International Journal of Food Microbiology 167 (2013) 153–160

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Review

Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit

Jia Liu [a,*], Yuan Sui [a], Michael Wisniewski [b], Samir Droby [c], Yongsheng Liu [a,d,**]

[a] School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei 230009, China
[b] U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS), Kearneysville, WV 25430, USA
[c] Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan 50250, Israel
[d] Ministry of Education Key Laboratory for Bio-resource and Eco-environment, College of Life Science, State Key Laboratory of Hydraulics and Mountain River Engineering, Sichuan University, Chengdu 610064, China

ARTICLE INFO

Article history:
Received 23 May 2013
Received in revised form 2 September 2013
Accepted 10 September 2013
Available online 18 September 2013

Keywords:
Antagonistic yeast
Mode of action
Postharvest disease
Stress tolerance

ABSTRACT

Significant losses in harvested fruit can be directly attributable to decay fungi. Some of these pathogenic fungi are also the source of mycotoxins that are harmful to humans. Biological control of postharvest decay of fruits, vegetables and grains using antagonistic yeasts has been explored as one of several promising alternatives to chemical fungicides, the use of which is facing increasingly more stringent regulation. Yeast species have been isolated over the past two decades from a variety of sources, including fruit surfaces, the phyllosphere, soil and sea water, and their potential as postharvest biocontrol agents has been investigated. Several mechanisms have been proposed as responsible for their antagonistic activity, including competition for nutrients and space, parasitism of the pathogen, secretion of antifungal compounds, induction of host resistance, biofilm formation, and most recently, the involvement of reactive oxygen species (ROS) in defense response. It has been recognized that a biocontrol system is composed of a three-way interaction between the host (commodity), the pathogen and the yeast, all of which are affected by environmental factors. Efficacy and consistent performance in controlling postharvest diseases are the hurdles that must be overcome if the use of yeast biocontrol agents and other alternatives are to be widely used commercially. Therefore, attempts have been made to combine alternative treatments in order improve their overall performance. The current review provides a brief overview of the topic of the use of yeasts as postharvest biocontrol agents and includes information on the sources from which yeast antagonists have been isolated, their mode of action, and abiotic stress resistance in yeast as it relates to biocontrol performance. Areas in need of future research are also highlighted.

Contents

1. Introduction . . . 153
2. Sources of yeast antagonists . . . 154
3. Mode of action . . . 155
4. Enhancing biocontrol efficacy . . . 157
5. Commercial application . . . 158
6. Conclusions . . . 158
Acknowledgments . . . 158
References . . . 158

1. Introduction

Postharvest losses of fruits, including pome fruit, stone fruit, berry fruit and citrus, can be quite significant if handling, processing, and storage conditions are not optimal. Losses representing up to 25% of total production in industrialized countries and more than 50% in developing

* Correspondence to: J. Liu, Hefei University of Technology, Tunxi Road No. 193, Hefei 230009, China. Tel.: +86 131 3551 9768; fax: +86 551 6291 9399.
** Correspondence to: Y. Liu, Hefei University of Technology, Tunxi Road No. 193, Hefei 230009, China. Tel.: +86 187 0983 2886; fax: +86 551 6290 1516.
E-mail addresses: liujia@hfut.edu.cn (J. Liu), liuyongsheng1122@hfut.edu.cn (Y. Liu).

0168-1605/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijfoodmicro.2013.09.004

countries have been reported (Nunes et al., 2012). The high levels of decay caused by fungal pathogens can be directly attributable to the large amount of nutrients and water, low pH, and the decrease in intrinsic decay resistance of fruit after harvest (Droby et al., 1992). Fungal species within the genera *Penicillium*, *Botrytis*, *Monilinia*, *Rhizopus*, *Alternaria*, *Aspergillus*, *Fusarium*, *Geotrichum*, *Gloeosporium* and *Mucor* represent postharvest pathogens responsible for many of the most important postharvest diseases (Barkai-Golan, 2001).

In addition to economic considerations, infected produce represents a potential health risk since several fungal genera, such as *Penicillium*, *Alternaria* and *Fusarium*, produce mycotoxins that pose a human health hazard. For example, *Penicillium expansum*, the causal pathogen of postharvest blue mold in a variety of fruits, produces a number of toxic secondary metabolites including patulin, citrinin, and chaetoglobosins, all of which are potential carcinogens (Andersen et al., 2004). Although the use of synthetic chemical fungicides is the principal method of controlling postharvest diseases and the resulting rots they cause, stricter regulatory policies are being imposed on their use. There is also strong consumer demand to reduce the use of potentially harmful chemicals in their food supply. For these reasons, alternative methods for managing postharvest diseases have been increasingly explored during the last 25 years. The reader is referred to the following reports for a comprehensive review of the subject: Droby et al. (1992, 2009), Janisiewicz and Korsten (2002), Sharma et al. (2009) and Wisniewski et al. (2007).

Biological control using microbial agents has been reported among several alternatives to be an effective approach, to the use of synthetic chemical fungicides for managing postharvest fruit decay (Droby et al., 2009; Spadaro and Gullino, 2004). In this regard, the use of antagonistic yeasts has been especially emphasized since the production of toxic secondary metabolites (antibiotics) is generally not involved in their inhibitory activity (Wisniewski and Wilson, 1992). Considerable information is also available with respect to the commercial scale production of yeast including fermentation, formulation, storage, and handling (Wisniewski et al., 2007).

The original source (fruit surfaces) for the isolation of yeast antagonists (Wilson and Wisniewski, 1989; Wilson et al., 1993) has expanded to other environments, such as sea water (Hernández-Montiel et al., 2010; Wang et al., 2008) and Antarctic soil samples (Vero et al., 2013). The main intent in exploring these new sources has been both to identify yeasts that can thrive in stressful environments and to discover isolates with novel modes of action.

Yeasts used to manage postharvest diseases in orchards and packinghouses encounter a variety of stressful conditions that can affect their viability and efficacy, including extreme temperature, low humidity, oxidative stress, lack of nutrients, and adverse pH. Therefore, abiotic stress tolerance is an essential attribute for yeasts used as biocontrol agents. Several methods, including physiological manipulation (Abadias et al., 2001; Mokiou and Magan, 2008), stress adaptation (Liu et al., 2011a, 2012), and the use of exogenous anti-stress substances (An et al., 2012; Liu et al., 2011b) have been employed to enhance stress tolerance and improve biocontrol efficacy.

A suitable formulation and large-scale testing are fundamental steps in the process of developing a commercial biocontrol product. Pilot scale studies of biocontrol yeasts using both liquid and dry formulations of yeasts have been conducted (Long et al., 2007; Patiño-Vera et al., 2005; Melin et al., 2007; Mokhtarnejad et al., 2011; Torres et al., 2003). In the current review, a brief overview of research that has led to a more comprehensive understanding of postharvest biocontrol systems is presented and discussed with an emphasis on the most recent literature. Information on methods and sources for isolating and screening yeast antagonists, the performance and efficacy of yeast under different environmental conditions, and the potential for commercializing yeast as postharvest biocontrol agents are presented. Areas in need of research are also highlighted.

2. Sources of yeast antagonists

In an early attempt to develop postharvest biocontrol agents, Pusey and Wilson (1984) reported the ability of a strain (B-3) of *Bacillus subtilis* to control brown rot on peach caused by *Monilinia fructicola*. It was subsequently discovered that the antibiotic, iturin, produced by the bacterium was the main factor responsible for the control of brown rot. The use of an antibiotic-producing microorganism on food raised significant concerns and so Wilson et al. (1993) developed a selection strategy for identifying potential postharvest biocontrol agents based on the in vivo selection of non-antibiotic producing yeast strains by applying wash water of fruits into surface wounds and isolating microbes from wounds that were protected against infection from artificially inoculated pathogens. This selection and screening strategy was later used by many different laboratories to identify potential yeast antagonists.

Once a promising yeast antagonist is isolated in pure culture, it is identified using morphological and physiological characterization (Chanchaichaovivat et al., 2007) and/or by DNA sequencing of conserved regions of ribosomal DNA (Kurtzman and Droby, 2001). A number of antagonists have been identified using this strategy and evaluated on the criteria presented in Table 1. Based on these selection criteria, the yeast antagonist, *Candida oleophila* (strain I-182), isolated from the surface of tomato fruit, became the first-generation of yeast-based postharvest biocontrol products commercialized under the trade name 'Aspire' by Ecogen, Inc. (Wisniewski et al., 2007). The product was available on the market for a few years but is no longer available; however, this potential of this yeast species has been explored by other researchers (Bastiaanse et al., 2010; Massart et al., 2005). A commercial yeast biocontrol product, based on *Metschnikowia fructicola*, is marketed in Israel under the trade name "Shemer" by Bayer, Inc.

Yeasts that are naturally present on and apparently endemic to fruit surfaces represent the major group of yeasts utilized to manage postharvest diseases. However, antagonists have also been isolated from other sources, such as the phyllosphere, roots, soil, and sea water. For example, the phyllosphere yeast, *Rhodotorula glutinis* (strainY-44), isolated from leaves of tomato, has been reported to suppress gray mold (*Botrytis cinerea*) on both leaves and fruits of tomato (Kalogiannis et al., 2006). *Kloeckera apiculata* (strain 34-9), isolated from citrus roots, has been reported to be effective in controlling *Penicillium italicum* and *B. cinerea* on citrus and grapes, respectively (Long et al., 2005). The psychrotrophic yeast, *Leucosporidium scottii* (strain At17), isolated from Antarctic soil, was identified as a good biocontrol agent against both blue and gray mold of apple caused by *P. expansum* and *B. cinerea*, respectively (Vero et al., 2013). Compared to yeasts isolated from fruit surfaces, marine yeasts typically have higher osmotolerance and therefore may potentially be more suitable for use under conditions where yeast are exposed to abiotic stress (Hernández-Montiel et al., 2010; Wang et al., 2010). The marine yeast, *Rhodosporidium paludigenum*, isolated from the East China Sea, effectively inhibits *P. expansum* on pear fruit, and *Alternaria alternata* on Chinese winter jujube (Wang et al.,

Table 1
Characteristics of yeasts for the biocontrol of postharvest spoilage fungi on fruits.
Adapted and modified from Barkai-Golan (2001) and Wilson and Wisniewski (1989).

Genetically stable
Effective at low concentrations
Not fastidious in its nutrient requirements
Capable of surviving under adverse environmental conditions (including low/high temperature, oxidative stress and controlled atmosphere storage)
Effective against a wide range of pathogens on a variety of fruits
Amenable to production on an inexpensive growth medium
Preparable in a formulation that can be effectively stored and dispensed
Compatible with commercial processing procedures
Resistant to pesticides, friendly to environment and non-pathogenic to host commodity
Not produce metabolites that are deleterious to human health
Unable to grow at 37 °C and not associated with infections in humans