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REVIEW

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Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters

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Abstract Among soil microorganisms, yeasts have received little attention as biocontrol agents of soil-borne fungal plant pathogens in comparison to bacterial, actinomycetes, and filamentous fungal antagonists. The mechanisms of action of potential antagonism by yeasts in relation to soil-borne fungal plant pathogens are expected to be similar to those involved with pathogens of aerial parts of the plant, including leaves and fruits. Several taxa of yeasts have been recorded as endophytes in plants, with a small proportion recorded to promote plant growth. The ability of certain taxa of yeasts to multiply rapidly, to produce antibiotics and cell wall-degrading enzymes, to induce resistance of host tissues, and to produce plant growth regulators indicates the potential to exploit them as biocontrol agents and plant growth promoters. More than ten genera of yeasts have been used to control postharvest diseases, especially of fruits. Suppression of classes of fungal pathogens of fruits and foliage that are similar to those associated with soil-borne fungal root pathogens, strongly suggests that yeasts also have potential for the biological control of diseases caused by soil-borne fungal plant pathogens, as is evident in reports of certain yeasts in suppressing some soil-borne fungal plant pathogens. This review explores the potential of soil yeasts to suppress a wider range of soil-borne fungal plant pathogens and to promote plant growth.

Key words Antagonism · Biological control · Endophytes · Plant growth regulators · Rhizosphere competence

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Introduction

Saprophytic yeasts are common occupants of leaf surfaces (Dickinson 1982; Andrews and Buck 2002; Buck 2002), bark (Buck et al. 1998), fruits (Wilson and Wisniewski 1989), flowers (Dickinson 1976), slime fluxes, necrotic tissues, tanning liquors of various plants (Spencer and Spencer 1997), and of soil and rhizosphere (Bab'eva and Belyanin 1966; Spencer and Spencer 1997; Slavikova and Vadkertiova 2003; El-Tarabily 2004).

The total soil yeast count is usually relatively low as compared with the numbers of bacteria and filamentous fungi (Phaff et al. 1978). Yeasts are common in soils of widely different texture, chemical composition, humidity, and pH values at various geographical locations and under diverse climatic conditions (Do Carmo Sousa 1969; Alexander 1977). They also dominate, albeit ephemerally, in soils subjected to fumigation (Sivasithamparam 1977). Orchard soils enriched by decaying windfalls may support populations of species of yeasts such as *Hanseniaspora* and *Kloeckera*. While many other yeast species are transient, residing temporarily in the soil, some yeasts are considered to be permanent residents in the soils (Phaff et al. 1978). Yeasts are particularly numerous on roots of certain plants such as cabbage, corn, sugar beet, and oat (Bab'eva and Belyanin 1966; Alexander 1977; Phaff et al. 1978).

Yeast populations are also affected by the depth where they occur in soil and are most numerous in the upper layers, from approximately 2 to 10 cm in depth (Phaff et al. 1978). The vertical distribution of yeasts in soil depends on such factors as compaction and porosity, rainfall, cultivation, burrowing animals, and the presence of soil-inhabiting insects that feed and breed on decomposing fruits (Phaff et al. 1978). The numbers of yeasts tend to be greater in summer (Spencer and Spencer 1997). The presence or absence of capsules on yeast species inhabiting soils, especially of the arid and semiarid types, may influence the ability of yeast cells to survive low moisture conditions (Spencer and Spencer 1997). The number of yeasts in soil depends greatly on the amount of available nutrients and is increased by the

addition of metabolizable substances. Most of the yeasts found in soil were nonfermentative (aerobic) species (Phaff and Starmer 1987).

Genera of soil yeasts most frequently isolated include *Candida*, *Cryptococcus*, *Debaryomyces*, *Hansenula*, *Lipomyces*, *Pichia*, *Aureobasidium*, *Rhodotorula*, *Saccharomyces*, *Schizoblastosporion*, *Sporobolomyces*, *Torulaspora*, *Torulopsis*, *Trichosporon*, *Kluyveromyces*, and *Zygosaccharomyces* (Alexander 1977; Spencer and Spencer 1997). Some of these genera, such as *Aureobasidium* and *Trichosporon*, can also have mycelial phases (Barnett et al. 1990).

Moawad et al. (1986) reported that population densities of yeast in soils correlated significantly with the organic carbon and organic nitrogen content of soils tested. di Menna (1962) concluded, from qualitative and quantitative surveys of the yeast flora of New Zealand soils, that yeast populations varied qualitatively from place to place with soil type and vegetation but not with season, whereas the density of yeast populations was different from place to place and also varied with season. Capriotti (1962) concluded from his qualitative studies on European and American soils that yeasts are more frequent in soils of warmer areas such as Italy and Spain than in soils of cold areas such as Holland, Sweden, and Finland.

A wide variety of yeast genera has been reported from the rhizosphere (Bab'eva and Belyanin 1966; Gomes et al. 2003) and to show a rhizosphere effect (Bab'eva and Belyanin 1966). Bab'eva and Belyanin (1966) found the colony-forming units of yeasts in the rhizosphere of cabbage, corn, sugar beet, and oats to be higher compared to normal bulk soils. Not only were the number of yeasts higher in the rhizosphere, but also the species compositions were more diverse (Bab'eva and Belyanin 1966; Moawad et al. 1986).

There is also some evidence that antibiotics produced by bacteria and actinomycetes may affect the distribution of yeast populations in soil (di Menna 1962). Falih and Wainwright (1995) suggested that soil yeasts might be used as inoculants to stimulate beneficial processes such as sulfur oxidation and phosphorus solubilization in soils. Soil amendments with the yeast *Yarrowia lipolytica* increased the native arbuscular mycorrhizal spore number and colonization in soil (Medina et al. 2004).

Endophytic yeasts have been isolated from inside living tissues of various plant species. The isolates obtained belong to the genera *Pichia*, *Rhodotorula*, *Cryptococcus*, and *Williopsis* (Nakamura et al. 1991; Larran et al. 2002; Nassar et al. 2005). Further work on the potential value of these endophytic yeasts in relation to biocontrol of soil-borne fungal plant pathogens is warranted and is addressed later in this review.

The main aim of this review is to present an overview on yeasts as potential biological control agents of soil-borne fungal plant pathogens and as promoters of plant growth. We do not attempt to review the extensive and exciting work that has been done on the biological control of postharvest diseases of fruits and vegetables (Wilson and Wisniewski 1989; Punja 1997; El Ghaouth et al. 2002), foliar diseases such as powdery mildews (Urquhart and Punja 2002), and the protection of stored grains from molding (Petersson et al. 1999; Druvefors et al. 2005). The successful use of yeasts in the biocontrol of postharvest and foliar diseases could be attributed to the fact that yeasts are a major component of the surfaces of leaves and other plant aerial parts of the plants (Wilson and Wisniewski 1989). In addition, they can be effective as biocontrol agents as they are naturally adapted to these niches and are able to rapidly and effectively colonize and compete for nutrients and space on aerial surfaces of plants (McLaughlin et al. 1990; Filonow 1998). Concerns regarding species or strains of yeasts hazardous to human health have been largely avoided by addressing strains with temperature optima well below 38°C (Smilanick 1994). Much of the research into the biological control of soil-borne fungal plant pathogens to date has concentrated on antagonists among bacteria, actinomycetes, and filamentous fungi. Relatively few studies have concentrated on the potential of yeasts as biological control agents of soil-borne fungal plant pathogens or for their potential as plant growth promoters and as biological fertilizers. The mechanisms of action of potential antagonism by yeasts in relation to soil-borne fungal plant pathogens, however, are expected to be similar to those involved in the control of postharvest and foliar diseases because many of the pathogens among soil-borne fungal plant pathogens belong to the same classes of fungi as those that occur on foliage, fruits, and other aerial parts of the plant.

Mechanisms of antagonism

The mechanisms of antagonism of yeasts involved in the biological control of fungal plant pathogens have been researched extensively in relation to pathogens associated with leaves (Fokkema et al. 1979; Sundheim 1986; Buck 2002; Urquhart and Punja 2002) and in relation to fruits (Wilson and Wisniewski 1989; Droby and Chalutz 1994; Filonow et al. 1996; El Ghaouth et al. 2002). These studies, although carried out in relation to pathogens of aerial parts, involved mechanisms that at functional levels are similar to those which occur in soil or crop residues or within seeds and roots and therefore should be relevant to the biological control of soil-borne fungal plant pathogens.

Mechanisms that have been reported to play a significant role in the biocontrol activity of these antagonistic yeasts against fungal pathogens of leaves and fruits include competition for nutrients and space (Fokkema 1984; Droby et al. 1989; Filonow 1998; Janisiewicz et al. 2000), production of cell wall-degrading enzymes such as β-1,3-glucanase and chitinase (Castoria et al. 2001; Masih and Paul 2002; Urquhart and Punja 2002), production of antifungal diffusible and volatile metabolites (Walker et al. 1995; Masih et al. 2001; Höfte et al. 2004), induction of host resistance (Wilson et al. 1994; Droby et al. 2002; El Ghaouth et al. 2003), and mycoparasitism (Wisniewski et al. 1991; Arras et al. 1998; El Ghaouth et al. 1998). The yeasts in such studies included the genera *Debaryomyces* (Santos et al.

2004), *Kloeckera* (McLaughlin et al. 1992), *Sporothrix* (Hajlaoui and Bèlanger 1993), *Saccharomyces*, *Zygosaccharomyces* (Suzzi et al. 1995), *Sporobolomyces* (Janisiewicz et al. 1994; Filonow 1998), *Metschnikowia* (Karabulut et al. 2004), *Tilletiopsis* (Ng et al. 1997), *Rhodotorula* (Lima et al. 1998; Sansone et al. 2005), *Cryptococcus* (Anderson et al. 1997; Lima et al. 1998), *Aureobasidium* (Castoria et al. 2001; Ippolito et al. 2005), *Pichia* (Masih and Paul 2002; Santos et al. 2004), and *Candida* (McLaughlin et al. 1992; Gamagae et al. 2004).

Understanding the modes of action of the antagonists among yeasts will help not only in the improvement of their performance resulting from the enhancement of their effectiveness as biocontrol agents but also in the development of criteria for rapid screening for superior biocontrol agents.

Competition for space and nutrients

Several investigations have been carried out in relation to competition for space and nutrition. Photographic evidence presented in certain studies (Mercier and Wilson 1994; Castoria et al. 1997; Arras et al. 1998) showed yeast colonies in close association with hyphae of fungal pathogens, indicating the attraction of these yeasts to the hyphal surfaces (hyphasphere) or to aggregates of mycelia where significant exudates or leakages from the filamentous pathogenic fungi could be expected to occur. This observation suggests not only the existence of chemoattraction of yeast cells towards the hyphae of fungal pathogens aggregated on surfaces of fruits, leaves, or roots, but that competition for space and nutrients is important.

Preemptive occupation by *Pichia guilliermondii* (Wisniewski et al. 1991) and *Candida oleophila* (Mercier and Wilson 1994) of fungal infection sites and the exclusion of the pathogens was proposed to be one of the mechanisms involved in their effectiveness for biocontrol of gray mold of apple caused by *Botrytis cinerea*. Nutrient competition has been studied extensively in the interaction models of *Rhodotorula glutinis* and *Cryptococcus laurentii* with *B. cinerea* or *Penicillium expansum* (Castoria et al. 1997), *Debaryomyces hansenii* with *Penicillium digitatum* (Droby et al. 1989), *C. laurentii* and *Sporobolomyces roseus* with *B. cinerea* (Filonow 1998), *Pichia guilliermondii* with *Penicillium italicum* (Arras et al. 1998), *C. oleophila* with *P. digitatum* (Brown et al. 2000), *Metschnikowia pulcherrima* with *B. cinerea* (Piano et al. 1997; Spadaro et al. 2002), and *Aureobasidium pullulans* with *B. cinerea*, *P. expansum*, *Rhizopus stolonifer*, or *Aspergillus niger* (Castoria et al. 2001). These studies established that competition for nutrients could be particularly relevant in relation to fruits where the presence of sugary exudates on the fruit surfaces are an attractive source of nutrients for both the pathogen and the yeast. Similarly, sugary root exudates probably enhance the antagonistic activities of yeasts in relation to their competence to colonize sugar beet roots (El-Tarabily 2004).

The rapid reproduction of yeasts helps them to outcompete fungal pathogens. For example, spore germination of *B. cinerea* was completely inhibited during cocultivation in vitro on a synthetic medium in the presence of cells of *M. pulcherrima*. However, culture filtrates and autoclaved suspensions of *M. pulcherrima* failed to reduce spore germination and were ineffective in reducing the extent of lesions on apple fruits caused by *B. cinerea*, *P. expansum*, or *Monilia* sp. This result suggests that living cells are necessary for effective biocontrol and that, in these instances, nutrient competition could be the main mechanism rather than antibiosis, despite the fact that this particular yeast was capable of producing antibiotics in vitro (Spadaro et al. 2002).

Antibiosis

Several reports on yeasts on fruits and leaf surfaces suggested the occurrence and the activity of antibiotics in the interaction with fungal pathogens. However, very few reports present details of the nature of the antibiotics produced, an exception being the heptadecenoic and methyl-heptadecenoic acids produced by the yeastlike fungus *Sporothrix flocculosa* (Choudhury et al. 1994; Benyagoub et al. 1996). Choudhury et al. (1994) isolated two novel secondary metabolites (4-methyl-7,11-heptadecadienal and 4-methyl-7,11-heptadecadienoic acid) with antimycotic and antibacterial activity from liquid cultures of *S. flocculosa* and *Sporothrix rugulosa*. Treatment of *B. cinerea* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* with the antibiotic produced by *S. flocculosa* greatly reduced spore germination and biomass production (Hajlaoui et al. 1994). Urquhart and Punja (2002) purified a fatty acid ester with antifungal activity from the yeastlike fungus *Tilletiopsis pallescens* and reported that a concentration of $130 \mu g\, ml^{-1}$ of the active fraction inhibited germ tube development of a powdery mildew fungus *Podosphaera xanthii*. Collapse of hyphae and conidiophores was also observed on mildewed leaves treated with the active fraction of the purified antifungal compound. The purified antifungal compound was also active against soil-borne fungal plant pathogens including *F. oxysporum*, *Phoma* sp., and *Pythium aphanidermatum* at a high concentration ($= 200 \mu g\, ml^{-1}$) (Urquhart and Punja 2002).

Many of the antibiosis studies involve either pairing of colonies on agar plates using different solid substrates and measuring inhibition zones (Suzzi et al. 1995; Walker et al. 1995; Spadaro et al. 2002), screening of the culture filtrates for inhibition of vegetative growth and spore germination of fungal pathogens in vitro, or utilizing artificially wounded fruits (Spadaro et al. 2002) or leaves (Masih et al. 2001) coinoculated with the antagonists and the pathogens.

Suzzi et al. (1995) reported that natural wine yeasts belonging to *Saccharomyces* and *Zygosaccharomyces* inhibited in vitro growth of soil-borne fungal plant pathogens including *Rhizoctonia fragariae*, *Sclerotinia sclerotiorum*, and *Macrophomina phaseolina*. Walker et al. (1995) also reported the potential ability of "killer yeasts" to inhibit strains of pathogenic fungi. These killer yeasts were shown to strongly inhibit the vegetative growth of *Heterobasidion*

annosum, *Rhizoctonia solani*, *Fusarium equiseti*, and a range of other plant pathogenic fungi. Although these studies were only carried out in vitro, there is a clear indication of the potential to use such yeasts for the biocontrol of soil-borne fungal plant pathogens, particularly as they have the potential to compete with other yeasts even in the soil environment. Ways to manipulate the environment to favor activity of yeasts on either root surfaces or seed surfaces need to be evaluated. For the biocontrol of soil-borne fungal plant pathogens, it may be necessary to identify and target soil-inhabiting yeasts with antagonistic characteristics, as such strains may have the best competency to be active in the soil, rhizosphere, and root cortical environments.

Masih et al. (2001) reported that when *B. cinerea*, the causal agent of gray mold of grapevine, was grown together with *Pichia membranifaciens* on the same agar plate, a small zone of inhibition appeared around the yeast inoculum. Hyphae developing in the vicinity of the inhibition zone failed to sporulate. However, when *B. cinerea* was grown together with *P. membranifaciens* in broth, the pathogen failed to germinate and to form colonies on fresh potato dextrose agar (PDA) plates, indicating a fungicidal effect. Microscopic examination of the fungus in contact with the antagonistic yeast showed extensive coagulation of its protoplasm, and many of the hyphal cells were observed to be devoid of contents (Masih et al. 2001). Payne et al. (2000) reported that *Saccharomyces cerevisiae* and *Debaryomyces* sp. significantly reduced radial growth of selected mold and stain fungi in vitro, solely through the liberation of volatile antifungal compounds.

Action of siderophores have been shown to be important in the biocontrol of fungal plant pathogens by bacteria (Buysens et al. 1996). Siderophores have also been reported to be produced by species of *Candida* (Ismail et al. 1985) and *Rhodotorula* (Calvente et al. 2001). Rhodotorulic acid, a siderophore produced by *Rhodotorula*, has shown ability to inhibit spore germination of various plant pathogens including *B. cinerea* (Calvente et al. 2001). On apple wounds, gray mold caused by *B. cinerea* was more effectively controlled by an antagonistic non-rhodotorulic acid-producing strain of *R. glutinis* in combination with rhodotorulic acid than by the antagonistic yeast alone (Sansone et al. 2005). It should be noted that these reports (Calvente et al. 2001; Sansone et al. 2005) only relate to strains of the yeast taxa that colonize aerial parts of the plant and not tested in soil or roots.

Cell wall-degrading enzymes and mycoparasitism

Although species of *Pichia anomala*, *P. membranifaciens*, *R. glutinis*, *C. laurentii*, *A. pullulans*, *Tilletiopsis albescens*, and *T. pallescens* have all been shown to produce β-1,3-glucanase, most of the tests have been conducted against fungal pathogens of either fruits or leaves; these include plant pathogens such as *B. cinerea*, *P. expansum*, *R. stolonifer*, *A. niger*, *Sphaerotheca fuliginea*, and *P. xanthii* (Urquhart et al. 1994; Castoria et al. 1997; Jijakli and Lepoivre 1998; Castoria et al. 2001; Masih and Paul 2002; Urquhart and Punja 2002).

Chitinase production has been also reported in cultures during the interaction of *A. pullulans* with *P. expansum* and also in apple wounds (Castoria et al. 2001), *T. albescens* and *T. pallescens* with *P. xanthii* (Urquhart and Punja 2002), and *Candida saitoana* with *B. cinerea* (El Ghaouth et al. 1998).

In relation to the lysis of cell walls of fungal plant pathogens, the β-1,3-glucanase produced by *P. anomala* has been studied, purified, and characterized on a medium supplemented with laminarin or *B. cinerea* cell wall fragments (Jijakli and Lepoivre 1998). The production of β-1,3-glucanase was found to be relatively higher when cell wall fragments of the pathogen were provided as the substrate for the induction of the enzymes in vitro (Jijakli and Lepoivre 1998). The purified β-1,3-glucanase from the culture filtrates of *P. anomala* showed, in vitro, a stronger inhibitory effect on germ tubes of *B. cinerea* than on conidial germination and caused physiological changes evident as leakage of cytoplasm and cell swelling (Jijakli and Lepoivre 1998). The enzyme was also detected on apples treated with *P. anomala*, and the addition of cell wall fragments of *B. cinerea* to a suspension of *P. anomala* stimulated both in situ β-1,3-glucanase activity and protective activity against *B. cinerea*, further strengthening the hypothesis that β-1,3-glucanase is one of the mechanisms of action involved in the suppression of *B. cinerea* by *P. anomala* (Jijakli and Lepoivre 1998). Castoria et al. (1997) and Masih and Paul (2002) also reported enhanced production of β-1,3-glucanase in the presence of cell walls of *B. cinerea* and *P. expansum*, indicating the induction and/or enhancement in the production of the cell wall-degrading enzymes by the yeasts. Masih and Paul (2002) also showed that β-1,3-glucanase produced by the yeast *P. membranifaciens* caused coagulation and leakage of the cytoplasm of *B. cinerea* hyphae. Clearly, the cell wall-degrading enzymes are involved in these interactions, and compared to the work carried out with soil-borne fungal plant pathogens, with fungal, bacterial, and actinomycete antagonists (Cook and Baker 1983; Whipps 2001), very few attempts (El-Mehalawy et al. 2004; El-Tarabily 2004) have been made to determine the involvement of cell wall-degrading enzymes produced by yeasts on soil-borne fungal plant pathogens either in vitro or in vivo.

Tenacious adhesion and attachment of living yeast cells to spores and hyphae of fungal pathogens were observed by many investigators (Wisniewski et al. 1991; Castoria et al. 1997; Cook et al. 1997; Arras et al. 1998; Spadaro et al. 2002), indicating a direct recognition and interaction between the yeast antagonists and pathogens. Rapid colonization of mycelia of *P. digitatum* by *Candida famata* with lytic and phagocytic activity against the hyphae has been reported by Arras (1996). Arras et al. (1998) reported the attachment of *P. guilliermondii* cells to the mycelium of *P. italicum* with subsequent degradation of the hyphal wall in proximity of the attached yeast cells. This finding may indicate growth of yeast at the expense of nutrients exuded by the hyphae, even though hyperparasitism by yeasts remains to be established. *B. cinerea* hyphae in close proximity to *C.*

saitoana exhibited severe cytological injury such as cell wall swelling and degeneration of protoplasm (El Ghaouth et al. 1998). The cell wall-degrading enzymes could also affect the integrity of cell walls of the fungal pathogen, allowing invasion by hyperparasites.

Induction of host resistance

Activation of host resistance has been reported following inoculation of fruits by antagonistic bacteria and yeasts (Wilson et al. 1994; Droby et al. 2002). This induced resistance has been related to the production of phenylalanine ammonia lyase (Droby et al. 2002), phytoalexins (Rodov et al. 1994; Arras 1996; Droby et al. 2002), peroxidases (Fajardo et al. 1998), and ethylene (Droby and Chalutz 1994; Droby et al. 2002) in plant tissues. *C. saitoana* induced chitinase activity and caused deposition of papillae on host cells in apple surface wounds (El Ghaouth et al. 1998). In apple wounds, *A. pullulans* caused transient increases in β-1,3-glucanase, chitinase, and peroxidase activities (Ippolito et al. 2000). The activation of host defenses against the invading pathogens in foliage and fruits reported to date is likely to also occur with root-infecting pathogens as well.

Biological control of soil-borne fungal plant pathogens using yeasts

Several reports on bacteria, actinomycetes, and filamentous fungi antagonistic to soil-borne fungal plant pathogens have been published (Cook and Baker 1983; Doumbou et al. 2001; Whipps 2001). There are, however, very few published reports on the use of yeasts as biocontrol agents of soil-borne fungal plant pathogens. Although a large array of yeast genera has been used to test the potential of yeasts for the biological control of postharvest diseases of fruits and vegetables (Wilson and Wisniewski 1989; Punja 1997; El Ghaouth et al. 2002; Janisiewicz and Korsten 2002), of molding of stored grains (Petersson et al. 1999; Druvefors et al. 2005), of wood-inhabiting fungi (Payne and Bruce 2001), and of foliar diseases such as powdery mildews (Urquhart and Punja 1997, 2002), only three reports exist in the literature in relation to the potential of yeasts to biologically control soil-borne fungal plant pathogens.

El-Tarabily (2004) reported that the application of three rhizosphere yeasts, namely *Candida valida*, *R. glutinis*, and *Trichosporon asahii* obtained from sugar beet rhizosphere, individually or in combination, significantly reduced postemergence damping-off of seedlings and crown and root rots of mature sugar beet caused by *R. solani* AG-2-2 under glasshouse conditions. These three yeasts showed different mechanisms of activity against *R. solani*. *C. valida* produced only β-1,3-glucanase with no evidence of chitinase, inhibitory volatile, or diffusible antifungal metabolites and degraded the hyphae of *R. solani* in vitro, causing hyphal plasmolysis and lysis of cell walls. *R. glutinis* produced only inhibitory volatiles, whereas *T. asahii* produced only diffusible antifungal metabolites, both inhibiting the vegetative growth of *R. solani* in vitro (El-Tarabily 2004). The specific mode of antagonistic activity of each of the three species indicates that individual species of the yeasts tested can, as with bacteria, actinomycetes, and filamentous fungi (Whipps 2001), employ different mechanisms to suppress a single pathogen. The three yeast species did not inhibit each other, and in fact a combination was the most effective treatment for protecting the sugar beet from diseases in the glasshouse trials (El-Tarabily 2004), demonstrating a synergistic effect among them. Whipps (2001) also reported that application of mixtures of antagonists enhanced biological control in comparison to individual candidates. Such mixtures increase the spectrum, efficacy, and reliability of the suppression of a disease without the need for genetic engineering (Janisiewicz 1996).

El-Mehalawy et al. (2004) reported that the application of *Candida glabrata*, *C. maltosa*, *C. slooffiae*, *Rhodotorula rubra*, and *Trichosporon cutaneum*, applied individually or as a mixture, significantly reduced the incidence of late-wilt disease of maize caused by *Cephalosporium maydis*. The mode of action of these yeasts, tested in vitro, was found to be the production of antifungal diffusible metabolites and cell wall-degrading enzymes, including chitinase and β-1,3-glucanase. El-Mehalawy (2004) also showed that the rhizosphere yeasts *Saccharomyces unispora* and *Candida steatolytica* significantly reduced the incidence of wilt disease of beans caused by *F. oxysporum* through the production of antifungal diffusible metabolites. Both reports concerned rhizosphere yeasts as biocontrol agents of soil-borne fungal plant pathogens and highlighted the activity of these soil yeasts in the rhizosphere, suggesting the importance of root exudates in their effectiveness as biocontrol agents.

Rhizosphere competence of yeasts

Biological control of soil-borne fungal plant pathogens requires biocontrol agents to protect seeds and roots of plants. Therefore, any agent introduced on seed needs to rapidly move to the roots, preferably before or as the roots become exposed to the pathogen. Rhizosphere competence confers on the antagonist a special ability to rapidly colonize plant roots (Ahmad and Baker 1987). Rhizosphere competence could be determined in two steps. Initially, an in vitro indicator root colonization plate assay (Kortemaa et al. 1994) could be carried out in a noncompetitive environment to determine whether the root could support the spread, survival, and activity of the yeast antagonists through its exudates. Plant species are known to produce various types and quantities of root exudates (Curl and Truelove 1986), which influence root colonization (Weller 1988). Promising isolates from this plate assay could then tested in a competitive environment to establish their competence in natural soils using the nonsterile sand tube method of Ahmad and Baker (1987).

In relation to yeasts, the value of rhizosphere competence has been emphasized in the work of El-Tarabily

(2004) where rhizosphere-competent yeasts were used to control *R. solani* diseases of sugar beet. In the root colonization plate assay, *C. valida* and *T. asahii* colonized 95% of roots by 6 days after radicle emergence, whereas *R. glutinis* colonized 90% of roots after 8 days. Root colonization abilities of the three yeast species tested by the sand-tube method showed that roots and soil particles attached to roots of 21-day-old sugar beet seedlings were colonized to different degrees by the three yeast species. Population densities showed that although the three species were found at all depths of the rhizosphere soil adhering to roots, population densities were significantly greater in the first 4 cm of the root system. Colonization frequency of the root segments and the rhizosphere soil was greater in plants treated with *C. valida* or *T. asahii* than with *R. glutinis*.

Although rhizosphere competence has been used as a criterion for selection of effective biocontrol agents among bacteria (Weller 1988), streptomycete actinomycetes (Kortemaa et al. 1994; Tokala et al. 2002), and fungi (Ahmad and Baker 1987; Al-Rawahi and Hancock 1997), it is noteworthy that very few attempts, with the exception of El-Mehalawy et al. (2004) and El-Tarabily (2004), have been made to screen yeasts for rhizosphere competence. Rhizosphere competence should be considered as a prerequisite for successful biological control of root diseases, and lack of reliable biological control observed in many studies may be related to the failure to adequately colonize roots (Weller 1988). Application methods for rhizosphere yeasts must take into consideration the need for the antagonists to be competent saprophytes both in soil and in the rhizosphere.

Methods of application and food base

As most yeasts are single-celled organisms and the nature of their reproduction is similar to that of many bacteria, production of biocontrol agents, or plant growth promoters, harvesting and preparation for material for field application could utilize methods similar to those used for bacteria and for single-celled actinomycetes. However, the methods that are commonly used for the preparation and application of inocula for filamentous fungi may not be suitable for yeasts, despite the fact that yeasts are also fungi.

As very few studies have involved biological control using yeasts, comparison cannot be made on various methods that are available for application specifically of yeasts. Food base or substrates added to seed as a coating or incorporated into soil with the antagonists should help plant growth promoters and biocontrol agents to survive and proliferate in the spermosphere and provide or supplement nutrients (Hoitink and Boehm 1999), even in the rhizosphere, for their biological activities.

In the work of El-Tarabily (2004), soybean bran was used as a food base. Its high nutrient content was expected to help in the vegetative growth, reproduction, and survival of the introduced yeasts. Amendment of soil with two microbiologically treated agricultural wastes (dry olive cake or sugar beet wastes) was used by Medina et al. (2004) in their work with the plant growth-promoting yeast *Y. lipolytica*. They reported that plant growth and nutrition and soil enzymatic activities were limited in nonamended soil where inoculations by yeast did not improve plant development. The effectiveness and performance of yeast inoculum was evident only in soil amended with food substrate (Medina et al. 2004).

As with certain other antagonists of soil-borne fungal plant pathogens (Cook and Baker 1983), the antagonists may be required to be established in the soil environment to be effective. El-Tarabily (2004) preincubated the yeast inoculum in the soil for 2 weeks before the addition of the pathogen during glasshouse studies. This step was expected to help the antagonists to establish in the soil before the exposure of the plant to the pathogen.

Endophytic yeasts

Recent work has established a wide variety of microorganisms to be endophytic in plant tissues (Hallmann et al. 1997; Stone et al. 2000; Sturz et al. 2000). Endophytic microorganisms have been defined as those that reside at some phases of their life cycle within living plant tissues without causing apparent damage to them (Petrini 1991) or which can be isolated or extracted from surface-disinfested plant material but do not visibly harm the plant (Hallmann et al. 1997).

Several recent studies have shown that the interaction between plants and certain endophytic microorganisms was associated with beneficial effects such as biological control of soil-borne fungal plant pathogens and plant growth promotion (Hallmann et al. 1997; Stone et al. 2000; Sturz et al. 2000; Narisawa et al. 2004). On the other hand, many endophytic microorganisms have failed to show any beneficial effects on the inoculated host plant (Sturz et al. 2000).

In addition to the yeasts present in the soil, rhizosphere, or on aerial plant parts (e.g., flowers, fruits, leaves, bark), there are significant numbers of endophytic yeasts present inside live plant tissues. Although endophytic yeasts isolated from inside live tissues of various plant species including the cordgrass *Spartina alterniflora* (*Pichia spartinae*) (Meyers et al. 1975), sugarcane leaves and stems (*Cryptococcus*, *Rhodotorula*, and *Debaryomyces* sp.) (Azeredo et al. 1998), tomato leaves (*Rhodotorula* sp.) (Larran et al. 2001), wheat leaves (*R. rubra* and *Cryptococcus* sp.) (Larran et al. 2002), banana roots (unidentified yeasts) (Cao et al. 2002), *Acrostichum aureum* rhizomes (unidentified yeasts) (Maria and Sridhar 2003), rice leaves (unidentified yeasts) (Tian et al. 2004), and tissue cultures of various plants (Bunn and Tan 2002) have been reported, they were not tested for their potential as biocontrol agents of soil-borne fungal plant pathogens or as plant growth promoters. Although endophytic yeasts have been shown to promote maize growth under gnotobiotic and glasshouse conditions (Nassar et al. 2005), no attempts to date have been made to use endophytic yeasts to protect plants from different soil-borne fungal plant pathogens.

The application of antagonistic endophytic yeasts as biocontrol agents or as plant growth promoters provides them with an advantage in the root region. In the rhizosphere, yeasts have to compete with other microbial occupants of the rhizosphere and therefore need to be applied in numbers large enough to compensate for this competition. Microbial endophytes, in contrast, are able to occupy the cortical tissues of roots where they can be very effective in the defenses against infection processes of invading pathogens (Sivasithamparam 1998). In addition, the cortex or the root tissues occupied by the antagonistic and plant growth-promoting endophytes clearly confer protection to these antagonists from the harsh environment of the bulk soil and the rhizosphere, where not only the biotic but also the abiotic environment can be inhospitable (Sivasithamparam 2002).

Plant growth-promoting yeasts and production of plant growth regulators

The role of rhizosphere microorganisms in the promotion of plant growth has received considerable attention (Glick 1995; Bashan et al. 2004). Plant growth-promoting rhizobacteria (PGPR) can affect plant growth directly or indirectly. Indirect effects are those related to the production of metabolites, such as siderophores (Buysens et al. 1996), antibiotics (Folman et al. 2004), which increase plant growth by decreasing the activities of pathogens or deleterious microorganisms. Direct effects reported include nitrogen fixation (Cocking 2003), production of plant growth regulators (PGRs) such as auxins, gibberellins, and cytokinins that directly promote plant growth (Bottini et al. 2004; Kuklinsky-Sobral et al. 2004), and by the enhancement of plant nutrient uptake (Glick 1995).

Although soil or rhizosphere filamentous fungi (Ousley et al. 1994; Wakelin et al. 2004) and bacteria including actinomycetes (Glick 1995; Doumbou et al. 2001; Bottini et al. 2004; Kuklinsky-Sobral et al. 2004) have been used to enhance plant growth, relatively few attempts have been made to use rhizosphere or soil yeasts as plant growth promoters. Bab'eva and Belyanin (1966) reported that germination of cabbage seeds was stimulated when they were soaked in the culture filtrates of nine strains of *Torulopsis* sp. isolated from cabbage rhizosphere. The application of *S. roseus* was reported by Perondi et al. (1996) to promote wheat yield by 16%–30%, and Abd El-Hafez and Shehata (2001) reported that a strain of *Rhodotorula* sp. was capable of increasing tomato growth and fruit yield. Soil inoculation with *C. valida*, *R. glutinis*, and *T. asahii* applied singly or in combination (El-Tarabily 2004) has been reported to promote sugar beet growth. It is interesting that the three yeast species reported by El-Tarabily (2004) were able to produce the highest level of growth promotion when they were combined together in glasshouse trials in the absence or presence of *R. solani*, possibly through a synergetic effect. El-Tarabily (2004) also showed that the presence of *R. solani* did not diminish the growth promotion effect evident in the plants exposed only to the yeast isolates. Inoculation with the yeasts has resulted in enhanced root and shoot production through the activity of indole-3-acetic acid (IAA), gibberellins (GA3), and possibly other PGRs, in addition to the potential of this promotion to mask or compensate for the damage caused by *R. solani* (El-Tarabily 2004).

The biological activities of the antagonistic yeasts could be independent of the activities of the pathogen. The yeasts could preemptively colonize the rhizosphere and root and may not have to directly compete for the same sites with the pathogen. There may also be involvement of induced resistance in the host following inoculation with yeasts. Many biocontrol agents suppressing soil-borne fungal plant pathogens have been recorded to promote plant growth in the presence or absence of a pathogen (El-Tarabily et al. 1997).

In a study by El-Mehalawy et al. (2004), the application of *C. glabrata*, *C. maltosa*, *C. slooffiae*, *R. rubra*, and *T. cutaneum*, when applied individually or in combination, significantly increased the growth of maize in the absence of *C. maydis*. El-Mehalawy (2004) also reported growth promotion of beans following the application of *S. unispora* and *C. steatolytica* in the absence of *F. oxysporum*. Medina et al. (2004) reported that the plant growth-promoting yeast *Y. lipolytica* increased plant phosphorus acquisition and promoted the growth of *Dorycnium pentaphyllum* (a legume) in a semiarid soil. Unfortunately, none of the studies involved assays to detect PGRs in tissues of plants treated with these yeasts.

There is at present considerable interest in the introduction or manipulation of endophytic microorganisms to increase the productivity of crops (Sturz et al. 2000). Although growth promotion by endophytic bacteria (Sturz et al. 2000; Bacon and Hinton 2002) and endophytic filamentous fungi (Sivasithamparam 1998; Mucciarelli et al. 2003) has been reported, only one report by Nassar et al. (2005) exists in the literature on the use of an endophytic yeast as a plant growth promoter. In their study, Nassar et al. (2005) found that an isolate of the yeast *Williopsis saturnus* endophytic in maize roots was capable of producing IAA and indole-3-pyruvic acid (IPYA) in vitro in a chemically defined medium amended with L-tryptophan (L-TRP) as a precursor for auxins. The introduction of *W. saturnus* to maize seedlings by the pruned root dip method significantly enhanced the growth of maize plants grown under gnotobiotic and glasshouse conditions in a soil amended with or without L-TRP (Nassar et al. 2005). This enhancement was evident from the increases in the dry weights and lengths of roots and shoots, and also from the significant increases in the levels of in planta IAA and IPYA compared with control plants grown in L-TRP-amended or nonamended soil. The plant growth promotion by *W. saturnus* was most pronounced in the presence of L-TRP as a soil amendment compared to seedlings inoculated with *W. saturnus* and grown in soil not amended with L-TRP (Nassar et al. 2005). The relevance of IAA and IPYA in their study was critical, as evidenced when an endophytic isolate of *R. glutinis* that was incapable of producing detect-

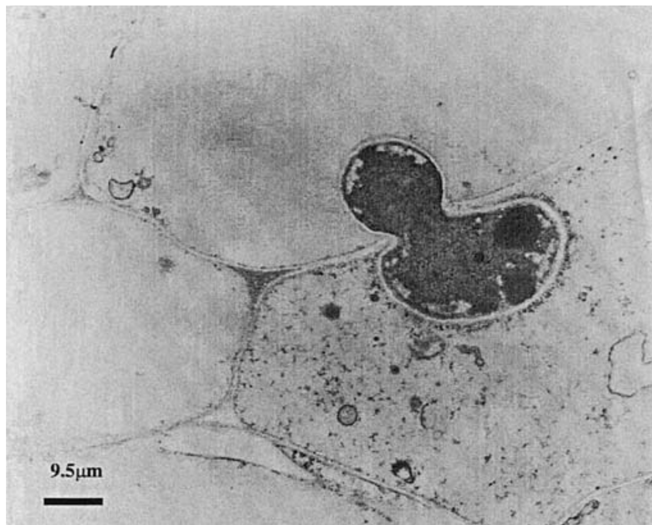

Fig. 1. Transmission electron micrograph of ultrathin sections of 2-week-old maize root inoculated with *Williopsis saturnus* showing penetration of a neighboring root cortical cell by a budding yeast cell. *Bar* 9.5 μm

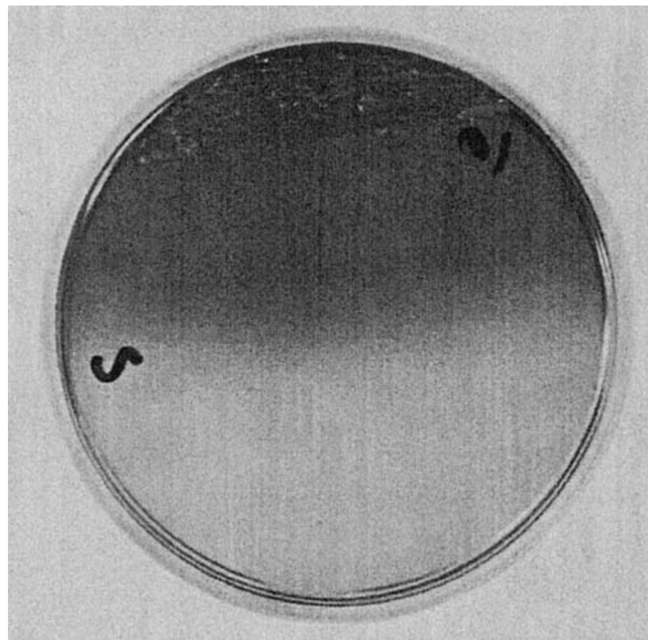

Fig. 2. Production of putrescine by *Williopsis saturnus* on Moeller's decarboxylase agar medium supplemented with L-arginine and phenol red as the pH indicator (Arena and Manca de Nadra 2001), 4 days after inoculation and incubation at 28°C. Note the change of phenol red indicator (evident as darkening of the agar around the yeast colony) due to the production of putrescine

able levels of IAA or IPYA failed to either increase the endogenous levels of IAA and IPYA or promote plant growth despite the extent of colonization of maize root tissues by *R. glutinis* being similar to that of *W. saturnus* (Nassar et al. 2005). Endophytic yeasts as natural residents of young root cortices (Fig. 1) are clearly more suited for plant growth-promoting activities compared to rhizosphere microflora.

Several reports exist detailing the production of PGRs such as auxins and gibberellins by yeasts in vitro. IAA and GA3 produced in vitro by yeasts have been reported to enhance growth and improve yields of host plants (El-Tarabily 2004). Yeasts have been reported to produce PGRs such as ethylene (Lynch 1972), GA_3 (Krassilnikov 1963; El-Tarabily 2004), IAA (Nakamura et al. 1991; Tuomi et al. 1993; El-Tarabily 2004; Nassar et al. 2005), and IPYA (Nassar et al. 2005). The reports by Krassilnikov (1963), Nakamura et al. (1991), and Tuomi et al. (1993) dealt only with the production of PGRs in vitro with no attempts to evaluate these yeast isolates for plant growth promotion under glasshouse or field conditions.

Polyamines such as putrescine, spermidine, and spermine have been implicated to play vital roles as modulators in a variety of growth, physiological, and developmental processes in higher plants (Galston and Kaur-Sawhney 1990). Polyamines are not considered to be plant hormones because they are very abundant, but they could be considered to be PGRs (Evans and Malmberg 1989). It has been reported that *Streptomyces griseoluteus* has been shown to promote bean growth through the production of polyamines (Nassar et al. 2003). Infestation of soil with *S. griseoluteus* resulted in a significant increase in the levels of endogenous putrescine, spermidine, and spermine and certain endogenous PGRs including IAA and GA_3. Polyamines were established to be the main cause of plant growth promotion because a polyamine non-producing mutant strain obtained from the wild-type isolate failed to produce polyamines and also failed to promote plant growth (Nassar et al. 2003). El-Tarabily and Sivasithamparam (unpublished data) have also found that an endophytic yeast isolate of *W. saturnus* promoting growth of maize was also capable of producing polyamines (Fig. 2). We are currently screening for strong polyamine production as a criterion for the selection of plant growth-promoting yeasts.

Conclusion

Considerable success has been achieved in the use of yeasts for the biocontrol of fungi involved in the postharvest diseases of plant products, especially fruits. Relatively few attempts have been made to evaluate yeasts as plant growth promoters and even fewer as biocontrol agents for the management of soil-borne fungal plant pathogens. Yeasts have shown promise as producers of PGRs, promoting plant growth, and in addition some are capable of being endophytic in plant roots. Characteristics of many of the isolates evaluated to date indicate the significant potential of yeasts as agents both for biocontrol of root diseases and for promotion of plant growth.

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