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Effect of water activity on the production of volatile organic compounds by *Muscodor albus* and their effect on three pathogens in stored potato

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ABSTRACT

Muscodor albus (Xylariaceae, Ascomycetes) isolate CZ-620 produces antimicrobial volatile organic compounds (VOC), which appear to have potential for the control of various postharvest diseases. The effect of water activity (Aw) on the production of VOC by *M. albus* culture, and their inhibitory effects on the growth of three pathogens of potato tuber (*Fusarium sambucinum*, *Helminthosporium solani*, and *Pectobacterium atrosepticum*) and the development of diseases caused by the three pathogens (dry rot, silver scurf, and bacterial soft rot, respectively) were investigated. Rye grain culture of the fungus produced six alcohols, three aldehydes, five acids or esters, and two terpenoids. The most abundant VOC were: isobutyric acid; bulnesene, a sesquiterpene; an unidentified terpene; 2 and 3-methyl-1-butanol; and ethanol. However, the level of each of those VOC varied with Aw of the culture. Emission activity occurred mainly at Aw above 0.75 and high emission of most VOC occurred only at Aw above 0.90. The aldehydes (2-methyl-propanal and 3-methyl-butanal) were the only VOC produced in quantities below an Aw of 0.90. An Aw value of 0.96 favored maximum emission of acids, esters, and terpenoids. There was a higher production of alcohols and a decrease in aldehydes with increase in Aw. Isobutyric acid, which has been the main *M. albus* VOC monitored in previous studies as an indicator of antifungal activity, had a rather narrow optimum, peaking at Aw of 0.96 and declining sharply above 0.98. Results showed that substrate Aw affects the production dynamics of each group of VOC by the fungus, and suggest that VOC production can be prolonged by maintaining *M. albus* culture at a constant optimum Aw. The VOC was inhibitory to *F. sambucinum*, *H. solani*, and *P. atrosepticum*; and biofumigation with *M. albus* significantly reduced dry rot and soft rot development, and completely controlled silver scurf in inoculated tubers incubated at both 8 °C and 22 °C. The results show that Aw of grain culture affects the production of VOC by *M. albus*; and that the VOC inhibit the growth of the tested pathogens and the diseases caused by them in potato tubers.

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Introduction

Muscodor albus (Xylariaceae, Ascomycetes) is a recently described endophytic fungus that produces a mixture of volatile organic compounds (VOC) with antimicrobial activity (Strobel *et al.* 2001; Worapong *et al.* 2001). The potency and types of VOC that include aldehydes, alcohols, acids, and esters, vary among isolates (Ezra *et al.* 2004; Strobel *et al.* 2007). Isolate CZ-620, the original *M. albus* isolate obtained from a cinnamon tree (Worapong *et al.* 2001), produces a fungicidal and bactericidal VOC that killed a wide spectrum of plant pathogens and other microorganisms *in vitro* (Strobel *et al.* 2001; Mercier & Jiménez 2004, 2007; Mercier & Smilanick 2005; Schotsmans *et al.* 2008; Goates & Mercier 2009). The VOC from this isolate was also shown to have nematicidal and insecticidal activity (Lacey *et al.* 2008; Riga *et al.* 2008). Because of those properties, the research on the development of *M. albus* as a biological fumigant has focused on isolate CZ-620 (Strobel 2006; Mercier *et al.* 2007). It has been widely tested for control of postharvest diseases of fruits in different handling systems such as individual storage boxes of grapes and peaches (Schnabel & Mercier 2006; Mercier *et al.* 2010) and storage room of lemons (Mercier & Smilanick 2005).

Before the discovery of *M. albus*, certain species of *Trichoderma* were the best known examples of VOC antibiosis in microbes (Dennis & Webster 1971). Recently, other microorganisms, fungi, and bacteria, have also been shown to produce antimicrobial VOC (Liu *et al.* 2008; Wan *et al.* 2008; Lee *et al.* 2009). VOC-producing microorganisms open new possibilities for the biological control of microbial decay in food and agriculture as biofumigation does not require physical contact with the product or commodity to be treated. Depending on the application, these microorganisms could be used directly as live biofumigant or be a model source of new chemical fumigants.

Optimizing the production of VOC by these microorganisms could help facilitate their use for biofumigation. In the case of *M. albus*, improving its VOC production, and the ability to scale up fumigation operation with the VOC would be of particular interest for large-scale fumigation of stored commodities and building materials (Mercier *et al.* 2007). The isolate and the growing medium affect the profile of VOC produced by *M. albus* (Ezra & Strobel 2003; Ezra *et al.* 2004). Desiccated rye grain culture of *M. albus* requires proper rehydration for reactivation, in a ratio approaching one part water for one part culture (Jiménez & Mercier 2005). Once reactivated, rye grain culture can produce VOC for several days at ambient room temperature (Mercier & Jiménez 2007, 2009). The incorporation of rye grain culture to soil or potting mix reduced the amount and duration of VOC production (Mercier & Jiménez 2009).

In this research, we examined closely how water availability affects VOC production by *M. albus*, focusing on the effect of water activity (Aw) of rye grain culture of isolate CZ-620. As a fumigant, *M. albus* has been mainly studied for the control of fruit decay (Mercier *et al.* 2007), but no information is available on its efficacy in the control of diseases in tuber or root crops. Thus we also investigated the effect of the VOC by *M. albus* on the *in vitro* growth and development of three important potato (*Solanum tuberosum* L.) storage pathogens and diseases, namely, *Fusarium sambucinum* (dry rot), *Helminthosporium solani* (silver scurf) and *Pectobacterium atrosepticum* (formerly *Erwinia carotovora* subsp. *atroseptica*) (bacterial soft rot).

Materials and methods

Microorganisms

Desiccated rye grains colonized with *Muscodor albus* (isolate CZ-620) mycelium were prepared as described by Mercier *et al.* (2007) and were stored at 4 °C until further use. For *in vitro* assays, the colonized grains were placed on potato dextrose agar (PDA; Difco Laboratories, Becton Dickinson, Sparks, MD) Petri plates to regenerate *M. albus* mycelium. *Fusarium sambucinum* and *Helminthosporium solani*, obtained from the Horticultural Research Centre culture collection (Université Laval, Québec, Canada), were grown at 22 °C in the dark on PDA and V8-agar (Difco), respectively. *Pectobacterium atrosepticum* (strain 1839) was provided by the Laboratoire de Diagnostic en Phytoprotection (MAPAQ, Québec, Canada). *Pectobacterium atrosepticum* was maintained in glycerol at −80 °C and cultivated on nutrient agar (NA; Difco) at 28 °C for further use.

Potatoes

Potato tubers (cv. 'Russet' and 'Dark Red Norland') were purchased locally. They were selected for freedom of defects, surface sterilized for 10 min by dipping in water containing 0.01 % sodium hypochlorite, rinsed twice in sterile water, and surface dried in ambient air.

Analysis of Muscodor albus *VOC production under different Aw*

Rye grain culture of *M. albus* (15 g) was moistened with water at 0—100 g of water/100 g grain culture to obtain different Aw and placed in closed containers (7.5 L) with a capillary pore for gas exchange, and were kept in the dark at room temperature. Aw was measured after 24 h of equilibration, where Aw of the moist grains remained quite stable. To obtain Aw values of grain culture above 0.80, the moistened or unmoistened grains were kept in containers where the air was humidified by placing water at the bottom of the container. Aw values of grains below 0.80 were obtained by keeping moistened or unmoistened grains in containers without humidification of air in the container. Before the analysis of the composition of VOC emitted by the culture, Aw of the grain cultures was measured with an Aw Meter (Aqualab Series 3, Decagon Devices Inc., Pullman, WA, USA).

Analysis of VOC was performed with a G1888 headspace sampler coupled to HP6890 GC and a 5973 N quadrupole MS detector (Agilent Technologies, Wilmington, DE, USA). A Zebron DB-Wax capillary column (Phenomenex, Torrance, CA, USA), 60 m length × 0.25 μm film thickness × 0.25 mm internal diameter, was used to separate compounds. Activated rye grain culture (5 g) was placed into headspace sampler vials, and the headspace sampler was set as follows: oven temperature: 90 °C, vial equilibration time: 7 min; vial