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Evaluation of *Muscodor cinnamomi* as an egg biofumigant for the reduction of microorganisms on eggshell surfaces and its effect on egg quality

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ABSTRACT

The presence of microorganisms on the eggshell surface is a factor of consideration in determining egg quality. These microorganisms can contribute to egg spoilage and can infect the egg. In this study, 18 morphotypes of microorganisms were isolated from eggshells. Morphological, biochemical, physiological and molecular analyses were used to identify these morphotypes into 7 species; *Bacillus drentensis*, *Staphylococcus arlettae*, *Stap. cohnii*, *Stap. kloosii*, *Stap. saprophyticus*, *Stap. sciuri* and *Stap. xylosus*. The potential of *Muscodor cinnamomi* to reduce the presence of microorganisms on eggshells by biological fumigation was investigated. The result showed that 16 strains of the tested microorganisms were inactivated after the exposure of the fungal volatile organic compounds. The most abundant compound was 2-methylpropanoic acid, followed by 3-methylbutan-1-ol. Our results indicated that a 24-h period of fumigation of 100 g rye grain culture of *M. cinnamomi* was the minimum dose that could significantly reduce the number of microorganisms on the eggshell surface. Fumigated eggs from both box and cabinet fumigation trials showed significantly lower microbial numbers on the eggshell than non-fumigated eggs during the storage period of 14 days. It was found that the values of the yolk index, albumen index and the Haugh unit of the eggs decreased during this storage time. However, those values of the fumigated eggs from both fumigation trials were found to be significantly higher than the non-fumigated eggs after the 24-h fumigation period and following storage for 5, 7 and 14 days. However, the values of the albumen index were not found to have significantly increased over 5 days of the box trial. This study is the first to report on mycofumigation activity for the purposes of reducing the presence of microorganisms on the surface of eggshells.

1. Introduction

Eggs have been promoted for worldwide consumption as a staple food and have been acknowledged as an important component in a balanced human diet (Bradley and King, 2005; Silversides and Scott, 2001; Stadelman and Cotterill, 1995). The Food and Agriculture Organization Statistical Database (http://faostat3.fao.org) has reported that China is the largest egg producer in the world, followed by the United States and India with global production in 2013 reaching almost 74 million tons. Indonesia leads Southeast Asia in egg production (1.5 million tons) followed by Thailand (1.1 million tons) and Malaysia (0.6 million tons). Determination of egg quality includes consideration of external characteristics (cleanliness and egg weight) and interior characteristics (albumen index, yolk index, Haugh unit and chemical composition), all of which are considered important in establishing acceptability to the consumer and the egg production industry (Adeogun and Amole, 2004; Silversides and Scott, 2001). Egg quality is greatly influenced by hen physiology, environmental conditions of the rearing system, as well as the production system and storage conditions (Jin et al., 2011; Scott and Silversides, 2000). The presence of microorganisms that contribute to egg spoilage is one of the significant factors of consideration in evaluating egg quality. Eggs that have been contaminated by microorganisms can indicate conditions that significantly affect poultry production and lead to the spreading of diseases. Generally, salmonellosis caused by *Salmonella* spp. is considered a major human disease that can spread *via* the surface of eggs. The foodborne diseases caused by *Bacillus* spp., *Escherichia coli* and *Staphylococcus* spp. are considered to be minor diseases (Gantois et al., 2009; Yang et al., 2001). Microorganisms can contaminate an egg during fertilization, collection, preservation, and during shipping. Contamination can also occur through contact with contaminated feces, and the contamination can pass into the inside of an egg (Al-Bahry et al., 2012; Berrang et al., 1999; Cason et al., 1994). Previous studies have reported

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that numerous bacterial strains *e.g. Bacillus* spp., *Enterobacter* spp., *Enterococcus* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp. and *Streptococcus* spp. can contaminate and infect eggs, and the consumption of infected eggs is a major factor in incidents of human illnesses, especially food poisoning (Al-Bahry et al., 2012; Chaemsanit et al., 2015; Kone et al., 2013). Therefore, reducing the microbial contamination on the eggshell can decrease the susceptibility to egg spoilage and the occurrence of infected eggs, which have become a food safety issue with regard to public health, as well as an economic issue.

Fumigation, spray applications, ultraviolet (UV) light and washing with an appropriate sanitizer are commonly applied practices for the elimination of microorganisms on eggshells (Coufal et al., 2003; Samberg and Meroz, 1995). There are many chemical disinfectants, *e.g.* alcohol compounds, formaldehyde, chlorhexidine compounds and hydrogen peroxide that have been used to eliminate microorganisms on eggshell surfaces. These products rely on certain active ingredients to kill different microorganisms (Mansour, 2001; Samberg and Meroz, 1995). However, these widely used chemical compounds have been associated with harmful issues that can negatively affect workers and consumers, as well as the environment (Galiş et al., 2013; Songur et al., 2010). For this reason, researchers have been interested in identifying biologically active volatile compounds acquired from plants and microorganisms that can be used to fumigate eggs and may offer the prospect of use against numerous infectious microorganisms (Copur et al., 2010; Kalemba and Kunicka, 2003; Krishnaswamy, 2008; Upadhyaya et al., 2015). Several endophytic fungi, *e.g. Hypoxylon* spp., *Muscodor* spp., *Nodulisporium* spp. and *Oxyporus latemarginatus*, have been reported to be producers of volatile compounds (Ezra et al., 2004; Lee et al., 2009; Strobel et al., 2001; Suwannarach et al., 2013a, 2013b). Mycofumigation is the use of antimicrobial volatile organic compounds (VOCs) produced by fungi for the control of other organisms (Stinson et al., 2003). Many previous research studies have focused on the development of the genus *Muscodor* as a mycofumigation biocontrol agent in postharvest technology (Goates and Mercier, 2009; Mercier and Jiménez, 2004; Strobel et al., 2001; Suwannarach et al., 2013a, 2015; Worapong and Strobel, 2009). However, there has not been any report on the use of volatile producing endophytic fungi in the reduction of microorganisms on eggshells. In this study, we aimed to evaluate the ability of the endophytic fungus *Muscodor cinnamomi* CMU-Cib461 as a potential biological agent to reduce the presence of microorganisms on eggshells. The overall egg quality of fumigated eggs was investigated and compared with non-fumigated eggs during different storage periods. Moreover, the VOCs of this fungus were identified by gas chromatograph and mass spectrometer (GC/MS). The knowledge gained can be used to develop *M. cinnamomi* CMU-Cib461 as a biocontrol agent for mycofumigation, which may replace the toxic chemical fumigants that are currently being used in the egg industry.

2. Materials and methods

2.1. Fungal strain

A volatile compound producing endophytic fungus, *Muscodor cinnamomi* CMU-Cib461, isolated from *Cinnamomum bejolghota* (Buch-Ham.) Sweet. (Suwannarach et al., 2010), was used in this study. The fungus was deposited in the Sustainable Development of Biological Resources (SDBR) Laboratory, Faculty of Science, Chiang Mai University,

Table 1
Morphological, physiological and biochemical characters of microorganisms isolated from eggshell and similarity search of 16S rRNA gene sequences.

	Microbial strain						
	CMU-BE01	CMU-BE02	CMU-BE03	CMU-BE04	CMU-BE05	CMU-BE06	CMU-BE07
Number of morphotype	1	2	1	3	7	3	1
Characteristic							
Gram stain	Gram-positive	Gram-positive	Gram-positive	Gram-positive	Gram-positive	Gram-positive	Gram-positive
Shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Rod
Spore forming	−	−	−	−	−	−	+
Colony pigment	+	−	−	−	+	−	−
Anaerobic growth	−	−	−	+	+	−	+
Aerobic growth	+	+	+	+	+	+	+
Staphylocoagulase test	−	−	−	−	−	−	ND
Catalase test	+	+	+	+	+	+	+
Alkaline phosphatase test	+	−	−	−	+	−	ND
Urease test	−	−	−	+	−	+	−
β-Glucosidase test	−	+	−	−	+	+	ND
β-Glucuronidase test	+	+	−	−	−	+	ND
β-Glactosidase test	−	+	−	+	−	+	ND
Nitrate reduction test	−	−	−	−	+	−	+
Novobiocin resistance	+	+	+	+	+	+	ND
Acid production from;							
D-Trehalose	+	+	+	+	+	+	+
D-Mannitol	+	+	−	−	−	+	−
D-Mannose	+	−	−	−	−	+	−
D-Xylose	+	+	−	−	−	+	−
D-Cellobiose	−	−	−	−	−	−	−
L-Arabinose	+	−	−	−	+	−	−
Maltose	+	−	+	+	+	+	+
Sucrose	+	+	−	+	−	+	+
N-Acetylglucosamine	−	−	−	−	−	+	−
Raffinose	+	−	−	−	−	−	+
GenBank accession number	KX235334	KX235335	KX235336	KX235337	KX235338	KX235339	KX235340
Matching search of 16S rRNA gene (% similarity)	*Staphylococcus arlettae* KU359263 (98%)	*Staphylococcus kloosi* JQ66023 (99%)	*Staphylococcus cohnii* JX501706 (99%)	*Staphylococcus saprophyticus* KF192274 (100%)	*Staphylococcus sciuri* KT260526 (99%)	*Staphylococcus xylosus* AF515587 (99%)	*Bacillus drentensis* KT719648 (98%)

(+) = Positive, (−) = Negative, ND = not determined.