Biological Control Of Postharvest Diseases: Theory And Practice

Biological Control

Expression of an Antifungal Peptide in *Saccharomyces*: A New Approach for Biological Control of the Postharvest Disease Caused by *Colletotrichum coccodes*

Richard W. Jones and Dov Prusky

First author: Vegetable Laboratory, Henry A. Wallace Beltsville Agricultural Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705; and second author: Department of Postharvest Science of Fresh Produce, Institute for Technology and Storage of Agricultural Products, The Volcani Center, Agricultural Research Organization, Bet Dagan 50250, Israel.
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ABSTRACT

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A cecropin A-based peptide inhibited germination of *Colletotrichum coccodes* at 50 µM. The DNA sequence encoding the peptide was cloned in pRS413, using the *Saccharomyces cerevisiae* invertase leader sequence for secretion of the peptide, and expressed in yeast. Yeast transformants inhibited the growth of germinated *C. coccodes* spores and inhibited decay development caused by *C. coccodes* in tomato fruits. Expression of the antifungal peptide in yeast therefore represents a new approach for the biological control of postharvest diseases.

Additional keywords: antifungal peptides, biocontrol mechanism.

Losses from postharvest pathogens on fruits and vegetables have been principally managed by the use of synthetic fungicides (10). Consumer concerns about possible risks associated with the use of fungicides, along with development of pathogen resistance to certain fungicides, have resulted in an intensive search for safer, more effective control options that pose minimal risk to human health and the environment. Significant progress has been made in developing potential biological alternatives to synthetic fungicides for the control of postharvest diseases of fruits and vegetables (27). The use of microorganisms, particularly yeasts occurring naturally on the surface of fruits or vegetables, usually has been preferred for the control of postharvest disease (6,8,15,16,18,25). Yeasts are suitable as biocontrol agents of postharvest diseases because they (i) rapidly colonize and survive on fruit surfaces for long periods of time under different conditions; (ii) use available nutrients to proliferate rapidly, limiting nutrient availability to the pathogen; and (iii) are generally unaffected by fungicides used commercially.

Several yeast antagonists have been reported to effectively inhibit the development of postharvest pathogens on various fruits (6–8,23). Among these yeasts, *Pichia guilliermondii* Wickerham and *Candida oleophila* Montrocher were developed into commercial products (9,14). However, one of the major problems with the use of those products is their insufficient and inconsistent performance under commercial conditions. Consequently, they are used in combination with low concentrations of postharvest fungicides (9) or by using preharvest treatments with an antagonist (1). Another significant problem is that the reported antagonists are mainly used to control wound pathogens but not for pathogens invading directly through the cuticle and causing quiescent infection (28).

Corresponding author: D. Prusky; E-mail address: prusky33@netvision.net.il

The current study was undertaken to investigate the possibility of expressing a DNA sequence in yeast to allow for the production of an antifungal peptide to produce an improved biocontrol organism (12). Small antibacterial peptides with lytic activity have been found in a broad variety of species (3). Two main structural patterns were identified: one characterized by a β-sheet structure with two or three intramolecular disulfide bonds, and a second group possessing an α-helical motif typical of cecropin families (2) that were used in the present work. Most of the work on the antimicrobial properties of cecropin A and B peptides has concentrated on their activities against plant-pathogenic bacteria. Consequently, these peptides have been studied with a view to engineering bacterial disease resistance in plants. However, antifungal activity was also reported for these peptides (5,21). In an effort to improve the control of postharvest decay by biological means, we have developed a new approach to control postharvest pathogens by expressing a lytic peptide in *Saccharomyces cerevisiae* Hansen. We chose the tomato fruit fungal pathogen *Colletotrichum coccodes* (Wallr.) S. J. Hughes as a target to demonstrate the potential of bioengineered yeast in disease control.

MATERIALS AND METHODS

Tomato fruit, fungal isolates, and growth media. Tomato fruit (*Lycopersicum esculentum* cv. Roma) were obtained from a local grocery store. A single-spore isolate of *C. coccodes* was obtained from a decayed tomato (cv. Roma). Three-week-old conidia were harvested from Mathur's medium (M_3S) plates (26) and used for culture and fruit inoculation. Spore production was estimated on 3-week-old colonies on M_3S plates at 20°C by counting spores with a haemacytometer (Brand, Wertheim, Germany).

Antifungal activity of the synthesized peptide. The antifungal peptide represents an internal portion of cecropin A. The peptide (WKLFKKIILKVL) was synthesized by Genosys (Genosys, The Woodlands, TX). Antifungal activity against *C. coccodes* was tested in water solutions at 0.5 µM and up to 5 mM of the pure peptide. Peptide stock solutions were prepared at twofold concentrations of the final concentrations tested and mixed at a 1:1 ratio

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