array of reactive species which are called together as reactive oxygen species (Anas et al., 2021). The high energy carrying ROS can kill bacteria in the proximity through oxidizing proteins, lipids and genetic materials and has been experimented successfully for killing pathogens in dental treatments, skin infections, veterinary medicine, food sterilization, disinfecting medical implants, and disinfection of water (Anas et al., 2021). Photodynamic therapy is comparable with antibiotics in terms of its fast action and broad spectrum, and the lack of resistance development is an additional advantage. Photodynamic antimicrobial chemotherapy with a wide range of activity profile against viruses and bacteria is advantageous for its applications in aquaculture (Abdulaziz et al., 2022; Malara et al., 2019).

The curcumin is a polyphenolic yellow pigment found in the root of turmeric (*Curcuma longa*), a commonly used herbal medicine in Asian countries. The killing of water associated pathogens of *Vibrio* spp. using photoexcited curcumin was reported earlier (Moideen et al., 2022). In the current study, we report the antibacterial activity of photoexcited curcumin to multiple antibiotic resistant *Aeromonas hyrophila* (MMRF 33), and its use in reducing the bacterial load associated with *Artemia* nauplii.

MATERIALS AND METHODS

Light source and photosensitizer

Two different LED units were designed for exciting curcumin for killing bacteria in plates and *Artemia* in experimental tanks. A light unit having a single LED of 3W (460 ± 30 nm,) for bacterial experiments and another one having 100 LEDs of 300 W (420 ± 30 nm) for exposing experimental tank were designed. In both cases, the light power of 11 mW cm^{-2} was confirmed.

Curcumin (AVT natural products or Himedia) was used as the photosensitizer in the current study. The stock solutions of curcumin (10 mM) was prepared in DMSO and working dilutions were prepared immediately before use in distilled water.

Bacterial isolates and live feed

A. hydrophila (MMRF 33) maintained at Marine Microbial Reference Facility, CSIR-National Institute of Oceanography was used for testing antimicrobial efficacy of photoexcited curcumin. The bacteria were retrieved from the lyophilized stock in Luria Bertani (LB) broth following standard microbiology protocols. *A. hydrophila* were transferred into LB agar slants and the purity was confirmed by Gram staining and 16S rRNA gene sequencing. For further experiments, the isolates were grown in LB broth for 24 h at 28 ± 2 ^{0} C.

Artemia nauplii was used as live feed in the current study, which were reared in artificial sea water by following standard protocols (Sorgeloos et al., 1977). Commercially available cysts (Ocean star international, USA) of *Artemia* (0.5 g) were dispensed in water (45 ml), aerated for one hour and were decapsulated by treating with equal volume of sodium hypochlorite for 5- 10 minutes. Decapsulated cysts were separated on a nylon mesh (100 µ pore size), washed copiously with water to remove residual chlorine. The cysts were dispensed in artificial seawater with continuous aeration and light exposure for 12 h. The nauplii were used immediately for the experiments.

Antibiotic susceptibility profile of *A. hydrophila*

The sensitivity of *A. hydrophila* towards twenty one antibiotics (Ampicillin - 10 mcg, Bacitracin - 10 units, Cefepime - 30 mcg, Cefoxitin – 30 mcg, Ceftazidime - 30 mcg, Ceftriaxone - 30 mcg, Cephalothin- 30 mcg, Chloramphenicol- 30 mcg, Erythromycin - 10 mcg, Gatifloxacin - 5 mcg, Gentamicin – 10 mcg, Imipenem – 10 mcg, Kanamycin - 5 mcg, Meropenem - 10 mcg, Moxifloxacin - 5 mcg, Nalidixic Acid - 30 mcg, Norfloxacin - 10 mcg, Penicillin G - 2 mcg, Streptomycin – 25 mcg, Tetracycline – 30 mcg, Vancomycin - 10 mcg.) were studied following disk diffusion assay. Muller Hinton (MH) agar plates were swabbed with overnight-grown bacterial suspension, commercially-available antibiotic discs (Himedia, India) were placed on the agar and incubated at 28 ±2°C for 24 h. The zone of inhibition was recorded using an antibiotic susceptibility scale (Himedia, India) and antibiotic resistance profile was calculated following the standard antimicrobial zone size interpretation chart (Bauer et al., 1966).

Photostability of curcumin

The photostability of curcumin (25 µM) was expressed as a measure of decrease in the absorption spectral peak on exposure to photoexcitation for 30 minutes. The absorption spectra (300-750 nm) of curcumin were measured at 10 minutes interval for 30 minutes in the presence and absence of photoexcitation. Further the FTIR spectrum of both curcumin and photoexcited curcumin was determined (Perkin Elmer Spectrum 100 FTIR Spectrometer) and the spectra were recorded at a scan range of 4000 cm^{-1} to 700 cm^{-1}.

Photodynamic killing of *A. hydrophila*

The efficiency of photoexcited curcumin to kill *A. hydrophila* was tested using standard death rate assay (Asok et al., 2012). A fixed concentration (10^6 cells ml^{-1}) of overnight grown *A. hydrophila* were dispensed in different tubes, mixed with different concentrations of curcumin (1nM- 5 µM) and exposed to blue light (11 $mWcm^{-2}$) from an LED for 30 minutes. The temperature of the solution was monitored using an iButton® temperature data logger (DS1922L/DS1922T, Maxim Integrated, USA) and confirmed as below 28 ± 2 ^{0} C. A duplicate set of tubes containing bacterial cells (10^6 cells ml^{-1}) and different concentrations of curcumin were incubated under dark conditions for 30 minutes as dark control. Bacterial solution (10^6 cells ml^{-1}) without curcumin and light exposure were maintained as negative control. The bacteria surviving the above treatments were enumerated by spreading 100 µl of the sample over the surface of LB agar medium. The plates were incubated at 28 ± 2^0 C for 24 h and the number of bacterial colonies appeared were counted. The survival of bacteria after photoexcitation and dark treatments were calculated as number of bacteria grown after each treatment in comparison with the initial count.

Interaction of bacteria with curcumin

The interaction of curcumin with bacteria were studied using UV-Visible spectroscopy and scanning electron microscopy. The UV-Visible spectra of bacteria incubated with different concentrations of curcumin (10, 50, 100 µM) were measured after 30 minutes of interaction. Here the overnight grown bacteria (10 ml) from a LB medium were centrifuged and resuspended in 2ml of phosphate buffered saline (pH 7.4±2). The tubes were supplemented separately with different concentration of curcumin and incubated at room temperature for 30 minutes under dark. A control tube containing curcumin solution (2 ml) was also maintained. Subsequently, the supernatant of the reaction mixture was separated by centrifugation and the absorbance was measured at 420 nm. The difference between the absorbance of supernatant of experimental and control tube was considered as the concentration retained with the bacterial cell. Further, the impact of curcumin and photoexcited curcumin on the cell wall integrity of *A. hydrophila* was studied using scanning electron microscopy (JEOL-JSM- 6490). The bacterial cells exposed to curcumin and photoexcited curcumin were separated by copious (3 times) washing with phosphate buffered saline (pH 7.4±2) and were treated with glutaraldehyde (3 %) for 60 minutes. The cells were washed and dehydrated by sequential exposure to different concentrations (30-100 %) of ethanol. The cells were placed over a glass slide, air dried and sputter-coated, and were imaged on a JEOL-JSM- 6490 microscope.

Photodynamic disinfection of live feed

Artemia nauplii (10 nos) were distributed into the wells of a sterile six – well plate carrying 10 ml of artificial sea water. Different concentration of curcumin (0, 5, 10, 20, 30, 40, 50, 100 µM) was added into separate wells and were exposed to

photoexcitation for 30 minutes. Subsequently, the plates were incubated at room temperature for 24 h. Control wells without photoexcitation was also maintained. The *Artemia* survived after 24 h was counted. A duplicate set of same experiment was maintained to understand the effect of photodynamic antimicrobial chemotherapy on the bacterial load associated with *Artemia*. Here the *Artemia* was harvested after 30 minutes exposure to photoexcitation, macerated with sterile saline (0.8 % NaCl) and spread over the surface of ZoBell's marine agar plate. The plates were incubated 28 ± 2 °C for 24 h in an incubator. The colonies formed were enumerated and the reduction in total bacterial population was calculated in comparison with the control group.

RESULTS AND DISCUSSION

Photostability of curcumin

The photostability of curcumin was expressed as a function of reduction in its absorption spectra with time on exposure to light. The absorption spectra of curcumin remain intact under dark conditions (Figure 1A) while it reduced significantly on exposure to photoexcitation (Figure 1B). Nearly 50 % reduction in the intensity of absorption peak at 420 nm was observed in the first 10 minutes of photoexcitation. The absorption spectra also showed a shift towards the blue region on photoexcitation with a blue shift in its peak from 420 nm to 370 nm. The FTIR spectrum of curcumin showed no significant variation (Figure 1C) and the prominent peaks due to the stretching vibrations of phenolic O-H group at 3374 – 3283 cm^{-1}, fused C=O and C=C group at 1636 cm^{-1}, asymmetric stretching of C-O-C bond at 1011 cm^{-1} and Benzoate trans –CH vibration at 950 cm^{-1} were found in both curcumin and its photoexcited form.

Susceptibility of *Aeromonas hydrophila* towards antibiotics and photoexcited curcumin

The *A. hydrophila* were found resistant to 9 out of 21 antibiotics tested in the current study. *A. hydrophila* was resistant to Bacitracin, Cefoxitin, Ceftazidime, Cephalothin, Erythromycin, Kanamycin, Penicillin G, Streptomycin, Vancomycin and were sensitive to Cefepime, Gatifloxacin, Gentamicin, Imipenem, Moxifloxacin, Meropenem, and Norfloxacin. On the other side, *A. hydrophila* was sensitive to micromolar concentrations of photoexcited curcumin (Figure 2). Nearly 40 % reduction in the survival was observed when *A. hydrohila* were exposed to 0.5 µM curcumin under photoexcitation for 30 minutes. The survival rate decreased further with the concentration of curcumin and reached a below 20 % with a combination of 2 µM curcumin and photoexcitation. All bacterial cells were survived up to 5 µM of curcumin in the absence of photoexcitation.

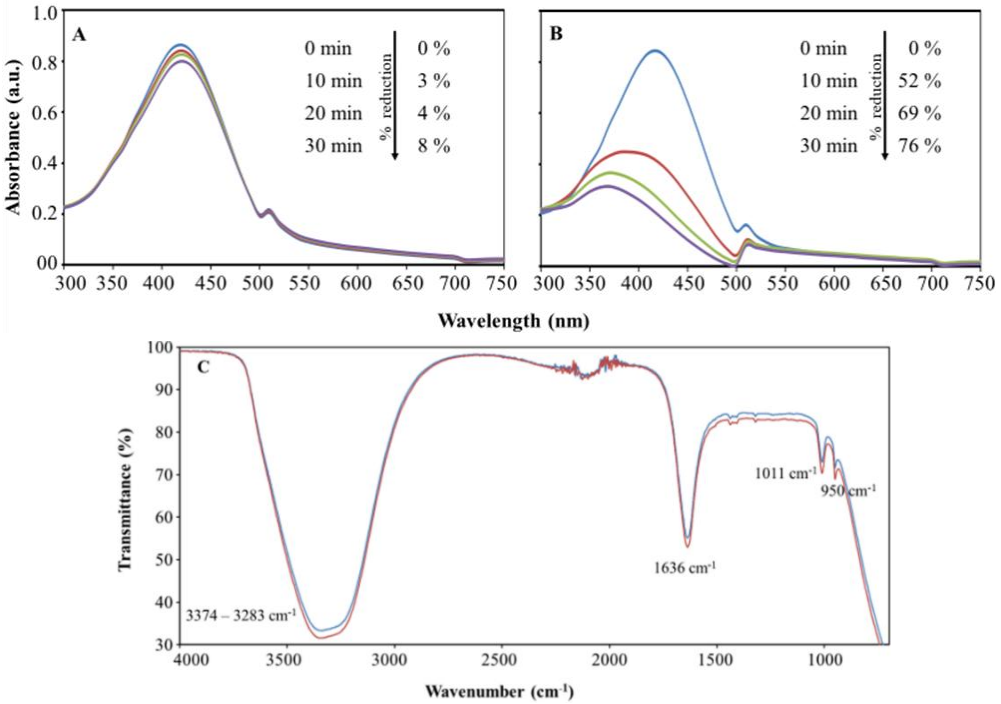

Figure 1. The absorption spectra of curcumin exposed to dark (A) and light (B) at different time intervals (blue – 0 minutes, red – 10 minutes, green – 20 minutes, violet – 30 minutes) and the FT-IR spectrum (C) of curcumin (blue) & photoexcited (red) curcumin.

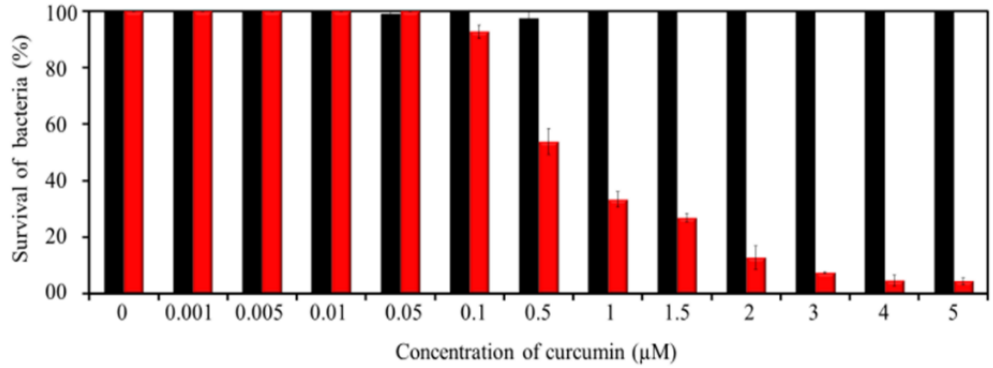

Figure 2. Survival of *Aeromonas hydrophila* exposed to different concentration of curcumin (black) and photoexcited curcumin (red).

Curcumin – bacteria interaction

Curcumin showed a strong interaction with the bacterial cell which increased with the concentration of curcumin (Figure 3A). Nearly 16 % of 10 µM curcumin was retained in the bacterial cell after 30 minutes of incubation, while it increased to nearly 70 % when the concentration of curcumin was increased to 100 µM. Further studies using scanning electron microscopy showed that the photoexcited curcumin induce damage to the cell wall of *A. hydrophila* (Figure 3B).

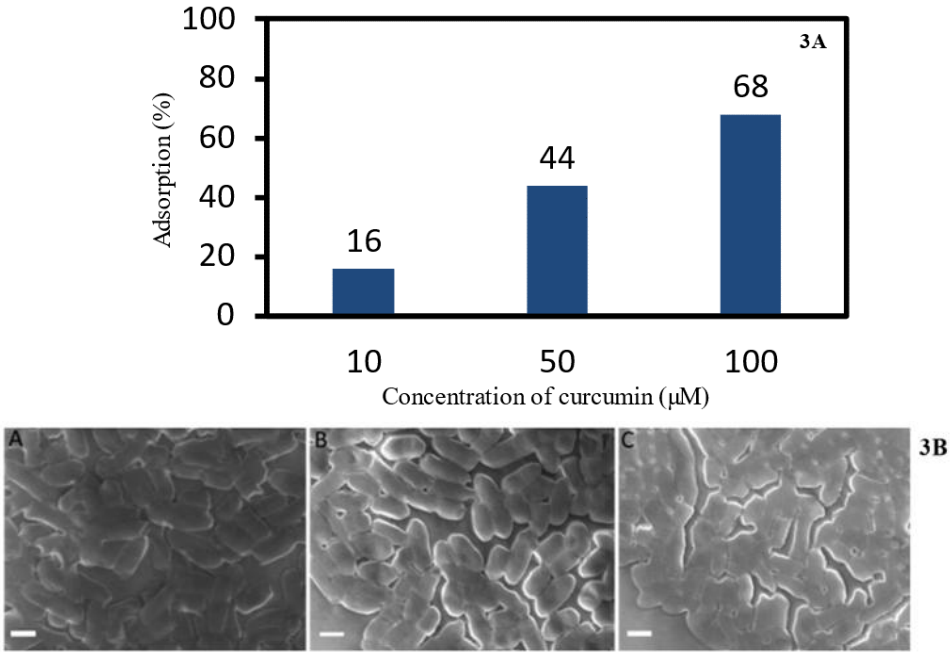

Figure 3. The attachment of increasing concentration of curcumin with *Aeromonas hydrophila* (3A) and the SEM images of *Aeromonas hydrophila* (3B) exposed to no treatment (A), treated with curcumin (B) and curcumin and light (C).

Efficiency of PACT in disinfecting live feed

Curcumin and photoexcited curcumin were safe to *Artemia* nauplii and there were no mortalities observed after 24 h exposure up to 50 µM concentrations (Figure 4A). On the other side, photoexcited curcumin reduced the abundance of bacteria associated with *Artemia* nauplii significantly with increasing concentration (Figure 4B). Under photoexcitation, 1 µM of curcumin could disinfect nearly 59% of bacteria which increases to 92% at 5 µM. Also, without photoexcitation, curcumin could not disinfect the bacterial population at the tested concentrations up to 50 µM.

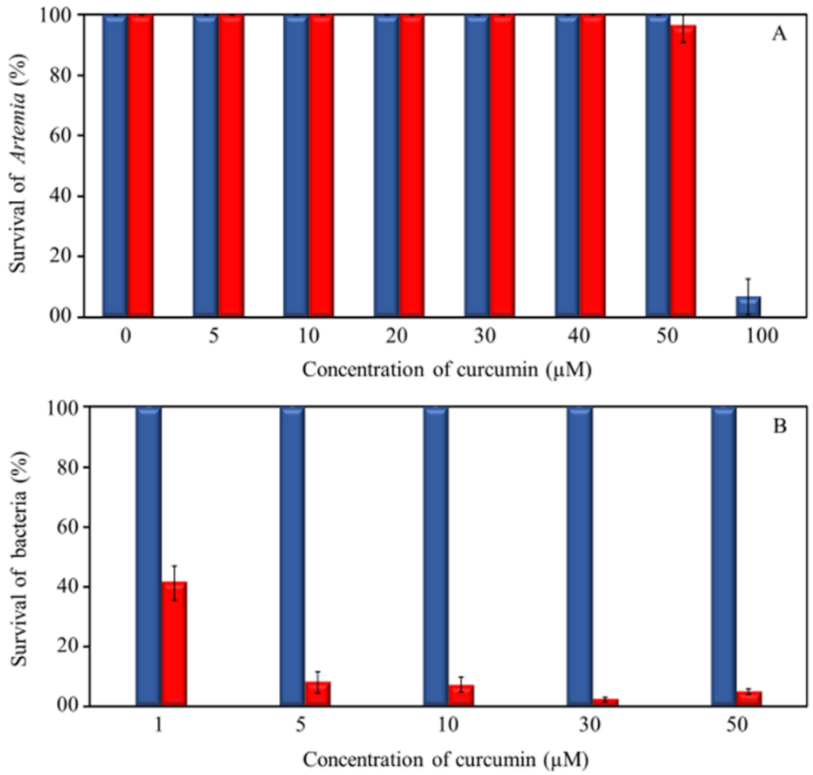

Figure 4. Survival of *Artemia* (A) and attached bacterial population (B) exposed to different concentration of curcumin (blue) and photoexcited curcumin (red).

The study findings revealed the potentials of photodynamic antimicrobial chemotherapy with a combination of curcumin and light source, in reducing the bacterial load associated with live feeds. Curcumin has been explored largely in ethnopharmacology for their biomedical properties including wound healing, antioxidant, and treating neurodegenerative diseases (Garodia et al., 2023; Kumari et al., 2022). Curcumin has a broad absorption spectrum with a peak at around 420 nm, which shifted towards the blue region with a reduction in the peak intensity on exposure to photoexcitation. The minor peak at around 530 nm is attributed to the formation of curcumin anion from the deprotonation of OH group in the phenolic backbone of curcumin (Priyadarsini, 2009). Structurally, curcumin have two O-methoxy phenols attached symmetrically with α, β-unsaturated β-diketone linker (Priyadarsini, 2009). The FT-IR spectrum of photoexcited curcumin also didn't show the formation of any toxic adducts and was similar to that of curcumin. However, the changes in the intensity of transmission indicates the degradation of curcumin on photoexcitation. The characteristic peaks at 3374 – 3283 cm^{-1} (due to stretching

vibrations of phenolic O-H group), 1636 cm^{-1} (due to the fused C=O and C=C group), 1011 cm^{-1} (due to asymmetric stretching of C-O-C bond) , and at 950 cm^{-1} (due the benzoate trans –CH vibration) are in agreement with the previous reports on the spectral properties of curcumin (Mohan et al., 2012). Curcumin is less stable with the photoexcitation and the degradative products such as vanillin, ferulic acid, cinnamic acid, and vanillic acid are non-toxic to the cells (Liao et al., 2016; Singh et al., 2010). Lack of cytotoxicity of photodegraded curcumin are attractive for aquaculture application as this avoid the side effects of degraded products, toxicity to animal cells due to excessive production of reactive oxygen species and the chances of resistance development due to remnants of photosensitiser.

Although the degradation of curcumin started within the first ten minutes of photoexcitation, the death rate assays, and live feed studies confirmed that the time was sufficient for killing bacteria in liquid medium or associated with live feed. Previous studies showed the killing of *V. parahaemolyticus, V. cholerae, V. harveyi, A. hydrophila, P. aeruginosa, S. typhimurium, E. coli* and *S. aureus* with different combinations of curcumin and light sources (Moideen et al., 2022; Penha et al., 2016; Rafeeq et al., 2020). The killing of bacteria is dependent on the uptake of curcumin, the cell wall characteristics of bacteria and the concentration of ROS generated. Current study recorded the uptake of ~70 % of 100 µM curcumin by *A. hydrophila* over 30 minutes of incubation. This was much higher compared to 25 – 30% of curcumin (0.5 - 5 mg/ml) by *Streptococcus* sp. (Soares et al., 2020). Based on the molecular weight and adsorption properties of curcumin, it could be assumed that the curcumin may not penetrate the cell wall of bacteria (Ratrey et al., 2020) and hence the uptake and photodynamic activities are restricted at the surface. The disruption of the cell wall of *A. hydrophila* by photoexcited curcumin was evident in the SEM images. Similar mode of action of curcumin and photoexcited curcumin were reported on other gram-negative bacteria such as *V. cholera, V. parahaemolyticus, V. harveyi* and *V. campbellii* (Moideen et al., 2022; Wu et al., 2016). A nuclear targeted PACT is possible on conjugating the photosensitizer with cell penetrating peptides, cationic molecules and membrane perturbing agents (Anas et al., 2021). The complexes of curcumin with Mn^{2+} were also able to exhibit bacterial cell wall permeabilization and induce oxidative damage to the genetic material (Saha et al., 2020). In another study, the abilities of curcumin to bind with the cytoskeleton protein FtsZ of *E coli* and *B. subtilis* to inhibit its assembly, was reported (Kaur et al., 2010).

Although the cells of *A. hydrophila* had shown resistance towards β – lactam and glycopeptide classes of cell wall synthesis inhibiting antibiotics such as Ceftazidime, Cephalothin, Penicillin G and Vancomycin (Bush, 2012), they were sensitive towards photoexcited curcumin. This could be because ROS have multiple targets in the cell wall of bacteria, while the antibiotics have specific target. The time required by PACT for disinfecting bacterial cells (30 min) is comparable with antibiotics.

However, the risky part of PACT is that the ROS is nonspecific and can also cause harm to the eukaryotic cells in the proximity. However, this is largely dependent on the ROS tolerance mechanisms of different organisms and can be controlled by selecting appropriate concentrations of curcumin and light source. The current study indicated that the photoexcited curcumin up to 50 µM did not induce any toxic effects to the live feed *Artemia* nauplii. Our previous studies reported irregular expression of neuroendocrine hormone genes in the post larvae of *Penaeus monodon* on exposure to photoexcited curcumin but there was no mortality even at 50 µM (Abdulaziz et al., 2022). The 5 µM concentration of curcumin could kill more than 90 % of the associated bacteria, without inducing any toxic effects to *Artemia* nauplii. Synthetic photosensitizer, Rose bengal (30 µM) and cationic porphyrin compound (TMPyP) (20 µM) were also found suitable for disinfecting live feeds *Artemia* nauplii and microalgae respectively, but the concentration and time required for getting a similar killing efficiency was higher compared to curcumin (Asok et al., 2012; Malara et al., 2019). These findings propose photodynamic antimicrobial chemotherapy as an efficient disinfection technique for live feeds, which is the major route of pathogen entry in aquaculture systems.

CONCLUSION

Current study proposes PACT as a propitious strategy to replace antibiotics in aquaculture. Although there is no report on bacterial resistance against PACT, caution is required in its use because of the possible toxicity of higher concentrations of ROS on host cells. Identification of appropriate combinations of photosensitizers and light sources are required to avoid the unintended effects of PACT. Our results indicate that the exposure to a combination of 5 µM of curcumin and light for 30 minutes can disinfect more than 90 % of bacteria associated with live feed. The combination may find applications in preparing bacteria free live feeds for aquaculture.

ACKNOWLEDGEMENT

The authors thank the Director, CSIR-National Institute of Oceanography, Goa for extending all the required support. This work was implemented with the financial support of the Department of Biotechnology, Govt of India (BT/PR26858/AAQ/3/880/2017). SKM is a recipient of the Junior Research Fellowship (UGC- Ref. No.: 935/ (CSIR-UGC NET JUNE 2017) of University Grants Commission, Govt of India. The support was extended by Mr. Sajin P. Ravi, Amrita Centre for Nano Sciences & Molecular Medicine, for taking SEM images.

REFERENCES

Abdulaziz, A., Pramodh, A.V., Sukumaran, V., Raj, D. and John, A.M.V.B. (2022). The Influence of Photodynamic Antimicrobial Chemotherapy on the Microbiome, Neuroendocrine and Immune System of Crustacean Post Larvae. *Toxics*, *11*(1): 36.

Anas, A., Sobhanan, J., Sulfiya, K.M., Jasmin, C., Sreelakshmi, P.K. and Biju, V. (2021). Advances in photodynamic antimicrobial chemotherapy. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, *49*, 100452. https://doi.org/https://doi.org/10.1016/j.jphotochemrev.2021.100452

Ansari, F.A., Guldhe, A., Gupta, S.K., Rawat, I. and Bux, F. (2021). Improving the feasibility of aquaculture feed by using microalgae. *Environmental Science and Pollution Research*, *28*(32), 43234-43257. https://doi.org/10.1007/s11356-021-14989-x

Asok, A., Arshad, E., Jasmin, C., Somnath Pai, S., Bright Singh, I.S., Mohandas, A. and Anas, A. (2012). Reducing *Vibrio* load in *Artemia* nauplii using antimicrobial photodynamic therapy: a promising strategy to reduce antibiotic application in shrimp larviculture. *Microbial Biotechnology*, *5*(1): 59 -68.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966). Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology*, *45*(4_ts): 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bush, K. (2012). Antimicrobial agents targeting bacterial cell walls and cell membranes. *Revue scientifique et technique (International Office of Epizootics)*, *31*(1): 43-56.

Das, P., Mandal, S. C., Bhagabati, S., Akhtar, M. and Singh, S. (2012). Important live food organisms and their role in aquaculture. *Frontiers in aquaculture*, *5*(4): 69-86.

Garodia, P., Hegde, M., Kunnumakkara, A.B. and Aggarwal, B.B. (2023). Curcumin, Inflammation, and Neurological disorders: How are they linked? *Integrative Medicine Research*, 100968. https://doi.org/https://doi.org/10.1016/j.imr.2023.100968

Hurtado, L., Miranda, C.D., Rojas, R., Godoy, F.A., Anazco, M.A., and Romero, J. (2020). Live feeds used in the larval culture of red cusk eel, Genypterus chilensis, carry high levels of antimicrobial-resistant bacteria and antibiotic-resistance genes (ARGs). *Animals*, *10*(3): 505.

Kaur, S., Modi, N. H., Panda, D. and Roy, N. (2010). Probing the binding site of curcumin in Escherichia coli and Bacillus subtilis FtsZ–a structural insight to unveil antibacterial activity of curcumin. *European journal of medicinal chemistry*, *45*(9): 4209-4214.

Kumari, A., Raina, N., Wahi, A., Goh, K. W., Sharma, P., Nagpal, R., Jain, A., Ming, L.C. and Gupta, M. (2022). Wound-Healing Effects of Curcumin and Its Nanoformulations: A Comprehensive Review. *Pharmaceutics*, *14*(11): 2288. https://www.mdpi.com/1999-4923/14/11/2288

Levican, A. and Avendaño-Herrera, R. (2015). Bacteria associated with mass mortality of post-larvae of red conger eel (Genypterus chilensis) cultured in a Chilean farm. *Bulletin of Europian Association of Fish Pathol*ogy, *35*: 162-169.

Liao, J.-H., Huang, Y.-S., Lin, Y.-C., Huang, F.-Y., Wu, S.-H. and Wu, T.-H. (2016). Anticataractogenesis Mechanisms of Curcumin and a Comparison of Its Degradation Products: An in Vitro Study. *Journal of Agricultural and Food Chemistry*, *64*(10): 2080-2086. https://doi.org/10.1021/acs.jafc.6b00430

Malara, D., Høj, L., Oelgemöller, M., Malerba, M., Citarrella, G. and Heimann, K. (2019). Sensitivity of live microalgal aquaculture feed to singlet oxygen-based photodynamic therapy. *Journal of Applied Phycology*, *31*(6): 3593-3606. https://doi.org/10.1007/s10811-019-01854-1

McIntosh, D., Ji, B., Forward, B. S., Puvanendran, V., Boyce, D. and Ritchie, R. (2008). Culture-independent characterization of the bacterial populations associated with cod (Gadus morhua L.) and live feed at an experimental hatchery facility using denaturing gradient gel electrophoresis. *Aquaculture*, *275*(1-4): 42-50.

Mohan, P.K., Sreelakshmi, G., Muraleedharan, C. and Joseph, R. (2012). Water soluble complexes of curcumin with cyclodextrins: Characterization by FT-Raman spectroscopy. *Vibrational Spectroscopy*, *62*: 77-84.

Moideen, S.K., Anas, A., Sobhanan, J., Zhao, H. and Biju, V. (2022). Photoeradication of aquatic pathogens by curcumin for clean and safe drinking water. *Journal of Photochemistry and Photobiology A: Chemistry*, *432*: 114104. https://doi.org/https://doi.org/10.1016/j.jphotochem.2022.114104

Penha, C.B., Bonin, E., Silva, A.F.d., Hioka, N., Zanqueta, E.B., Nakamura, T.U., Filho, B.A. d.A., Campanerut-Sa, P.A.Z. and Mikcha, J.M.G. (2016). Photodynamic inactivation of foodborne and food spoilage bacteria by curcumin. *LWT - Food Science and Technology* 1-5. https://doi.org/10.1016/j.lwt.2016.07.037

Priyadarsini, K.I. (2009). Photophysics, photochemistry and photobiology of curcumin: Studies from organic solutions, bio-mimetics and living cells. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, *10*(2): 81-95.

Rafeeq, S., Shiroodi, S., Schwarz, M.H., Nitin, N. and Ovissipour, R. (2020). Inactivation of Aeromonas hydrophila and Vibrio parahaemolyticus by curcumin-mediated photosensitization and nanobubble-ultrasonication approaches. *Foods*, *9*(9):1306.

Ratrey, P., Dalvi, S.V., and Mishra, A. (2020). Enhancing aqueous solubility and antibacterial activity of curcumin by complexing with cell-penetrating octaarginine. *ACS omega*, *5*(30): 19004-19013.

Saha, T., Kumar, P., Sepay, N., Ganguly, D., Tiwari, K., Mukhopadhyay, K. and Das, S. (2020). Multitargeting Antibacterial Activity of a Synthesized Mn2+ Complex of Curcumin on Gram-Positive and Gram-Negative Bacterial Strains. *ACS Omega*, *5*(27): 16342-16357. https://doi.org/10.1021/acsomega.9b04079

Samat, N.A., Yusoff, F.M., Rasdi, N.W. and Karim, M. (2020). Enhancement of Live Food Nutritional Status with Essential Nutrients for Improving Aquatic Animal Health: A Review. *Animals*, *10*(12): 2457. https://www.mdpi.com/2076-2615/10/12/2457

Singh, U., Verma, S., Ghosh, H.N., Rath, M.C., Priyadarsini, K.I., Sharma, A., Pushpa, K.K., Sarkar, S.K. and Mukherjee, T. (2010). Photo-degradation of curcumin in the presence of TiO2 nanoparticles: Fundamentals and application. *Journal of Molecular Catalysis A: Chemical*, *318*(1): 106-111.

Soares, J.M., Silva, K.O.O., Inada, N.M., Bagnato, V.S. and Blanco, K.C. (2020). Optimization for microbial incorporation and efficiency of photodynamic therapy using variation on curcumin formulation. *Photodiagnosis and Photodynamic Therapy*, *29*: 101652.

Sorgeloos, P., Bossuyt, E., Laviña, E., Baeza-Mesa, M., and Persoone, G. (1977). Decapsulation of Artemia cysts: A simple technique for the improvement of the use of brine shrimp in aquaculture. *Aquaculture*, *12*(4): 311-315.

Wu, J., Mou, H., Xue, C., Leung, A. W., Xu, C. and Tang, Q.-J. (2016). Photodynamic effect of curcumin on *Vibrio parahaemolyticus*. *Photodiagnosis and photodynamic therapy*, *15*, 34-39.