Exploitation of Yeasts as an Alternative Strategy to Control Post Harvest Diseases of Fruits-A Review

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Abstract: There is an increasing concern about the environmental effects and safety of chemical pesticides and fungicides all over the world. Microbial biocontrol agents possess a number of important advantages over traditional chemical pesticides which make their commercial outlook particularly promising and can be commercially developed with relative ease to control post harvest diseases of fruits. Biological control of different plant diseases was focused primarily using bacteria or filamentous fungi and so, application of yeasts as biocontrol agents acts as a new trend against different pathogens. Various observations suggest that competition for space and nutrients between yeasts and pathogens, antibiosis, parasitism, induction of host resistance and also resistance to oxidative stress are likely to be the main mechanisms of action. In order to enhance biocontrol activity of antagonists against fungal pathogens, certain strategies, such as adding calcium salts, carbohydrates, amino acids and other nitrogen compounds to biocontrol treatments are proposed and can prove more beneficial than using yeasts alone. A more thorough understanding of the mechanisms of action, interactions of fruit tissue-pathogen-biocontrol agent and effective formulation techniques are still needed to develop these yeasts into potential candidates in the management of post harvest diseases of fruits.

Key words: Biological control • Fruits • Post-harvest diseases • Yeasts

INTRODUCTION

Post harvest diseases of fruits caused by pathogens pose a major challenge throughout the fruit growing areas of the world accounting to about 20-25% of the harvested during postharvest handling [1-4] in developed countries and even more exasperating in the developing countries, where it often exceeds over 35%, due to inadequate storage, processing and transportation facilities [5]. A side from direct economic considerations, diseased produce poses a potential health risk. In most cases, fresh produce which is obviously diseased would not be consumed. A number of fungal genera such as Aspergillus, Penicillium, Alternaria and Fusarium are known to produce mycotoxins under certain conditions. Generally speaking, the greatest risk of mycotoxin contamination occurs when diseased fruit produce is used in the production of processed food. Losses due to postharvest disease are affected by a great number of factors including: commodity type, the postharvest environment (temperature, relative humidity, atmosphere composition, etc.), produce handling methods, post harvest hygiene, produce maturity and ripeness stage, cultivar susceptibility to postharvest diseases and treatments used for disease control. Fungicides are commonly used to control these post harvest decays. However, though harvested fruits treated with fungicides retard post harvest diseases, there is a greater likelihood of direct human exposure to them. Synthetic chemicals are also discouraged due to their carcinogenicity, teratogenicity, high and acute residual toxicity, environmental pollution and their effects on food and side effects on humans [6-7]. Besides these, phytotoxicity and off odour effects of some fungicides have limited their use. Development of resistance to commonly used fungicides within populations of post harvest pathogens has also become a significant problem. For example, acquired resistance by Penicillium italicum and Penicillium digitatum to fungicides used for treating citrus fruits has been reported [8]. This calls for a new

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paradigm shift from the use of synthetic fungicides to a safer and environmentally friendly alternative for reducing the postharvest decay in fruits and vegetables [9-10].

In the last few years, biological control of postharvest diseases of fruits has been developed as a promising alternative to chemical control [11-12]. Among the replete of bio-control approaches, the use of the microbial antagonists like yeasts, fungi and bacteria is quite promising and gaining popularity [2, 13-19]. Among the antagonistic microorganisms, natural yeasts have been widely used as biological control agents [20]. Yeast based technologies and products (e.g Aspire® containing Candida oleophila, Yield Plus® containing C. albidus) for post harvest disease management of fruits are already practiced and developed in foreign countries. Unfortunately, no such technology or product (s) have been developed in India although it is the major fruit producing country in the world and very limited research work on this aspect has been carried out.

The objective of this review is to critically assess the use of yeasts as microbial antagonists for controlling postharvest diseases of fruits, in order to reduce the use of chemical agents that may be harmful to humans and the environment.

**What are yeasts?:** Yeasts are a heterogenous group of fungi that superficially appear to be homogeneous. They grow in a conspicuous unicellular form that reproduces by fission, budding, or a combination of both. True yeasts reproduce sexually, developing ascospores or basidiospores under favourable conditions. The majority of ascomycetous and basidiomycetous yeasts isolated in laboratories go unrecognized because most of them are heterothallic. In most instances, only one of the mating types is isolated and therefore no asc or basidia are produced. Yeast-like fungi (imperfect yeasts) reproduce only by asexual means. The identification of these fungi is based upon a combination of morphological and biochemical criteria. Morphology is primarily used to establish the genera, whereas biochemical assimilations are used to differentiate the various species. Several important properties of yeasts make them stand out among others for biological control purposes:

(i) Yeasts do not produce allergenic spores or mycotoxins as many mycelial fungi do or antibiotic metabolites as possibly produced by bacterial antagonists

(ii) They generally have simple nutritional requirements and are able to colonize dry surfaces for long periods of time.

(iii) They rapidly utilize available nutrients and can sustain many of the pesticides used in the post-harvest environment and can grow rapidly on cheap substrates in fermenters and are therefore easy to produce in large quantities. The suggested modes of action of biocontrol yeasts are not likely to constitute any hazard for the consumer. Furthermore, yeast cells contain high amounts of vitamins, minerals and essential amino acids and several reports on the beneficial effect of yeast addition in both food and feed can be found in the literature [21-23].

**Mechanisms of Yeasts in Biological Control:**

The mechanism by which the yeasts inhibits the target pathogen is not clear and still incomplete due to the difficulties encountered during the study of the complex interactions between host, pathogen, antagonist and other microorganisms present making it extremely difficult to construct experiments that can exclude all other possible mechanisms in the complex biocontrol environment. However, several modes of action have been suggested and so far no one single mechanism has been shown to be responsible for the whole biocontrol effect [15].

**Competition for Space and Nutrition:** Competition for space and nutrients is the primary mechanism of action of most of the biocontrol agents. Rapid colonization of fruits by biocontrol agents is critical for decay control and manipulations leading to improved colonization enhance biocontrol [24]. They should be able to grow more rapidly than the pathogen and should have the ability to survive even under conditions that are unfavorable to the pathogen. The biocontrol activity of some yeasts increase with their increasing concentrations and decreasing concentrations of pathogens. For example, Candida saitona was effective at a concentration of 10⁷ CFU/ml for controlling Penicillium expansum on apples fruits and less effective when concentrations were decreases [25]. In another study, it was reported that for Candida saitona, a concentration of 10⁶ CFU/ml was better in controlling Penicillium expansum on apples [26]. This qualitative relationship, however, is highly dependent on the ability of the antagonists to multiply and grow at the wound site. This was demonstrated by using a mutant of Pichia guilliermondii, which lost its biocontrol activity against Penicillium digitatum on grapefruit and against Botrytis cinerea on apples, even when applied to the wounds at concentrations as high as 10¹⁰ CFU/ml [14]. The cell population of this mutant remained constant at the wound sites during incubation.
period, while that of the wild type increased 10- to 20-fold, within 24 h hours. Competition for nutrients has been suggested as the mode of action of several yeasts like _Pichia guilliermondii_ against _Penicillium digitatum_ [27], _Candida guilliermondii_, _Cryptococcus laurentii_ and _Metschnikowia pulcerima_ against _Botrytis cinerea_ and _Penicillium expansum_ [13, 28-31]. It was demonstrated that addition of exogenous nutrients reduced the efficacy of _P. guilliermondii_ against _P. digitatum_ [27]. The antagonistic yeasts _Candida lauretii_ and _Sporobolomyces roseus_ had stronger sugar consumption than the pathogen _B. Cinerea_ by blocking fungus conidial germination due to the deprivation of nutrients [32]. However, no differences in sugar consumption were observed between yeasts with and without biocontrol activity, suggesting that additional factors are part of the inhibiting mechanisms. In fruit wounds, competition for nutrients is probably extended to other nutrients, such as nitrogen compounds present in low concentration. Both organic and inorganic sources of nitrogen can be utilized by yeast for growth. Amino acids, amines and urea are all suitable sources of nitrogen for all yeasts, as inorganic ammonium salts while nitrate utilization is confined to a certain species or genera of yeasts and this is a valuable diagnostic character used for identification purposes too. Although very few species can hydrolyse proteins extracellularly, short peptides can be transported into the cell and utilized intracellularly [33]. In a tissue culture plate system with membrane inserts separating the organism, Janisiewicz et al. [34] were able to show that the yeast _Aureobasidium pullulans_ consumes the amino acids and inhibits germination of _P. expansum_ in apple juice. A non-destructive method using tissue culture plates: a defusing membrane at the lower end of cylindrical inserts to study the competition for nutrients separated from the competition for space has been developed [34].

**Antibiosis:** Production of antibiotic substances is a commonly suggested mode of action for bacterial biocontrol agents [35]. It has been shown that the yeast _Pseudozyma flocculosa_ produces extracellular fatty acids that are detrimental to powdery mildew [36]. Biocontrol agents producing antibiotic metabolites could be effective against a wide range of target organisms, including pathogens that have occurred prior to the application of the biocontrol agent, as well as against latent infections. However, given the current debate about the antibiotic resistance of human pathogens it is doubtful that an antibiotic-producing biocontrol agent would be registered for use on food or feed. Moreover, similar problems with resistant pathogen strains, as experienced today with fungicides, would probably rapidly occur if a one-substance effect was the only mechanism involved.

**Parasitism:** Parasitism by yeasts has been suggested as a fungus-inhibiting mechanism. The yeast _Pichia guilliermondii_ inhibits _B. cinerea_ and adheres strongly to the fungal mycelium [37]. It has also been shown that _P. anomala_ strain K has a strong production of β-1,3-glucanase enzyme that degrades the fungal cell wall [38]. _Aureobasidium pullulans_ in apple wounds produces extracellular exochitinase and β-1,3-glucanase which could play a role in the biocontrol activity [39].

**Induction of Host Resistance:** The characteristic defense response in fruit includes production of inhibitors of cell wall-degrading enzymes of the pathogen, activity of antifungal compounds (such as phenolic compounds and phytolaexins), active oxygen species and reinforcement of the cell wall of the host [40]. The resistance induction is due to the antagonist ability to elicit host plant defense responses. It involves several chemical or biochemical reactions in the host tissue, including changes of tissue structure and production of pathogenesis-related proteins, which can be expressed locally or systemically [41-42]. _P. guilliermondii_ has been shown to stimulate the production of ethylene in grapefruit [37]. _Candida famata_ (F35) stimulates the production of the phytoalexins, scoparone and scopeol in the wound site of oranges [43]. _Candida oleophila_ was found to induce resistance to _P. digitatum_ when applied in the surface of both wounded and unwounded grapefruit [44]. Some _Candida_ strains are able to cause chemical and osmotic changes in apple tissues and thus favouring antagonist settlement [25]. _P. guilliermondii_ strain has been shown to stimulate the production of ethylene, a hormone in grapefruit able to activate the phenylalaninammoniumlyase, an enzyme involved in the synthesis of phenols, phytoalexins and lignins [37]. Accumulation of phytoalexins, i.e., scoparon and scopeol has been observed in citrus fruits treated with yeast cells [45].

**Resistance to Oxidative Stress:** Resistance to oxidative stress is another suggested mode of action of some yeast. A model system consisting of two yeasts with higher (_Cryptococcus laurentii_ LS-28) or lower (_Rhodotorula glutinis_ LS-11) antagonistic activity against the postharvest pathogens _Botrytis cinerea_ and
Penicillium expansum was analysed and found that Cryptococcus laurentii LS-28 exhibited faster and greater colonization of wounds than Rhodotorula glutinis LS-11 [46]. In contrast to LS-28, the number of LS-11 cells dropped 1 and 2 h after application and then increased only later. In vitro, LS-28 was more resistant to ROS-generated oxidative stress. The combined application of biocontrol yeasts and ROS-deactivating enzymes in apple wounds prevented the decrease in number of LS-11 cells mentioned above and enhanced colonization and antagonistic activity of both biocontrol yeasts against B. cinerea and P. expansum.

To summarize, the activity of a biocontrol agent seems to be primarily dependent on its ability to rapidly colonize the wound site and compete for nutrients, but may also depend on its ability to attach firmly to hyphae of the pathogen, produce cell wall degrading enzymes or volatile compounds, or induce host resistance. An example: it has been demonstrated that direct attachment, competition for nitrogen and carbon sources, secretion of hydrolytic enzymes and induction of host resistance play a role in the biocontrol mechanisms of P. guilliermondii M8 against B. cinerea [47].

Yeasts as a Biological Control Candidate in the Management of Post Harvest Diseases: Yeasts are particularly attractive as biological control candidates and are encouraged by the fact that these microorganisms are frequently associated with foods and might be more acceptable to consumers than exotic bacteria [48] and also there is considerable information available with respect to techniques for genetic manipulation, production and storage of yeast cells [49]. Moreover, yeasts as a major component of the epiphytic microbial community on the surfaces of fruits and vegetables [50], may be a more effective biocontrol agent because they are phenotypically adapted to this niche and, thus, are able to more effectively colonize and compete for nutrients and space on fruit surfaces. In the last two decades, several scientific studies have demonstrated the efficiency of yeasts as biocontrol agents against a wide variety of phytopathogens [51-60]. Among yeasts, Candida spp. have been found to be highly effective against different fungal pathogens [25], Hanseniaspora uvarum against Rhizopus stolonifer and Botrytis cinerea [61], Cryptococcus spp. against grey mould, blue mould and Mucor rot [62]. Yeast strains from species, Pichia guilliermondii, Debaryomyces hansenii and Kloeckera apiculata have been shown to have the ability to control post-harvest diseases of several fruits [25, 63-64].

Commercially Available Yeast Biocontrol Products: There are currently three commercial yeast biocontrol products available on the market for combating post-harvest decays in fruit. Aspire® (Ecogen, Inc., Langhorne, Pa) is based on the yeast Candida oleophila and is used as a spray or dip against post-harvest diseases on pome and citrus fruits [15, 65]. The product was introduced commercially in the United States in 1996. The commercial product Yield Plus® with Cryptococcus albidus as the active antagonist was introduced commercially on the South African market in 1997 by Anchor Yeast. Yield Plus® is used as a biocontrol product against Botrytis, Penicillium and Mucor on apples and pears and is also under evaluation for other crops (Lisa Picard, Anchor Yeast, Cape Town, South Africa, pers. comm.). The recent product Shemer®, registered in Israel, is based on a newly identified yeast Metschnikowia fructicola [66] and is effective against a wide range of pathogens of grape, strawberry and sweet potato.

Combination of Some Safe Compounds with Yeasts: Biological means cannot at the moment solve all the problems of postharvest rots during fruit storage and they must be considered instruments to be used in combination with other methods in an integrated vision of disease management. For example, biocontrol agents can be combined with waxes and fungicides applied not only in post but also in pre-harvest [67]. In order to enhance biocontrol activity of antagonists against fungal pathogens, certain strategies, such as adding calcium salts, carbohydrates, amino acids and other nitrogen compounds to biocontrol treatments, are proposed [68-71]. Many researchers have shown that calcium plays an important role in the inhibition of postharvest decay of fruits [72-73]. Postharvest calcium treatment of apples provided broad-spectrum protection against the postharvest pathogens of Penicillium expansum and Botrytis cinerea [74]. The addition of CaCl₂ (2% w/v) to the formulation of the yeast biocontrol agent, Candida oleophila, enhanced the ability of this yeast to protect apples against postharvest decay [75]. The efficacy of controlling grey mould and blue mould rots in apples was enhanced when Trichosporon sp., even at a low concentration of 10⁵ CFU mL⁻¹, was applied in the presence of CaCl₂ (2% w/v) in an aqueous suspension [76]. However, application of 68 mM CaCl₂ to grapefruit reduced the incidence of green mold decay by 43% and in combination with an antagonist P. guilliermondii strain US-7 by 97% [77]. Other compounds like sodium bicarbonate and ammonium molybdate exhibit...
broad-spectrum antifungal activity [78-79]. Chitosan and its derivatives, including glycolchitosan, were also reported to inhibit fungal growth and to induce host-defense responses in plants and harvested commodities [80]. Combining 0.2% glycolchitosan with the antagonist *Candida saitoana* was more effective in controlling green mould of oranges and lemons, caused by *P. digitatum* and gray and blue molds of apples than either treatment alone [81]. The addition of ammonium molybdate to the yeast *Candida sake*, significantly enhanced its biocontrol efficacy in controlling postharvest decay in pear fruits [82]. However, there was little reported use of sodium bicarbonate as an additive to improve the efficacy of yeasts against fungal diseases in fruits.

**Challenges for the Future and Concluding Remarks:**

Over the past 20 years, biocontrol research has evolved toward being more integrated into a production systems approach with more awareness of industry concerns. As noted in this review, more research is needed in many aspects of the science and technology of yeast postharvest biocontrol and in integrating the agents into combined pre- and postharvest production and handling systems and combining with safe chemicals in preventing postharvest diseases and can be used to control mixed populations of fungicide-sensitive and fungicide-resistant pathogens [83]. Although several mechanisms of action have been suggested for postharvest biocontrol agents, a deeper understanding of the tritrophic interactions of fruit tissue-pathogen-biocontrol agent is still needed. Induced resistance has been postulated to be one of the mechanisms of action of yeasts as postharvest biocontrol agents. However, information about elicitors/effectors of the antagonist involved and our ability to genetically and physiologically manipulate them is still lacking. Questions related to the effect of host physiology on bio-control activity are also unresolved. More research effort is needed in order to address the need to lower the effective biomass and the inherent production costs of antagonistic microorganisms to be used in practical applications and to enhance the efficacy of these beneficial microbes. Suitable formulations of these agents could play a crucial role in their effectiveness by increasing their dispersion and colonization on fruit skin, by prolonging their survival in practical conditions and by enhancing the mechanisms of action underlying their biological activity. The biological control of postharvest diseases is viewed with caution and skepticism by many in the agricultural community. Unlike the control of tree, field crop or soil-borne diseases, successful commercial control of postharvest diseases of fruit and vegetables must be extremely efficient, in the range of 95-98%. As of today, such levels of control can be reliably reached by bio- fungicides only when supplemented with low levels of synthetic chemical fungicides. A more thorough understanding of the microbial ecology of fruit surfaces will help us figure out which problems to work on, how to approach them, when and where to apply the biocontrol agent and predict situations in which biocontrol would not be expected to work.

**REFERENCES**


