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Antifungal activity and mechanism of monocaprin against food spoilage fungi

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ABSTRACT

Monocaprin is generally recognized as a safe food additive and widely used as a functional emulsifier in food industry. In this work, the preservative ability of monocaprin against three food spoilage fungi, *Saccharomyces cerevisiae*, *Aspergillus niger* and *Penicillium citrinum* was investigated to evaluate the potential use of monocaprin as a potent food preservative. The minimum inhibitory concentrations (MIC) of monocaprin against these fungi were 0.31, 0.63 and 0.63 mg/mL, and the minimum fungicidal concentrations were 1.25, 2.50 and 2.50 mg/mL, respectively. The MICs remained unchanged when the pH values were increased from 3 to 9. After being exposed to monocaprin at MIC, the growth of *A. niger* or *P. citrinum* was completely inhibited for one week, and *S. cerevisiae* began to degrade at 100 h. These results indicated that monocaprin may be a potential preservative independent of pH. The mechanism of action was then investigated by the measurement of damage of cell walls, permeability of cell membrane, release of cellular contents and morphological observation. Results indicated that monocaprin may kill *S. cerevisiae* by disrupting the cell wall and plasma membrane, resulting in the release of cellular contents. Monocaprin inhibited *A. niger* spore and *P. citrinum* by increasing the permeability of the cell membrane and release of cellular contents, but not involving cell structure alteration.

1. Introduction

Many kinds of foods, including meats, fruits and cereals, are extremely susceptible to fungal spoilage. Fungal growth has been reported to be responsible for food spoilage, which leads to significant economic losses (Cabral, Pinto, & Patriarca, 2013; Moss, 2008). *Penicillium*, *Aspergillus* and yeasts are common fungi which can be easily isolated from spoiled food, especially fresh fruits, vegetables and grains, and produce many toxins. To keep food fresh and extend the shelf life, synthetic additives, such as propionate, sorbate and benzoate have been widely used in food and other products, and have been proved to effectively inhibit food spoilage fungi (Beuchat, 1981; Chakrabarti & Varma, 2000; Heydaryinia, Veissi, & Sadadi, 2011). However, the uses of chemical preservatives are extremely restricted by new resistant fungal strains, their toxicity, poor solubility and low potency (Jing et al., 2014). Therefore, novel and safe alternatives are urgently needed for extending the shelf life of food.

Monocaprin is an extensively used food emulsifier containing intermediate-length C_{10} fatty acids (Park et al., 2010), and is generally recognized as a safe food additive (Kyungmin, Ohtaek, Seonmin, Jaehwan, & Chang, 2009; Park, Kwon, Ahn, Lee, & Chang, 2010). Because it's an energy source easily absorbed by human body, monocaprin has been used as an ingredient in foods

Abbreviations: DIZ, Diameter of inhibition zone; MFC, Minimum fungicidal concentration; MIC, Minimum inhibitory concentration; PBS, Phosphate buffer saline; PDA, Potato dextrose agar; PDB, Potato dextrose broth.

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designed for infants, children, the elderly, and the sick (Marten, Pfeuffer, & Schrezenmeir, 2006). Moreover, monocaprin has been reported to exhibit a broad spectrum of inhibitory effects against food-borne bacteria, including *Campylobacter jejuni*, *Salmonella* spp., *Bacillus cereus*, *Salmonella typhimurium* and *Escherichia coli*, and fungi like *Penicillium* and *Aspergillus* species, as well as virus, such as HIV, HSV, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Buňková et al., 2010; DolezÁlková, Máčalík, Butkovičová, Janiš, & BunKová, 2012; Neyts, Kristmundsdóttir, De, & Thormar, 2000; Nitbani, Jumina, Siswanta, & Sholikhah, 2016; Thormar & Hilmarsson, 2010; Thormar, Hilmarsson, & Bergsson, 2006; Thorgeirsdóttir, Kristmundsdóttir, Thormar, Axelsdóttir, & Holbrook, 2006). It has been reported that monocaprin killed *Chlamydia trachomatis* by disrupting the membrane(s) of the elementary bodies (Bergsson, Arnfinnsson, Karlsson, Steingrímsson, & Thormar, 1998). Monocaprin killed *Candida albicans* by disrupting or disintegrating plasma membrane, and Gram-positive cocci was killed by disintegration of the cell membrane, leaving the bacterial cell wall intact (Bergsson, Arnfinnsson, & Thormar, 2001).

Although the antibacterial activity and mode of action of monocaprin against food pathogens and spoilage organisms have been reported, the information about antifungal efficiency and persistence of monocaprin is still scarce. Most importantly, to our knowledge, little has been known about the mechanism by which monocaprin may act on the growth of food spoilage fungi. Therefore, in this study, we investigated the inhibitory ability of monocaprin against *S. cerevisiae*, *A. niger* and *P. citrinum* as compared with potassium sorbate and sodium benzoate, and to further evaluate the possible underlying mechanism of action for monocaprin against these fungi.

2. Materials and methods

2.1. Chemicals

Monocaprin was synthesized from camphor tree seed kernel oil and glycerol using the lipase from porcine pancreas as a catalyst (Zeng et al., 2012). Monocaprin was then purified by short-path vacuum distillation, and the purity of monocaprin (95.8%, w/w) was determined by comparing characteristic peak area with standard monocaprin (Sigma-Aldrich Co, China, purity > 99%, w/w) via gas chromatography (GC).

Potassium sorbate and sodium benzoate (food grade) were from Tianjing Dongda Chemical Group Co. (China). D-sorbitol solution (AR, 98.0%) and Tween-80 (cell culture grade) were purchased from Shanghai Aladdin Bio-Chem Technology Co. (China). All other chemicals used in this study were of analytical grade.

2.2. Fungal strains and culture conditions

S. cerevisiae ACCC 20167 and *A. niger* ACCC 30117 were obtained from Agricultural Culture Collection of China, *P. citrinum* AS3.2788 was purchased from Guangdong Huankai Microbial Sai. & Tech. Co. (China). Prior to use, *S. cerevisiae* was maintained on potato dextrose broth (PDB) at 28 °C for 48 h, while *A. niger* and *P. citrinum* were cultured on potato dextrose agar (PDA) at 28 °C for approximate 4–7 days. Cells of *S. cerevisiae* were harvested via centrifugation and washed with sterilized water three times, while spores of *A. niger* and *P. citrinum* were harvested in sterilized water using a sterile inoculation loop and gentle agitation. The initial concentration of cells or spores was adjusted to approximately $1–2 \times 10^6$ CFU/mL.

2.3. Determination of inhibitory effect

The inhibitory effect of monocaprin against *S. cerevisiae*, *A. niger* or *P. citrinum* was determined according to Cizeikiene, Juodeikiene, Paskevicius, and Bartkiene (2013). Briefly, the appropriate volume of sterile PDA was poured onto Petri dishes, and then cooled to 45 °C. After solidifying, 100 μL of the indicator strain suspension (10^6 CFU/mL) was overlaid and evenly spread onto the agar plates. Then, 100 μL of monocaprin solution (0, 2.5, 5 and 10 mg/mL) was added to each well (8 mm in diameter) punched in the PDA plates and incubated for 48 h at 28 °C. Potassium sorbate and sodium benzoate at equal concentrations were used as positive controls. The inhibitory effects of these antifungal agents against tested fungi were evaluated by classifying the diameter of inhibition zones (DIZ). All assays were conducted in triplicate.

2.4. Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC of monocaprin was determined using a two-fold dilution method (Bomfim et al., 2015) with slight modifications. Briefly, serial dilutions of monocaprin were prepared with PDB medium in sterile tubes, with final concentrations at 10.00, 5.00, 2.50, 1.25, 0.63, 0.31, 0.16, 0.08, 0.04 and 0.02 mg/mL, respectively. Fungal suspensions were mixed with the above medium to give a volume of 10 mL and a final concentration of approximately 10^4 CFU/mL. Potassium sorbate and sodium benzoate, with final concentrations at 80.00, 40.00, 20.00, 10.00, 5.00, 2.50, 1.25, 0.63, 0.31 and 0.16 mg/mL, respectively, were used as controls. The medium was cultured in a shaker at 28 °C for 48 h. The MIC was defined as the lowest concentration of antifungal agents that inhibited visible growth of fungi tested after incubation. To determine the MFC, 100 μL suspension obtained from the above tubes was evenly coated onto a PDA plate and incubated at 28 °C for 48 h. The lowest concentration of an antifungal agent that no colony appeared on the plate was considered as MFC.

2.5. Measurement of antifungal activity of monocaprin

2.5.1. Effect of pH on the antifungal potency of monocaprin

The effect of pH on antifungal activity was determined using a method by Argus Cezar, Marcelo, and RobsonMarcelo (2015) with modifications. PDB solutions with different concentrations of antifungal agents were prepared, the pH values of the mixture were adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 or 9.0 by the addition of 0.05 N HCl or 2 N NaOH. Meanwhile, PDB media without antifungal agents at the same pH values described above were used as controls. The MIC of antifungal agents under different pH values was calculated to determine the effect of pH on antifungal activity. The experiment was repeated three times.

2.5.2. Efficacy of antifungal activity

The efficacy of antifungal agents on fungi was determined according to Luo et al. (2014). Briefly, the photoelectric turbidimetry test was used to determine the inhibition efficacy against *S. cerevisiae*. 1 mL of cell suspension (10^6 CFU/mL) was added to 99 mL PDB with MIC of monocaprin. Potassium sorbate and sodium benzoate were used as positive controls, and the medium without an antifungal agent was prepared as a negative control. The media were cultured at 28 °C and 120 rpm. Every 20 h, the A_{600} nm was measured by TU-1950 Double-beam UV–Vis spectrophotometer (Beijing Purkinje General Instrument Co., China). The inhibition (I %) to *S. cerevisiae* was calculated using the following formula:

$$I\% = [(A_{t,CK} - A_{0,CK}) - (A_{t,I} - A0,I)] / [A_{t,CK} - A_{0,CK}] \times 100\%$$