Biological Control 79 (2014) 24–35

Contents lists available at ScienceDirect

Biological Control

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

Biological control of *Botrytis* bunch rot in Atlantic climate vineyards with *Candida sake* CPA-1 and its survival under limiting conditions of temperature and humidity

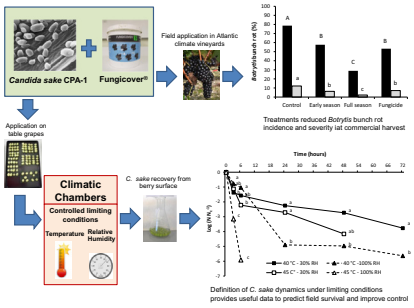
C. Calvo-Garrido [a], N. Teixidó [b], J. Roudet [c], I. Viñas [a], J. Usall [b], M. Fermaud [c,*]

[a] Food Technology Department, Lleida University, XaRTA-Postharvest, Agrotecnio Center, Rovira Roure 191, 25198 Lleida, Catalonia, Spain
[b] IRTA, XaRTA-Postharvest, Scientific and Technologic Parc of Gardeny, FRUITCENTRE building, 25003 Lleida, Catalonia, Spain
[c] INRA, UMR1065 Santé & Agroécologie du VignoblE, ISVV, Université de Bordeaux, CS 20032, 33882 Villenave d'Ornon cédèx, France

HIGHLIGHTS

- *C. sake* field treatments reduced *Botrytis* bunch rot in Atlantic climate vineyards.
- *C. sake* maintain high population density on berries after field applications.
- Survival pattern of *C. sake* under different T and RH limiting conditions is described.
- An incubation period, prior to exposure to limiting conditions, increased BCA survival.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 March 2014
Accepted 30 May 2014
Available online 16 June 2014

Keywords:
Abiotic factors
Extreme conditions
Fatty acids
Fungal disease
Grapevine
Vitis vinifera

ABSTRACT

Candida sake CPA-1 is an antagonistic yeast that has previously been shown to effectively control *Botrytis* bunch rot in grapes. The efficacy of biological control agents is dependent on their survival, which may also depend on climatic conditions. However, few studies have evaluated the effect of abiotic factors affecting the survival of biological control agents, such as temperature (T) or relative humidity (RH). In this study, efficacy of *C. sake* (5×10^7 CFU mL^{-1}), which was applied with the additive Fungicover (FC; 50 g L^{-1}), was tested against BBR in the laboratory and in field trials under the Atlantic climate conditions of the Bordeaux region (France). The study also evaluated the survival of *C. sake* under T and RH regimes simulated in climatic chambers. Two or five applications of *C. sake* plus FC during the growing season significantly reduced BBR severity at harvest by 48% and 82%, respectively, when compared to the control. Similar reductions were achieved after inoculation with selected virulent *Botrytis cinerea* strains (75% compared to control) in laboratory experiments. *C. sake* populations showed minimal decreases between field applications and were favored by simulated Atlantic climate conditions. The survival pattern of *C. sake* exposed to 40 and 45 °C combined with 30% and 100% of RH was described, demonstrating a sharp decrease during the first 24 h. Allowing 48 h for *C. sake* to incubate and become established on fruits prior to the exposure to 40 °C and 30% RH increased survival ($P < 0.05$). These results confirm the efficacy of treatment with *C. sake* plus FC under favorable climatic conditions for BBR development, while survival studies may help to improve the survival and efficacy of yeast BCAs, such as *C. sake* CPA-1.

Abbreviations: T, temperature; RH, relative humidity; FC, Fungicover; BCA, biological control agent; BBR, *Botrytis* bunch rot; Rf, accumulated rainfall.

* Corresponding author.
E-mail address: fermaud@bordeaux.inra.fr (M. Fermaud).

http://dx.doi.org/10.1016/j.biocontrol.2014.05.011
1049-9644/

1. Introduction

Biological control by antagonistic microorganisms has been extensively studied in recent decades and is regarded as a promising alternative to the use of synthetic fungicides to control fruit pathogens (Droby et al., 2009; Nicot, 2011). Among these pathogens, *Botrytis cinerea* Pers.:Fr., the causative agent of *Botrytis* bunch rot of grapes (*Vitis vinifera* L.), is an important disease in vineyards, causing substantial economic losses in wine and table grapes. In the Bordeaux winegrowing region (France), for example, BBR quantitatively reduces the yield if meteorological conditions are conducive to epidemic development. Moreover, it has recently been shown to cause significant loss of wine sensory quality, perceptible from a disease threshold as low as 5% of rotted berries at harvest (Ky et al., 2012). The epidemiological development of BBR is complex because it is dependent on multiple infection pathways (Elmer and Michailides, 2004). Among the key factors determining disease development are the following: (i) the genetic structure of the *B. cinerea* population (Martinez et al., 2008, 2005); (ii) the grape berry ontogenic resistance associated with the fruit developmental stage (Deytieux-Belleau et al., 2009); (iii) the grapevine susceptibility related to plant vegetative vigor (Valdés-Gómez et al., 2008) and important interactions between the pathogen and insect vectors such as *Lobesia botrana* (Denis & Schiffermüller), the European Grape Berry Moth "EGBM" (Fermaud and Lemenn, 1992) and *Thrips obscuratus* (Crawford) (Fermaud and Gaunt, 1995).

During the past few decades, promising studies have reported effective preharvest disease control by commercially available biological control agents and other microorganisms at various developmental stages (Elmer and Reglinski, 2006; Nally et al., 2012; Parry et al., 2011). Furthermore, postharvest applications of several BCAs have also been demonstrated to be effective during storage of table grapes (Romanazzi et al., 2012). The antagonistic yeast *Candida sake* CPA-1, applied in combination with the additive Fungicover®, significantly reduced BBR incidence and severity at harvest in field experiments in dry Mediterranean climate conditions (Calvo-Garrido et al., 2013; Cañamás et al., 2011). It effectiveness was also reported against postharvest diseases of pome fruit through pre- and post-harvest applications (Teixidó et al., 1998b, 1999). Spatial and nutrient competition on the fruit surface was considered to be the main mechanism by which *C. sake* CPA-1 mediates disease suppression, as described for other antagonistic yeast and yeast-like fungi (Droby et al., 2009; Filonow, 1998; Jijakli, 2011; Lima et al., 1997). This mode of action requires the presence and persistence of BCA cells at high concentrations on fruit tissues, and a minimal concentration of 10^4 CFU cm^{-2} is needed to maintain consistency of control, making the survival of BCA populations a crucial factor (Andrews, 1992).

BCA survival is affected by abiotic factors such as temperature, relative humidity and UV radiation (Lahlali et al., 2011; Magan, 2001; Teixido et al., 2010). The influence of these factors can be reduced during controlled postharvest storage. However, under field conditions, populations are subjected to daily fluctuations of temperature and RH, as well as periods of limiting conditions interfering with BCA survival. Especially in hot and dry summer climates, days with maximum T over 35 °C are frequent and RH can drop below 30% during the day. These conditions may affect the survival of a yeast BCA like *C. sake*, which has an optimal growth temperature of 25 °C in culture medium (Teixidó et al., 1998c).

In addition, differences in T and RH during the growing season are known to be key factors determining BBR epidemic development (Elmer and Michailides, 2004). Performance of BCAs may also be modified by environmental conditions, as suggested by studies investigating the effects of RH and T on the relationship between a pathogen and a BCA (Agra et al., 2012).

The population dynamics of antagonistic yeast and yeast-like BCAs have been assessed during storage, following postharvest applications on a variety of fruit commodities: mainly in pome fruit (Jijakli, 2011; Lima et al., 2003; Manso and Nunes, 2011; Tian et al., 2004; Viñas et al., 1998) but also in sweet cherries (Ippolito et al., 2005; Tian et al., 2004) and citrus (Teixidó et al., 2001). Other studies have also evaluated the field survival of these BCAs for controlling diseases during the growing season (Guetsky et al., 2002; Lima et al., 2002) or following pre-harvest applications to control fruit pathogens during storage (Benbow and Sugar, 1999; Ippolito and Nigro, 2000; Lahlali et al., 2009; Teixidó et al., 1998b). On grapes, BCA population studies include the evaluation of field survival after postharvest applications of *Aureobasidium pullulans* and *Candida oleophila* (Lima et al., 1997), *Candida guillermondii* and *Acremonium cephalosporium* (Zahavi et al., 2000), *Metschnikowia fructicola* (Karabulut et al., 2003), *Metschnikowia pulcherrima* and *Pichia guilliermondii* (Kinay and Yildiz, 2008), *Cryptococus laurentii* (Meng and Tian, 2009), the developing yeast-like fungi BCA-L1 (Parry et al., 2011) and *C. sake*, evaluated in both conventional and organic-managed vineyards (Calvo-Garrido et al., 2013; Cañamás et al., 2011).

However, few reports have studied the direct effects of key abiotic factors such as T and RH on BCA survival. The *in vitro* response of *C. sake* to water, temperature and pH stress has been studied (Teixidó et al., 1998c). Lahlali et al. (2008) established a model for the survival of *Pichia anomala* (strain K) and *C. oleophila* (strain O), exposing populations on treated apples to different temperatures (5, 15 and 25 °C) and RH (75% and 98%). Furthermore, the efficacy and survival of *C. oleophila* (strain O) was also tested in extreme conditions of water activity and RH (Lahlali and Jijakli, 2009). However, there are no similar studies on grapes, and none has evaluated the *in vivo* survival of a BCA under selected T and RH regimes in controlled conditions. This information could be valuable for predicting survival and hence improving the efficacy of BCA treatments in the future.

The aims of this work were (1) to test the efficacy of *C. sake* CPA-1 plus Fungicover applications against *B. cinerea*, in the laboratory and in the field under the climatic conditions of Bordeaux vineyards, (2) to evaluate populations of the BCA under these field conditions, and (3) to investigate the effects of different regimes of simulated climatic conditions and limiting conditions of T and RH on *C. sake* CPA-1 survival on grape berries.

2. Materials and methods

2.1. Yeast and fungal material

Three pathogenic *B. cinerea* strains (213, 344 and 351) were selected from the collection of INRA (UMR 1065 SAVE), Bordeaux. The strains have been characterized as II-*vacuma* for strain 351 and II-*transposa* for strains 213 and 344. They were selected based on their marked aggressiveness, ranging from high virulence (213) to intermediate-high virulence (351 and 344), compared to other *B. cinerea* strains from the same collection (Martinez et al., 2003). Stock cultures were maintained on solid malt agar (MA) medium (15 g L^{-1} Cristomalt, Materne, France and 20 g L^{-1} of agar) and then subcultured on MA at 21 °C (±1 °C) in the dark.

The strain CPA-1 of *C. sake* was deposited in the Colección Española de Cultivos Tipo (CECT-10817) at the University of Valencia, Burjassot, Spain. *C. sake* was used for experiments as a