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Integrated control of crown rot of banana with *Candida oleophila* strain O, calcium chloride and modified atmosphere packaging

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ABSTRACT

An integrated approach for biological control of crown rot of banana was studied. The efficacy of three control measures, applied alone or in various combinations, was evaluated under conditions highly conducive to the development of crown rot (artificial inoculation of *Colletotrichum musae* at 10^4 conidia/ml). The studied measures were: application of an antagonistic yeast (*Candida oleophila* strain O at 1.10^7 cfu/ml), treatment with 2% (w/v) calcium chloride, and modified atmosphere packaging of fruit (MAP) in non-perforated polyethylene bags. *C. oleophila* was able to grow under MAP, maintaining a large population (7.10^6 to 7.10^7 cfu/g crown) throughout the 13 days of storage. Both treatment with the antagonistic yeast and storage under MAP, applied separately, reduced crown rot significantly (by 22% and 20%, respectively, as compared to untreated controls). The effect of the yeast was the same whether it was produced in Petri dishes or in a fermentor. Calcium chloride treatment alone had no effect on *C. musae*. The antagonistic yeast showed a 16% higher biocontrol activity (from 26% to 42%) when applied together with 2% (w/v) calcium chloride, and the presence of this adjuvant made it possible to achieve the same protective effect with a lower yeast concentration. The highest efficacy (53%) was achieved by the combination of the three alternatives means of control and a synergistic relation has been detected between the yeast, calcium chloride and MAP. Considering the severe conditions of screening, the consistency of the results obtained in this study indicates that the integrated strategy has great potential for control of crown rot of banana under commercial conditions.

1. Introduction

Crown rot is one of the most damaging postharvest diseases of exported bananas (Slabaugh and Grove, 1982; Reyes et al., 1998; Krauss and Johanson, 2000). It is caused by a large complex of fungal pathogens varying according to the geographic location and time of year (Meredith, 1971). Among the organisms composing this complex, *Colletotrichum musae* is often regarded as the most pathogenic (Greene and Goos, 1963; Lukezic et al., 1967; Shillingford, 1976; Finlay and Brown, 1993; Lassois et al., 2008). First symptoms may be observed after shipment, increasing rapidly during fruit ripening (Slabaugh and Grove, 1982). The rot begins at the cut surface of the crown and proliferates through the crown tissue, causing blackening and softening. In severe cases, the rot can spread through the pedicels of individual fingers and infect the banana pulp (Muirhead and Jones, 2000). This decay makes the fruit difficult to handle and unappealing to consumers, and a high incidence of crown rot can cause premature ripening during transit rendering the fruits unmarketable (Greene and Goos, 1963).

Control of crown rot of bananas involves an integrated combination of sanitation practices at field and packing station level, postharvest fungicide treatments and modified atmosphere packaging (MAP) (Slabaugh and Grove, 1982; Krauss and Johanson, 2000; Lassois et al., 2008). Despite these management practices, high crown rot levels are still encountered in the marketing of bananas. In the UK, for instance, losses exceeding 10% have been observed for fruits harvested in the Windward Islands during the rainy season (Krauss and Johanson, 2000). Furthermore, the increasing public demand for a strongly reduced pesticide use, stimulated by greater awareness of environmental and health issues, and the emergence of *C. musae* strains resistant to the key fungicides (Griffee and Burden, 1976; Johanson and Blazquez, 1992; de Lapeyre de Bellaire and Dubois, 1997), limit the postharvest application of chemicals to banana. Yet for banana from the Philippines, for example, losses as high as 86% have been recorded when the fruits were not subjected to any postharvest chemical treatment (Alvindia et al., 2000). These facts constitute a strong

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incentive to develop alternative strategies for the control of crown rot of banana.

The use of biological control agents (BCAs) for the management of postharvest diseases of fruits appears as a realistic approach for three main reasons: (i) the regulation of the environmental parameters during fruit storage is suitable for BCAs, (ii) the application sites of BCAs are limited to the fruit and (iii) the high retail value of fresh fruits can justify the potentially higher cost of biological treatments as compared to chemical methods (Wilson and Wisniewski, 1989; Janisiewicz and Korsten, 2002). Biological control of crown rot of banana has been evaluated with several BCAs, including notably fungi such as *Glyocladium* sp., *Pythium* sp., *Trichoderma* sp., *Verticillum* sp. (Krauss et al., 1998; East and Kenyon, 1998), bacteria of the *Burkholderia cepacia* complex (de Costa and Erabadupitiya, 2005) and several yeast strains of the species *Rhodotorula glutinis*, *Cryptococcus laurentii* and *Cryptococcus albidus* (Postmaster et al., 1997). A previous study identified *Candida oleophila* strain O, a naturally occurring saprophytic yeast isolated from the surface of apple fruit (Jijakli et al., 1993b), as a promising agent for the control of crown rot of banana (Lassois et al., 2008). This strain was initially selected for its high and reliable antagonistic activity against *Botrytis cinerea* and *Penicillium expansum*, two important postharvest wound pathogens of apples (Jijakli et al., 1993b). Its potential as a BCA for use against crown rot of banana has been demonstrated on the basis of its antagonistic activity against an artificial fungal complex composed of *C. musae*, *Fusarium moniliforme* and *Cephalosporium* sp. In these experiments, the level of protection against the fungal complex appeared to depend on the incubation time before inoculation of the pathogens and on the fruit susceptibility to the fungal complex (Lassois et al., 2008). The highest protection level (54.4%) was achieved when the strain was applied to crowns at the concentration of 10^8 colony-forming units per milliliter (cfu/ml). Data also demonstrated an antagonistic activity of *C. oleophila* strain O against *C. musae* inoculated alone, with a protective level ranging from 33.4% to 47.2% at the concentrations tested (10^6, 10^7 and 10^8 cfu/ml). Nevertheless, the biological treatments tested were always significantly less effective than the fungicide treatments.

Many BCAs provide a partial protection against postharvest diseases of many fruits, but most of them, when used alone, did not offer the level of control of synthetic fungicides. As biological means cannot currently solve all the problems of rots during fruit storage, they must be considered as a tool to be used in combination with other approaches in the framework of integrated disease management (Janisiewicz and Korsten, 2002). The combination of a BCA with organic and inorganic salts has been proposed as a safe and effective means of improving the biocontrol activity of the BCA against postharvest diseases of fruit. Prior studies have shown that the addition of calcium chloride improves the efficacy of *C. oleophila* against *B. cinerea* and *Penicillium expansum* on apple (McLaughlin et al., 1990; Jijakli et al., 1993a; Wisniewski et al., 1995), of *Pichia guillermondii* against rots of grapefruits (Droby et al., 1997) and of *Aureobasidium pullulans* against postharvest rots of sweet cherries (Ippolito et al., 2005). Improvement of biocontrol with additives may result from direct inhibition of the pathogen, elicitation of systemic acquired resistance in the host tissue and/or stimulation of the antagonistic activity. Moreover, since additives can make it possible to use a lower antagonist concentration without affecting the protection level (Lima et al., 2005), the presence of specific additives in the formulation of BCAs appears as an essential prerequisite to the commercial success of BCAs.

The successful commercial use of a BCA also depends on its compatibility with postharvest practices. Modified atmosphere packaging (MAP) of banana in non-perforated polyethylene bags called banavac is commonly used in international transport. Alteration of the gaseous environment, due to fruit respiration and specific permeability of the plastic bags to gases, extends the storage life of bananas and maintains important quality attributes (Thompson, 1998). MAP of fruits in banavacs is also known to suppress spore germination and to reduce postharvest decay of bananas (Goos and Tschirsch, 1962; Slabaugh and Grove, 1982; Chillet and de Lapeyre de Bellaire, 1996; Thompson, 1998). By combining a BCA with calcium chloride and MAP of fruit it might be possible to overcome the shortcomings of each measure used separately and thus to obtain a satisfactory level of protection.

The present study was undertaken to develop an integrated strategy for controlling crown rot of banana, based on the combined use of the biocontrol agent *C. oleophila* strain O, calcium chloride and fruit storage under MAP of fruit in 20-μm-thick polyethylene banavacs. We first investigated the population dynamics of the antagonistic yeast during fruit storage under a reduced-O_2, elevated-CO_2 atmosphere, as the protective effect of *C. oleophila* strain O seems to be closely related to its ability to colonize apples (Jijakli and Lepoivre, 1993). Next we tested some technological properties of *C. oleophila* strain O, comparing the protective activity of the fresh yeast produced in a Petri dish with that of the lyophilized yeast produced in a fermentor. We then evaluated the efficacy of BCA, calcium chloride and MAP applied as stand-alone treatments and in combination under conditions highly conducive to the development of crown rot through artificial inoculation with *C. musae*.

2. Materials and methods

2.1. Fruits

Experiments were conducted with healthy-looking mature and unripe banana fruits (*Musa acuminata* AAA, cv Grande Naine, Cavendish subgroup) obtained (a) for monitoring the yeast population dynamics, from a commercial source in Belgium just before the ethylene treatment or (b) for the biocontrol assays, from the second and third hands of 20 bunches, just harvested at the same physiological age of 900 degree-days (Chillet et al., 2006) and collected in a packing shed in Cameroon (Dia-Dia, "Plantations du Haut Penja" PHP, Njombé).

For all experiments, hands were separated into 4-finger clusters, each randomly assigned to one assay modality. The external fingers on each hand were systematically eliminated. Smoothly cut crowns were obtained with a sharp knife leaving as much crown tissue as possible. Latex from crown tissue was dried with absorbent paper after 30 min and the crowns were surface-sterilized by submersion in 50% ethanol. The crowns were then dried during 2 h at ambient temperature before use.

2.2. Microorganisms and chemical compounds

Fifteen days before use, *C. musae* cultures were initiated from cryotubes (stored at −20 °C in a 30% glycerol solution) by placing 100 μl of suspension on a modified Mathur culture medium (per liter: 2.5 g $MgSO_4 \cdot 7H_2O$, 2.7 g KH_2PO_4, 1 g peptone, 1 g yeast extract, 10 g sucrose, 15 g agar) at 25 °C for 5 days. The culture was then transferred to fresh Mathur medium and incubated at 25 °C for 10 days. The spore suspension was prepared by flooding the culture with sterile distilled water and filtering through a 45 μm sieve. The concentration of the conidial suspension was determined with a Mallassez cell and adjusted to 10^4 conidia/ml. The *C. musae* strain inoculated is sensitive to bitertanol and thiabendazole.

The antagonistic yeast *C. oleophila* strain O was isolated from the surface of apple fruit (Jijakli et al., 1993b) and used at the concentration of (a) 10^8 cfu/ml in order to monitor the yeast population dynamics or (b) 10^7 cfu/ml in the three biocontrol experiments.