

Biological Control of Postharvest Diseases of Fruits and Vegetables: Recent Advances

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Although it is difficult to determine the full extent of postharvest losses due to diseases, conservative estimates place losses to fruits and vegetables from spoilage at $\approx 24\%$ of the harvested crop in the United States (U.S. Dept. of Agriculture, 1965) and 50% in underdeveloped, tropical countries (Coursey and Booth, 1972).

Fungicides are a primary means of controlling postharvest diseases (Eckert and Ogawa, 1985). Their use worldwide is variable, comprising 26% of the pesticide market in Europe and Asia and 6% in the United States (Jutsum, 1988). However, as harvested fruits and vegetables are commonly treated with fungicides to retard postharvest diseases, there is a greater likelihood of direct, human exposure to them than to other pesticides that are applied solely to protect foliage.

Public and scientific concern about the presence of synthetic chemicals in our food supply and in the environment has been increasing in the past decade. A report from the National Academy of Sciences (NAS) (1987) indicated particular concern about the health risks associated with the use of fungicides. As a direct result of these mounting concerns, real or perceived, several fungicides (e.g., captan and benomyl) have been banned by the U.S. Environmental Protection Agency or voluntarily withdrawn from the market for some or all postharvest use. This action has the potential of greatly diminishing our ability to control postharvest diseases of many commodities. The NAS report clearly indicated this possibility by stating, "For certain crops in certain regions, the loss of all oncogenic compounds—particularly fungicides—would cause severe short-term adjustments in pest control practices because of the lack of economically viable alternatives."

Despite this situation, the trend to restrict or ban the use of current, synthetic fungicides for postharvest use is continuing. A recent report in *Postharvest News and Information* (Rendall-Dunn, 1991) indicated that the European Parliament has voted in favor of a total ban on postharvest treatment of

fruits and vegetables with pesticides as soon as this practice becomes feasible. As a further indication of this trend, Technical Insights, Inc., in their *Twenty-five Predictions for the New Century* (1991), has stated that "the Agricultural Chemical Industry as we know it today will begin to disappear. Biological products will become cost competitive with chemicals due to new environmental-impact taxes."

There is clearly an urgent need to develop new and effective methods of controlling postharvest diseases that are perceived as safe by the public and pose negligible risk to human health and the environment. The use of nonchemical techniques and nonselective fungicide treatments have and will in the future answer a part of this need. Inoculum reduction achieved through sanitation and exclusion (Bancroft et al., 1984), the use of nonselective fungicides (sodium carbonate, sodium bicarbonate, active chlorine, and sorbic acid), and heat treatments can significantly lower the disease pressure on harvested commodities (Eckert, 1991). Harvesting and handling techniques that minimize injury to the commodity, along with storage conditions that are optimum for maintaining host resistance (Sommer, 1985), will also aid in suppressing disease development after harvest. In addition to the above methods, however, considerable attention has also been placed on assessing the potential of biological control of postharvest diseases of fruits and vegetables as a viable alternative to the use of present-day, synthetic fungicides (Wilson and Wisniewski, 1989; Wilson et al., 1991). In the following report, we will review recent advances in the use of microbial antagonists to control postharvest diseases (especially of fruit) and to address their commercial potential.

Biological control-A new climate

Biological control agents have had a difficult time making it from the laboratory to the marketplace. This difficulty has been largely due to problems of ineffectiveness when the biocontrol agents are subjected to the "uncontrolled" environment of the field and to the lack of economic incentive to develop the technology necessary for their effective use. Lack of support for widespread, sustained research in this area has been largely due to the effectiveness and perceived safety of chemical fungicides. However, because of the changing socioeconomic climate and

recent advances in genetic engineering, interest in biological control as a meaningful approach to pest and disease management has been rejuvenated (Jeffries and Jeger, 1990; Wilson and Wisniewski, 1989, 1992). Recent changes in U.S. patent laws also have contributed to an atmosphere that is more conducive to the development of marketable biological control agents. Several venture-capital companies or subsidiaries of well-known agrochemical companies, with a focus on biological control, have arisen in response to this new climate. Therefore, the outlook for developing economically viable biological control products looks very promising.

Postharvest biological control-A unique environment

Two basic approaches are available for using microorganisms to control postharvest diseases: use and management of the beneficial microflora that already exist on fruit and vegetable surfaces or the artificial introduction of antagonists against postharvest pathogens. Our knowledge of methods to manipulate naturally occurring populations of mixed species of microorganisms in a beneficial manner, however, is meager (Wilson, 1989), and the greatest use of biological control (pre- and postharvest) has come through the artificial introduction of large numbers of a known antagonist (Wilson and Wisniewski, 1989).

Several factors indicate that postharvest biological control with the use of artificially introduced antagonists may prove to be an effective technology. First, environments for the storage of harvested commodities are often controlled and maintained. This control should lessen the problem of introducing the biocontrol agent into an unpredictable and highly variable environment, which previously has been a limiting factor in field-released biocontrol measures. Second, the ability to target the biocontrol agent to the site needed for activity is enhanced in postharvest application. Third, the high value of harvested commodities may make the application of elaborate biological control procedures more cost-effective than similar procedures in the field. Fourth, for some commodities harvested for fresh-market consumption, protection from postharvest diseases is only needed for a short duration.

Although it appears that the postharvest environment may be especially favorable for

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the development of biocontrol agents, a considerable investment of time and money is required to establish whether any particular organism has commercial potential. Therefore, the isolation, screening, and selection of potential antagonists should receive careful deliberation. The characteristics of an "ideal" antagonist are listed in Table 1. As indicated by Roberts (1991), any potential antagonist must have the ability to colonize and persist on the commodity at effective levels; be compatible with other postharvest practices, processes, and chemicals; and be effective under cold and, in some cases, controlled-atmosphere conditions. Additionally, the organism must be amenable to large-scale production using low-cost substrates. Given these constraints, several antagonists for control of postharvest diseases of fruit have been identified for their commercial potential.

Antagonistic organisms-Identification and characterization

In their book on biological control, Cook and Baker (1983) presented only one example of the biological control of a postharvest disease of a fruit or vegetable. This was the biological control of botrytis rot of strawberry (*Fragaria × ananassa*) with *Trichoderma* sp. (Tronsmo and Dennis, 1977). Since that time, there has been a wealth of research conducted in this area, mostly on fruit diseases, and several antagonists have been identified. The topic has also been the subject of several reviews (Janisiewicz, 1988a; Jeffries and Jeger, 1990; Wilson and Pusey, 1985; Wilson and Wisniewski, 1989, 1991; Wilson et al., 1991).

Microbial antagonists have been reported to control several rot pathogens on diverse commodities (Table 2). Of particular interest among these antagonists are yeasts and yeast-like organisms, such as *Pichia guilliermondii* Wickerham, isolated and developed by Wilson and Chalutz (1989) and subsequent co-workers (Droby et al., 1989, 1991; McLaughlin et al., 1990a, Wisniewski et al., 1991), for control of postharvest rots of citrus and other fruits; *Acremonium breve* (Sukapure and Thiramalachar) W. Gams, isolated by Janisiewicz (1988b); and several species of *Cryptococcus*, isolated by Roberts (1990a, 1990b), for control of postharvest rots of apple (*Malus domestica* Borkh.) and pear (*Pyrus communis* L.). As indicated by Janisiewicz (1988a), yeasts can colonize a surface for long periods under dry conditions, produce extracellular polysaccharides that enhance their survivability and restrict both colonization sites and the flow of germination cues to fungal propagules, use available nutrients rapidly and proliferate, and are impacted minimally by pesticides. These are features that can greatly enhance the effectiveness of any antagonist that is identified for its potential use in the postharvest environment. The effectiveness of the yeast *Pichia guilliermondii*, previously classified as *Debaromyces hansenii* (McLaughlin et al., 1990a), is highly effective in controlling botrytis rot

of apples and penicillium rot of oranges *ICitrus sinensis* (L.) Osb.] when applied to wounded and inoculated fruit (Fig. 1).

In addition to the above yeasts, the bacteria *Bacillus subtilis* (Ehrenberg) Cohn and *Pseudomonas cepacia* Burk. have shown potential in controlling a wide range of rot organisms on several commodities (Janisiewicz and Roitman, 1988; Pusey and Wilson, 1984; Singh and Deverall, 1984; Utkehede and Sholberg, 1986). Their activ-

ity, however, appears to rely on the production of potent antibiotics by the antagonists. This feature may be of special concern when evaluating their commercial potential.

In general, the mode of action of many of the antagonists that have been identified for use in controlling postharvest diseases is poorly understood. In the absence of the production of antibiotics, it appears that the mode of action involves a complex syndrome of characters (Droby et al., 1989, 1991; Wisniewski et al., 1989, 1991), including nutrient competition, site exclusion, attachment of the antagonist to the pathogen, induced resistance, and, perhaps, direct parasitism. Undoubtedly, many of the evolving concepts for biocontrol of plant diseases also would apply to the postharvest arena. Additionally, as Baker (1987) has indicated, many undiscovered mechanisms have evolved and are functioning in the natural world. As research on biological control of postharvest diseases continues, our knowledge likely will increase rapidly by drawing from and building on the base of information developed in other areas of biocontrol and phytoplane research. This base of knowledge should allow the development of more reliable procedures for the effective application of known antagonists and also provide a rationale for efficiently selecting other effective antagonists (Wilson and Wisniewski, 1989).

Table 1. Characteristics of an "ideal antagonist" for the postharvest environment (adapted from Wilson and Wisniewski, 1989).

Genetically stable.
Effective at low concentrations.
Not fastidious in its nutrient requirements.
Ability to survive adverse environmental conditions (including low-temperature and controlled-atmosphere storage).
Effective against a wide range of pathogens on a variety of fruits and vegetables.
Amenable to production on an inexpensive growth medium.
Amenable to a formulation with a long shelf life.
Easy to dispense.
Does not produce metabolites that are deleterious to human health.
Resistant to pesticides.
Compatible with commercial processing procedures.
Nonpathogenic to host commodity.

Table 2. Reports of postharvest biological control.

Commodity	Disease	Biocontrol agent	Reference
Apple	Blue mold	<i>Pseudomonas syringae</i>	Janisiewicz, 1987
	Blue mold	<i>Pseudomonas cepacia</i>	Janisiewicz and Roitman, 1988
	Blue mold	<i>Cryptococcus</i> spp.	Roberts, 1991
	Blue mold	<i>Pichia guilliermondii</i>	McLaughlin et al., 1990b
	Gray mold	<i>Pichia guilliermondii</i>	Wisniewski et al., 1988; McLaughlin et al., 1990b
Citrus	Gray mold	<i>Pseudomonas cepacia</i>	Janisiewicz and Roitman, 1988
	Gray mold	<i>C. laurentii</i>	Roberts, 1990a
	Gray mold	<i>C. flavus</i> , <i>C. albidus</i>	Roberts, 1991
	Gray mold	<i>Acremonium breve</i>	Janisiewicz, 1988b
	Mucor rot	<i>Pseudomonas cepacia</i>	Janisiewicz and Roitman, 1987
	Green mold	<i>Pichia guilliermondii</i>	Chalutz and Wilson, 1990; Wilson and Chalutz, 1989
Citrus	Green mold	<i>Bacillus subtilis</i>	Singh and Deverall, 1984
	Blue mold	<i>Pichia guilliermondii</i>	Chalutz and Wilson, 1990
	Sour rot	<i>Pichia guilliermondii</i>	Chalutz and Wilson, 1990
	Sour rot	<i>B. subtilis</i>	Singh and Deverall, 1984
	Sour rot	<i>Trichoderma</i> sp.	De Matos, 1983
	Stem end rot	<i>B. subtilis</i>	Singh and Deverall, 1984
Pear	Blue mold	<i>Pseudomonas cepacia</i>	Janisiewicz and Roitman, 1988
	Gray mold	<i>Pseudomonas cepacia</i>	Janisiewicz and Roitman, 1988
	Gray mold	<i>Pseudomonas gladioli</i>	Mao and Cappellini, 1986
	Mucor rot	<i>C. laurentii</i> , <i>C. flaws</i> , <i>C. albidus</i>	Roberts, 1990b
	Nectarine	Brown rot	<i>B. subtilis</i>
Peach	Brown rot	<i>B. subtilis</i>	Pusey and Wilson, 1984
Peach	Rhizopus rot	<i>Enterobacter cloacae</i>	Wilson et al., 1987
Apricot	Brown rot	<i>B. subtilis</i>	Pusey and Wilson, 1984
Plum	Brown rot	<i>B. subtilis</i>	Pusey and Wilson, 1984
Cherry	Alternaria rot	<i>E. aerogenes</i>	Utkehede and Sholberg, 1986
	Brown rot	<i>B. subtilis</i>	Utkehede and Sholberg, 1986
Grape	Gray mold	<i>Trichoderma harzianum</i>	Dubos, 1984
	Gray mold	<i>Pichia guilliermondii</i>	Chalutz et al., 1988
	Rhizopus rot	<i>Pichia guilliermondii</i>	Chalutz et al., 1988
Tomato	Gray mold	<i>Pichia guilliermondii</i>	Chalutz et al., 1988
	Alternaria rot	<i>Pichia guilliermondii</i>	Chalutz et al., 1988
Strawberry	Gray mold	<i>Trichoderma</i> sp.	Tronsmo and Dennis, 1977
Pineapple	Penicillium rot	Attenuated strains of <i>Penicillium</i> sp.	Tong-Kwee and Rohrbach, 1980
		<i>Pseudomonas putida</i>	Colyer and Mount, 1984

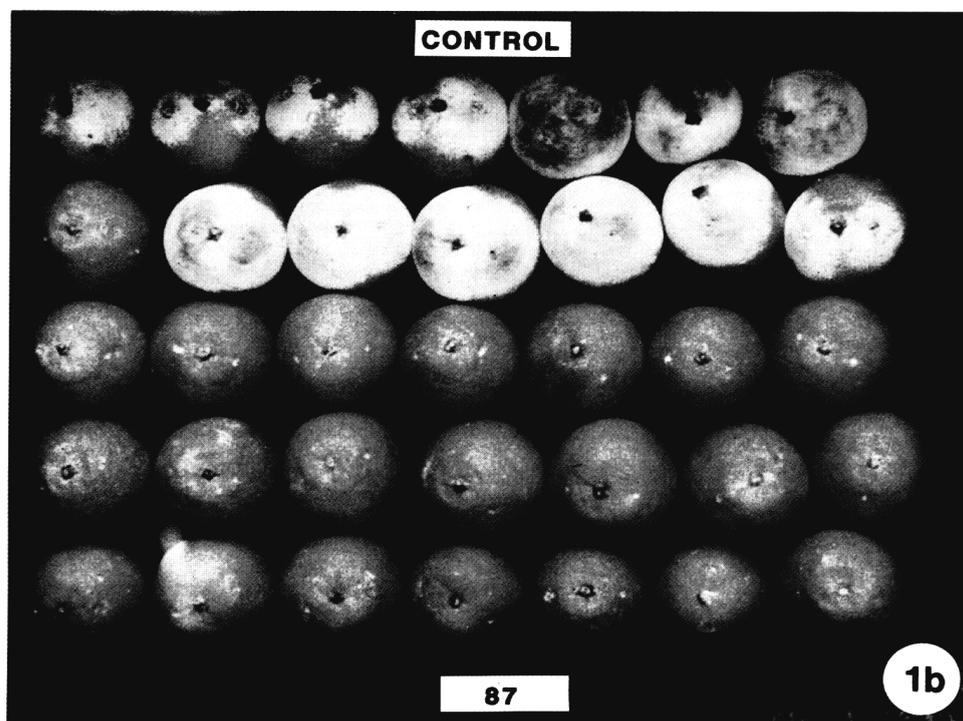
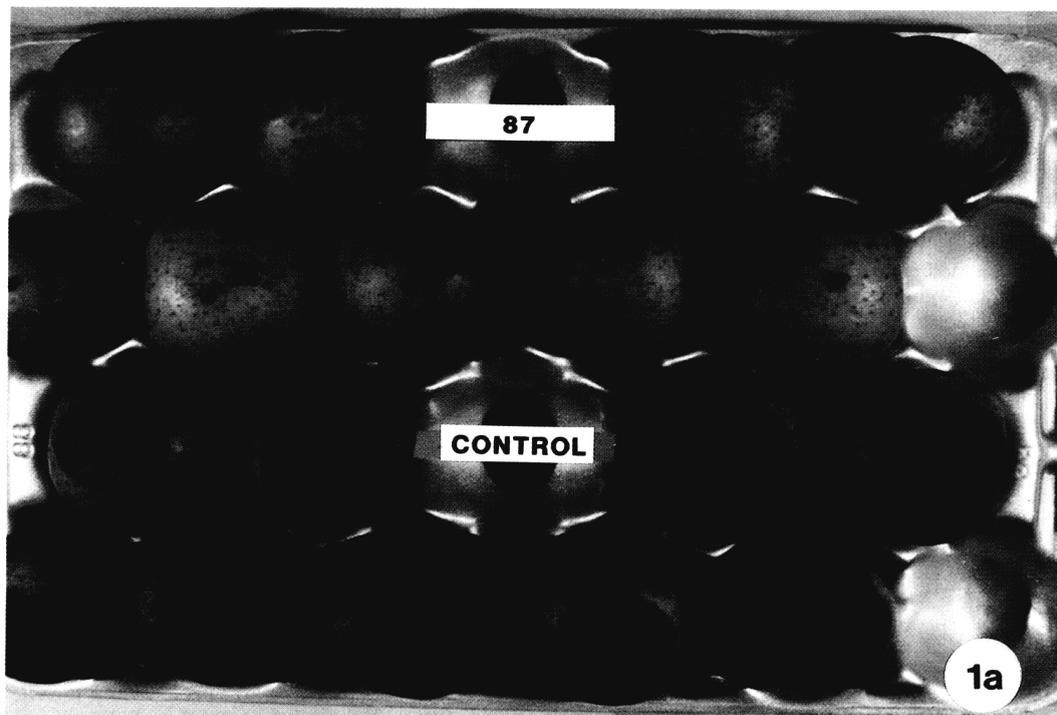


Fig. 1. Biological control of gray mold (*Botrytis cinerea* Pers.:Fr) on apple (1a) and green mold [*Penicillium digitatum* (Pers.:Fr) Sacc.] on oranges (1b) with the use of the antagonistic yeast *Pichia guilliermondii* (strain 87). Photographs were taken 1 week after wounding and application of the yeast and pathogen to the wound site.

Postharvest biological control-Commercialization

Commercialization of an antagonist requires the integration of biological control measures with current handling practices. Pusey et al. (1986, 1988) were able to incorporate the antagonist *B. subtilis* into the fruit wax used commercially to treat peaches

on the packing line. In these pilot tests, the use of the antagonist gave a level of protection similar to that obtained with wax plus the fungicide methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl).

McLaughlin et al. (1990b) demonstrated that the addition of 2% calcium chloride to the yeast suspension greatly enhanced the ability of the yeast *P. guilliermondii* to con-

trol postharvest diseases of apples. This allowed a significant reduction in the amount of yeast biomass needed to achieve control. Further, initial pilot tests of *P. guilliermondii* on citrus (Hofstein et al., 1991) have indicated that biocontrol activity of the yeast can be significantly enhanced with the addition of 10% of the normally recommended rate of the fungicide thiabendazole. These

reports suggest that biocontrol procedures can be integrated into the commercial post-harvest operation.

As Wilson and Wisniewski (1992) have indicated, the application of antagonistic microorganisms to food that is to be consumed presents special problems. Public reaction to the application of "living fungicides" to food is yet to be determined. The use of antagonists that produce antibiotics as their principal mode of action will also raise additional concern. The potential exists that exposure of human and animal pathogens to such antibiotics may cause resistance to develop to potentially effective therapeutic compounds. Possible pathogenicity to man and other animals, as well as a wide range of harvested commodities, must be considered. In selecting antagonists as biological control agents on food, attention should be given to these potential problems. However, microorganisms have been used since ancient times to pickle and ferment foods to preserve them (Gilliland, 1985). Among the wide array of antagonists available, selection of safe and effective biocontrol agents should be possible.

The public's demand for reduced pesticides in our food and the environment has caused an energetic debate over the safeness of our present control practices for post-harvest diseases. As researchers, we have the challenge and opportunity to develop safe and effective alternatives to present-day synthetic fungicides. The climate for support of biological control research is now excellent. There is every indication that significant advances will be made and commercially available products will be available for postharvest use in the near future.

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