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Evaluation of monolaurin from camphor tree seeds for controlling food spoilage fungi

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ABSTRACT

The preservative ability between monolaurin and potassium sorbate or sodium benzoate against *Saccharomyces cerevisiae*, *Aspergillus niger* and *Penicillium glaucum* was compared to determine the potential use of monolaurin as a novel food preservative. The mode of action for monolaurin has also been investigated. The minimum inhibitory concentrations (MIC) of monolaurin against *S. cerevisiae*, *A. niger* and *P. glaucum* were determined to be 0.32, 0.32 and 0.16 mg/mL, respectively, and the minimum bactericidal concentrations (MBC) were 1.25, 2.50 and 0.63 mg/mL, respectively, and monolaurin began to degrade in the cultures at 100 h, 3rd day and 2nd day, respectively. The results showed that monolaurin had better inhibition abilities against these fungi than potassium sorbate and sodium benzoate. Furthermore, unlike potassium sorbate or sodium benzoate, monolaurin was independent of pH. The exposure of *S. cerevisiae* cells, *A. niger* and *P. glaucum* spores to monolaurin at MIC resulted in the release of intercellular ions, nucleic acids and proteins with molecular weights of 15–100, 15–70 and 35–70 kDa, respectively. Monolaurin was found to be incorporated into the membrane of bacteria, leading to the changes in its permeability and fluidity. In addition, the mode of monolaurin's action may involve inhibition of cell respiration.

1. Introduction

Approximately 20% of food in the world is wasted by spoilage and intoxication caused by mold, food-borne pathogens and bacteria. *Saccharomyces cerevisiae*, *Aspergillus niger* and *Penicillium glaucum* are common fungi causing food spoilage via secreting lipase and proteinase. Food preservatives can inhibit the growth of bacteria to extend shelf life of food, and have been widely used in the food industry. With the increasing concern of food safety issues related to teratogenicity and carcinogenicity on the usage of conventional chemical preservatives such as nitrite, benzoate and sorbate, natural anti-microbial agents have received great attention due to their well-documented safety profiles.

Monolaurin naturally exists in the plants and the milks of humans and animals. It has been reported that monolaurin can provide energy for the body, reduce blood fat and cholesterol levels, lower blood pressure and prevent body fat accumulation (Takeuchi, Noguchi, Sekine, Kobayashi, & Aoyama, 2006; Tholstrup et al., 2004). Monolaurin has been widely used as a food emulsifier in many countries for its bioactivities.

Monolaurin was reported to be active against major food-borne pathogens including *Escherichia coli*, *Staphylococcus aureaus*, *Salmonella typhi*, *Helicobacter pylori*, *Vibrio cholerae* and *Listeria monocytogens*, food-spoilage organisms such as *Penicillium* spp., *Aspergillus* spp., *Bacillus subtilis* and *Herpes simplex virus* (Altieri, Bevilacqua, Cardillo, & Sinigaglia, 2009; Bunkova, Krejci, & Janis, 2010; Kristmundsdóttir, Ranado'ttir, Bergsson, & Thormar, 1999; Ruzicka, Velclova, Janis, & Krejci, 2003; Thormar, Isaacs, Brown, Barshatzky, & Pessolano, 1987; Zeng, Zhao, Luo, & Zhou, 2012). Monoglycerides are derivates of fatty acids, many researchers thought its mode of action may be similar to fatty acids. At the

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present, the mode of action for monoglycerides and fatty acids can be summarized as follows: 1) Most researchers thought that the effects of monoglycerides and fatty acids on bacterial cells can be attributed to their detergent properties due to their amphipathic structure that allows them to interact with the cell membrane and create transient or permanent pores of variable sizes. Tangwatcharin et al. (2012) investigated the combined effect of monolaurin and lactic acid on *S. aureaus*, and found that the combination changed cell wall structure and resulted in the loss of cytoplasm. We have also found that the combination of monolaurin and monocaprin damaged the intactness of cells and membrane of food-borne pathogens and spoilage bacteria (*Escherichia coil*, *S. aureaus* and *B. subtilis*), and caused the release of intercellular proteins, ions and nucleic acids (Zeng et al. 2012). 2) Fatty acids can affect the production of energy via interferring the electron transport chain and disrupting oxidative phosphorylation (Beck et al. 2007; Greenway & Dyke, 1979). 3) Monoglycerides can inhibit the transcription of toxin and antibiotics resistant genes (Projan, Brownskrobot, Schlievert, Vandenesch, & Novick, 1994; Ruzin et al. 1998). However, the mode of action for monoglycerides is still unclear. The reported conclusions were different with different organisms tested and methods used. It was reported that the inhibition was a complicated process and not a result of one mode but combination of several modes (Chamberlain et al. 1991).

In this study, a comparative test on the inhibition ability of monolaurin with potassium sorbate and sodium benzoate against *S. cerevisiae*, *A. niger* and *P. glaucum* was conducted to determine its potential use as a novel preservative. Furthermore, the mode of action for monolaurin against these fungi has also been investigated.

2. Materials and methods

2.1. Bacteria and reagents

S. cerevisiae, *A. niger* and *P. glaucum* were purchased from Agricultural Culture Collection of China. Monolaurin (purity 96.07%, w/w) was synthesized from camphor tree seed oil by our laboratory. All culture media, purchased from Sinopharm Chemical Reagent Co. (Tianjing, China) were of analytical grade. The kit of SDS-PAGE was from Beijing Solarbio Co., China. The pre-stained protein ladder was purchased from Themor Fisher Scientific Inc (Waltham, America).

2.2. Analysis of antimicrobial activity

2.2.1. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The potato dextrose broth was prepared at twice the final concentration. Monolaurin was added into glass tubes to make the sample media with the final concentrations at 0.02, 0.04, 0.08, 0.16, 0.32, 0.63, 1.25, 2.50, 5.00 and 10.00 mg/mL, respectively. Positive control culture media for potassium sorbate or sodium benzoate with the final concentrations at 0.63, 1.25, 2.50, 5.00, 10.00, 20.00, 40.00 and 80.00 mg/mL, respectively, were prepared as the sample media. Negative control sets were simultaneously run without the addition of an antimicrobial agent. An appropriate volume of inoculum (6.0 log CFU/mL) was added to the media to make the final cell concentration at approximately 4.0 log CFU/mL. After 48 h culture at 28 °C and 120 rpm, the concentration in the transparent tube without the pellet containing the least antimicrobial agent was determined as the MIC. Then, 0.1 mL suspension obtained from the above transparent tubes was coated to a potato dextrose agar (PDA) plate. After 48 h culture at 28 °C, the concentration of inoculated suspension containing the least antimicrobial agent which led to no colony appearing on the plate was determined as the MBC.

2.2.2. Effect of pH on antimicrobial activity

The pH values of sample and positive control media containing different levels of antimicrobial agents were adjusted to 3, 4, 5, 6, 7, 8 and 9, respectively. Negative control was prepared without the addition of an antimicrobial agent. The MIC values of antimicrobial agents under different environmental pH values were calculated to determine the effect of pH on antimicrobial activity.

2.2.3. Efficacy of antimicrobial activity

2.2.3.1. S. cerevisiae. Photoelectric turbidimetry test was used to determine the efficacy of antimicrobial activity of an antimicrobial agent to *S. cerevisiae*. Sample, positive control media with MIC of antimicrobial agent and negative control media without addition of an antimicrobial agent were prepared. Then seed suspensions were inoculated into the media to make the initial cell density (OD_{600}) of approximately 0.05, which was equivalent to approximately 4.0 log CFU/mL. The media were cultured at 28 °C and 120 rpm. Every 20 h, the A_{600nm} was measured by 765pc Uv–vis spectrophotometer (Shanghai Spectrum Co., Ltd, China). The inhibition ($I\%$) to *S. cerevisiae* was defined as the reduction to bacterial growth, and was calculated using the following formula:

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

where $A_{0,CK}$ represents the A_{600nm} of negative control at initial time; $A_{t,CK}$ represents the A_{600nm} of negative control at indicated time; $A_{0,I}$ represents the A_{600nm} of sample at initial time; $A_{t,I}$ represents the A_{600nm} of sample at indicated time; $I\% = 100\%$ represents no bacterial growth, and $I\% > 100\%$ represents bacteria that have started to die.

2.2.3.2. A. niger and P. glaucum. The efficacy of antimicrobial activity against *A. niger* or *P. glaucum* was evaluated by measuring the increase of dry weight of mycelium. Sample, positive control media with MIC of antimicrobial agent and negative control media without addition of an antimicrobial agent were prepared. Then, an appropriate volume of inoculum (6.0 log CFU/mL) was added to the media to make a final spore concentration of approximately 4.0 log CFU/mL. Then, the media were cultured at 28 °C and 120 rpm. Each day, the mycelium was filtered, washed and dried to the constant weight. The inhibition ($I\%$) to *A. niger* or *P. glaucum* was defined as the reduction to mycelium growth and calculated using the following formula:

$$I\% = \left[100 - (W_{t,I}/W_{t,CK})*100\right] \times 100\%$$

where $W_{t,I}$ represents the dry weight of mycelium filtered from the sample and positive media at indicated time; $W_{t,ck}$ represents the dry weight of mycelium filtered from negative control media at indicated time.

2.3. Measurement of cell respiration rate

Cell respiration rate was measured as described previously (Chen, Huang, Gao, & Ning, 1994). Briefly, 25 mM phosphate buffer (pH 7.2) containing 0.03 μM glucose and MIC of monolaurin was prepared. The control was prepared without the addition of monolaurin. The seed suspension was inoculated into the system to make the final concentration of cells or spores at approximately 4.0 log CFU. The inoculated mixture was added into the well of O_2 electrode and kept at 28 °C. The well was immediately closed after bubbling enough O_2 into it. The consumption of O_2 was measured by OXY041A Clark-type polarographic O_2 electrode (Rank Bros Ltd., Bottisham, England). The respiration rate (R, μmol O_2/g/min) was defined as velocity of O_2 consumption of cells or spores in the