Postharvest Biology and Technology 68 (2012) 54–63

Contents lists available at SciVerse ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Effects of sodium bicarbonate on pathogenicity of *Colletotrichum musae* and potential for controlling postharvest diseases of banana

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ARTICLE INFO

Article history:
Received 19 May 2011
Accepted 11 February 2012

Keywords:
Biological control
Colletotrichum musae
B. spinosa
GRAS compounds
Fruit peel characters

ABSTRACT

The effects of sodium bicarbonate ($NaHCO_3$) on pathogenicity of *Colletotrichum musae* and its potential to control postharvest diseases of bananas were determined. Addition of $NaHCO_3$ reduced mycelial growth, spore production, spore germination and appressoria production of *C. musae*, in vitro by increasing pH (from 6.9 to 8.7) of the culture medium (PD broth). The pH of 8.59, created by 100 mM $NaHCO_3$ completely inhibited spore production. Postharvest dip treatment in 300 mM $NaHCO_3$ for 10 min reduced the lesion area of anthracnose on artificially inoculated banana fruit. Natural infections of anthracnose, crown rot and blossom end rot were also reduced significantly in fruit that were treated with 300 mM $NaHCO_3$ for 10 min. Efficiency of integrating $NaHCO_3$ with a bacterial antagonist, *Burkholderia spinosa* for controlling postharvest diseases of bananas was also determined. Dipping banana fruit in 300 mM $NaHCO_3$ solution for 10 min followed by dipping in *B. spinosa* suspension in nutrient broth (cell concentration 1×10^8 cfu/mL) effectively controlled anthracnose, crown rot and blossom end rot of bananas (var. Kolikuttu). Dipping bananas in 300 mM $NaHCO_3$ increased pH, total soluble solids and thickness of the fruit peel which may have an indirect or cumulative effect on the reduction of postharvest disease development in bananas.

1. Introduction

Postharvest diseases cause varying degrees of economic loss to harvested perishables produced all over the world, and control of postharvest diseases has been traditionally achieved by pre- and post-harvest applications of fungicides (Eckert and Ogawa, 1988). However, producers have been compelled to seek alternative methods due to increased global demand for chemical-free fresh produce (Korsten, 2006), development of resistant strains of plant pathogens against currently used fungicides (Spotts and Cervanntes, 1986) and higher costs involved with synthetic fungicides. Among the currently available non-fungicidal approaches, use of soft chemicals, natural chemicals, disinfectants, calcium applications, growth regulators, chemical elicitors to induce natural host defences, biological agents, hypobaric pressure, irradiation, hot water, modified atmosphere storage, special packaging and genetic manipulation of fruit have been practiced with varying degrees of success (Coates and Johnson, 1997; Barkai-Golan, 2001; Janisiewicz and Korsten, 2002; Korsten, 2006).

Bicarbonates and carbonates are common food additives used for leavening, pH control, taste and texture modification and spoilage control (Corral et al., 1988). In addition, the ability of these chemical salts in controlling postharvest decay of bell pepper (Fallik et al., 1997), citrus (Arimoto et al., 1995) and melon (Aharoni et al., 1997) and anthracnose of papaya (Sivakumar et al., 2002) has been documented. Regulatory barriers for the use of these chemicals are few, as most are classified as generally recognized as safe (GRAS) chemicals by the US Food and Drug Administration. Moreover, bicarbonates have been exempted from residue tolerance on all agricultural commodities by the US Environmental Protection Agency and the US Department of Agriculture has classified many carbonates and bicarbonates as approved ingredients on products labelled "organic" (Smilanick et al., 1999). Treating produce with bicarbonates and carbonates is inexpensive and less sophisticated in comparison with other non-chemical alternatives such as biological control and heat treatment. Furthermore, the chemical is easily available and the control measures can be implemented without much professional expertise.

Use of biological antagonists has already been shown to be an efficient method for controlling postharvest diseases (Chalutz and Wilson, 1990; Smilanick and Denis-Arrue, 1992; Vinas et al., 1998; Fan and Tian, 2001; Sharma et al., 2009). Our previous investigations revealed the potential of *Burkholderia spinosa*, a bacterial antagonist, originally isolated from the peel of banana fruit for controlling several postharvest diseases of banana caused by *Colletotrichum musae* (De Costa and Erabadupitiya, 2005; De Costa et al., 2008). We identified *C. musae* as the causal organism

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doi:10.1016/j.postharvbio.2012.02.002

of quiescent infections of anthracnose and blossom end rot of several dessert banana varieties grown in Sri Lanka (De Costa and Erabadupitiya, 2005; De Costa et al., 2008) and also as one of the causal organisms of crown rot (De Costa and Subasinghe, 1998). In these studies, the anthracnose symptoms (i.e. necrotic lesions with salmon-pink colour spores) developed at the ripe stage on the bottom tip region of the fruit were defined as blossom end rot. Crown rot was defined as the rotting, which starts at the tip of the stem-end of an individual banana finger and spreading downwards. Integration of sodium bicarbonate and other carbonates with different types of biological control agents such as bacteria and yeast for improvement of biocontrol efficiency of postharvest pathogens has been documented previously (El-Ghaouth et al., 2004; Yao et al., 2004; Sharma et al., 2009).

In the present study, the potential of sodium bicarbonate (commonly known as baking soda) was evaluated with the objective of determining its effects on pathogenicity of *C. musae*, the causal organism of banana anthracnose and the potential to control of postharvest diseases of banana (i.e. anthracnose, crown rot and blossom end rot) caused by *C. musae*.

2. Materials and methods

2.1. Fungal culture and growth conditions

C. musae was isolated from the dessert banana variety Kolikuttu (AAB), a highly susceptible variety to anthracnose, showing typical anthracnose symptoms. A pure culture of *C. musae* with cinnamon colour colony morphology was obtained from a single cell culture and maintained in potato dextrose agar (PDA). The fungal culture was confirmed as *C. musae* based on colony and spore characteristics as described by Photita et al. (2005). Long-term storage was done using PDA discs of the fungal culture in sterile distilled water at 4 °C.

2.2. Effects of $NaHCO_3$ on growth and spore production of *C. musae*

By adding different volumes from a 1 M $NaHCO_3$ stock solution, potato dextrose (PD) broths having final concentrations of 2.5, 5.0, 10.0, 25.0, 100, 200 and 300 mM $NaHCO_3$ were prepared. Broth culture pH was measured by a pH meter (TOA pH METER HM-20SB, Japan). A disc of *C. musae*, diameter 1 cm, was obtained from a culture grown on PDA medium and introduced to each of the glass culture tubes containing 50 mL of potato dextrose broths with different final concentrations of $NaHCO_3$. Three replicates were maintained for a given final concentration of $NaHCO_3$. The control treatment contained only potato dextrose broth with no addition of $NaHCO_3$. The broth cultures were incubated at 25 °C for four days with shaking at 150 rpm. Growth of the fungal mass in different concentrations of $NaHCO_3$ was quantified according to Derakhshan et al. (2008). Briefly, the mycelial mass grown under different $NaHCO_3$ concentrations (i.e. different treatments) were harvested by filtration using a sterile Buchner funnel fitted with filter paper (Whatman No. 42, Maidstone, England) under suction. The mycelial mass was oven-dried at 70 °C until a constant weight was obtained and the dry weight was recorded. Mean spore production under different treatments was quantified by measuring the spore concentration of five aliquots taken from each replicate using a haemocytometer.

2.3. Effects of $NaHCO_3$ on spore germination and appressoria formation

A spore suspension of *C. musae*, concentration 1×10^6 spores/mL, was prepared from a 12 to 13 d-old culture. 1 mL of spore suspension was added separately to sterilized Petri dishes each containing 10 mL of potato dextrose broth having final $NaHCO_3$ concentrations of 100, 200 and 300 mM. These concentrations were found to be the least favourable for growth and spore production of *C. musae*. An aliquot of 0.5 mL of the spore suspension was mixed with potato dextrose broth having different final concentrations of $NaHCO_3$ and the Petri dishes were incubated under 80–90% RH in a closed container. Number of spores with germtubes and number of spores with appressoria per unit area (0.5 mm^2) were counted using 90–100 spores by a light microscope under 10×40 magnification at 2, 8, 24 and 48 h after incubation at 25 °C. For counting the number of germinated spores and spores with appressoria, equal aliquots were taken at different incubation times from the spore suspension under incubation. Percentage spore germination and percentage appressoria formation were quantified. Each $NaHCO_3$ concentration was replicated three times according to a completely randomized design. Three control treatments (i.e. PD broth + spores of *C. musae*, PD broth added with a 3% sterilized sugar solution + spores of *C. musae* and sterilized distilled water + spores of *C. musae*) were also maintained with three replicates.

2.4. Effects of $NaHCO_3$ on anthracnose development on artificially inoculated banana

Dessert banana varieties Embon and Kolikuttu were used for artificial inoculation of *C. musae* grown under different pH conditions. Banana fruit at mature unripe green stage (Stage 1 according to standard colour chart, http://ripening-fruit.com/banana) were purchased from a grower as bunches of the same inflorescence and surface sterilized by 10% commercial bleach solution, washed with sterile distilled water and air-dried. A spore suspension was obtained by growing *C. musae* in PD broth. Spore suspensions were mixed separately with $NaHCO_3$ solutions having final concentrations of 100, 200 and 300 mM and incubated for 1 h. The *C. musae* spore concentration in these $NaHCO_3$ solutions was 10^6 spores/mL. The above concentrations of $NaHCO_3$ were selected as they gave the least mycelial growth, spore production, spore germination and appressoria formation based on previous investigations (see Sections 2.2 and 2.3). Inoculation of the pathogen and treatment conditions of this experiment were as follows: (1) *C. musae* grown in PD broth with 100 mM $NaHCO_3$; (2) *C. musae* grown in PD broth with 200 mM $NaHCO_3$; (3) *C. musae* grown in PD broth with 300 mM $NaHCO_3$; (4) *C. musae* grown in PD broth without $NaHCO_3$; and (5) sterilized distilled water only. Three points per fruit were inoculated on the fruit peel with a 20 μL drop of the suspension, containing 10^6 spores/mL, taken from the PD broths having different $NaHCO_3$ concentrations using a syringe. The same volume of sterilized distilled water without $NaHCO_3$ was applied to banana fruit as the control treatment. Each treatment was replicated five times and the treated fruit were arranged according to a completely randomized design. Inoculated fruit were incubated in a glass chamber with a mean temperature of 28.5 °C and RH of 80%. Days taken for anthracnose symptoms to appear were recorded and disease severity was quantified as area of lesion development from each point of inoculation in each fruit. Area of lesion development was measured by tracing the lesion area (15 lesions per treatment (i.e. three point inoculations × five replicates) on to a polythene sheet and then estimating the area using a 1 mm^2 graph paper. The experiment was conducted three times (i.e. initial experiment and two repetitions).

2.5. Treatment of banana with $NaHCO_3$ to control natural infections

Effects of dip treatment of $NaHCO_3$ in controlling natural infections of postharvest diseases of banana were determined by