International Journal of Food Microbiology 406 (2023) 110360

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Review

Identification of antagonistic yeasts as potential biocontrol agents: Diverse criteria and strategies

M. Sipiczki

Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary

ARTICLE INFO

Keywords:
Antimicrobial antagonism
Yeasts
Biocontrol
Bioprotection
VOC-mediated antagonism

ABSTRACT

Plant pathogenic and food spoilage microorganisms cause serious losses in crop production and severe damage during food manufacturing, transportation and storage. Synthetic antimicrobial agents are commonly used to control their propagation and harmful activities. However, the recent trend is shifting from chemicals towards safer and more eco-friendly alternatives. The use of antagonistic microorganisms as biological antimicrobial agents is becoming popular throughout the world to replace chemical agents. High numbers of microorganisms have turned out to exert adverse/inhibitory effects on other microorganisms including pathogens and spoiling strains. However, most of them are only active under laboratory conditions and their activity is sensitive to environmental changes. Only a small number of them can be used to manufacture biological protective products on an industrial scale. Therefore, there is a great need to identify additional antagonists. Yeasts have come to the forefront of attention because antimicrobial antagonism is fairly widespread among them. In the recent years, numerous excellent review articles covered various aspects of the phenomenon of antimicrobial antagonism of yeasts. However, none of them dealt with how antagonistic yeasts can be sought and identified, despite the high number and diverse efficiency of screening and identification procedures. As researchers working in different laboratories use different criteria and different experimental set-ups, a yeast strain found antagonistic in one laboratory may prove to be non-antagonistic in another laboratory. This review aims to provide a comprehensive and partially critical overview of the wide diversity of identification criteria and procedures to help researchers choose appropriate screening and identification strategies.

1. Introduction

Microorganisms sharing the same niche interact with each other as animals and plants. The interaction between two microorganisms can be synergistic (mutually beneficial) or antagonistic (beneficial for one partner but harmful for the other). Yeasts can antagonistically interact with other microorganisms by a plethora of mechanisms. The most common modes are competition for nutrients (yeast cells utilise, consume a particular nutrient faster than the other microorganism), physical (contact) inhibition (e.g. the dimorphic yeast forms a dense mycelium or pseudomycelium and biofilm that halts the growth of the other microorganism), inhibition by the secretion of cell-wall lytic enzymes, killer factors or agents that immobilise certain nutrients (e.g. pulcherriminic acid), by releasing volatile organic compounds, etc. Numerous excellent review articles have been published in the recent years that covered various aspects of the phenomenon of antimicrobial antagonism of yeasts (e.g. Mukherjee et al., 2020; Zhang et al., 2020; Di Canito et al., 2021; Gil-Rodríguez and Garcia-Gutierrez, 2021; Hernandez-Montiel et al., 2021; Reyes-Bravo et al., 2021; Gianvito et al., 2022; Li et al., 2022; Ma et al., 2023). Some reviews provided more or less comprehensive overviews of the mechanisms of antagonism, others focused on specific groups of antagonistic yeasts, and still others surveyed the experience gained during the application of antagonistic yeasts to the biological protection of certain commodities and plants. However, none of them dealt with how antagonistic yeasts can be sought and identified, despite the fact that many procedures of various specificities and sensitivities have been developed and many procedures lead to inconsistent results. A yeast strain found antagonistic in one test may prove to be non-antagonistic in other tests. Since published studies frequently used different identification and test methods, it is difficult to compare their results. Researchers of different laboratories often worked with different strains of the same species, different types of culture media, various substrates and also different culture modalities. This review aims to provide a comprehensive and partially critical overview of the wide diversity of identification procedures. Such an overview can be very useful for researchers at selecting appropriate procedures to

E-mail address: gecela@post.sk.

https://doi.org/10.1016/j.ijfoodmicro.2023.110360
Received 21 June 2023; Received in revised form 30 July 2023; Accepted 7 August 2023
Available online 10 August 2023

identify and test novel antagonistic yeasts that meet the new (and ever-changing) expectations of food industry and agriculture for biological control agents suitable for replacing chemicals in protecting plants and food from harmful microorganisms.

2. Terminology and article structure

In the procedures covered by this review, yeasts are tested for antagonistic activities by the examination of their effect(s) on other microorganism(s). If the effect is adverse (e.g. causing cell death, reduced spore germination or growth rate), the yeast is considered antagonistic, at least against the organisms involved in the examination. As the response of the latter organisms indicates whether the yeast has or does not have an antagonistic effect, they will be referred to as "indicator organisms" throughout this article. Alternatively, they can be called test organisms or testers. However, the term "target organisms", which is quite commonly used in the literature, is less appropriate because it assumes that they will be attacked by the yeast which, however, may not take place.

For the sake of easier orientation, the presented procedures are grouped in the following major categories: tests on agar plates, tests in liquid media, tests on natural substrates, microscopic methods, tests for VOCs production and combination of methods.

3. Plate assays (tests on agar plates)

In these methods, the yeast is tested for antagonism on laboratory culture media solidified with agar. The composition of the medium is adapted to the nutritional demands of the yeast and the indicator organism.

3.1. Yeast on indicator (YOI)

The yeast is tested for antagonism by inoculating it onto an agar (test) plate spread by cells (or conidia) of the indicator organism. If it has an inhibitory (antagonistic) effect, a halo of no-growth or reduced growth will be seen in the lawn of the indicator around the yeast colony (Fig. 1A and B).

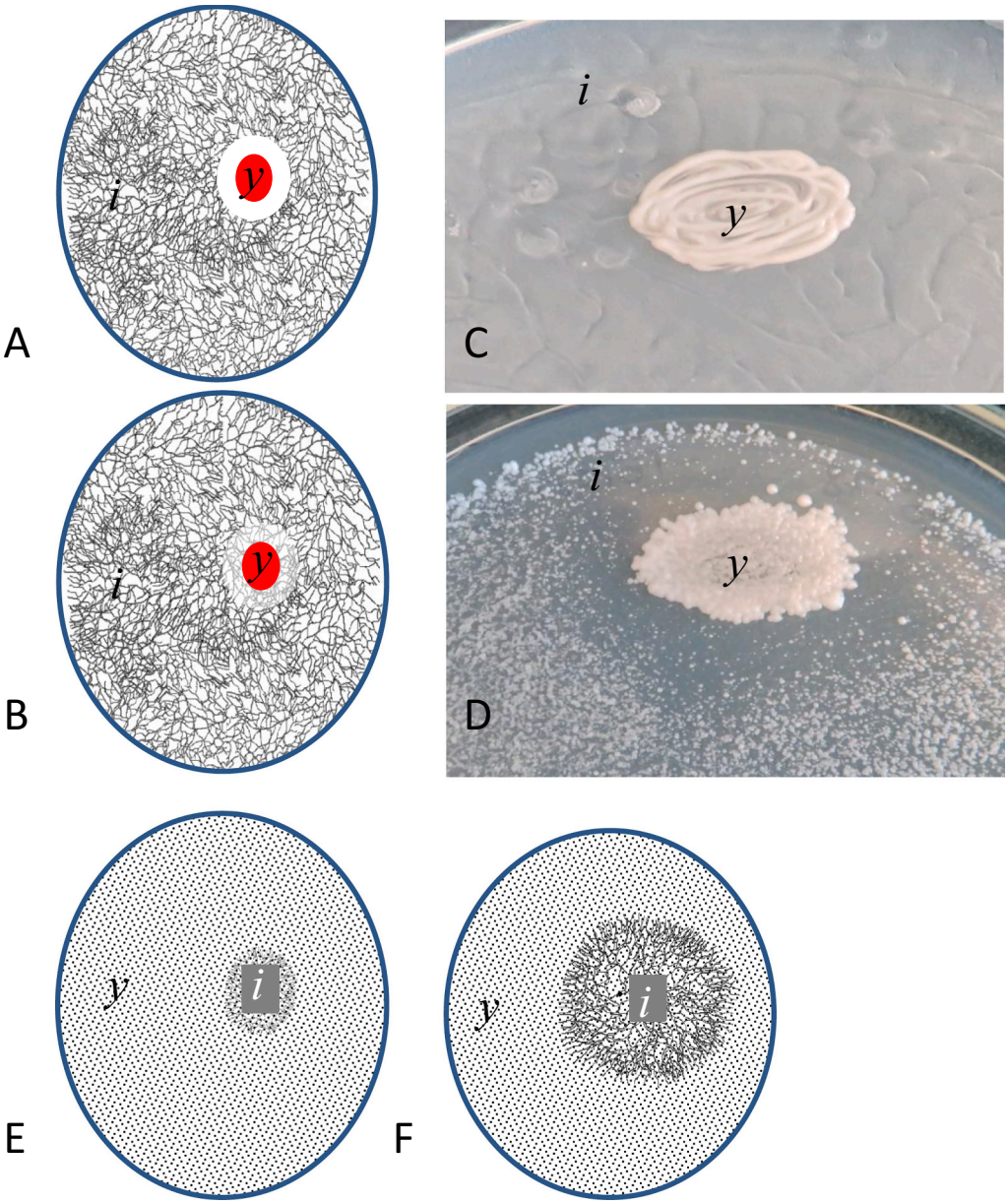

Fig. 1. Plate assays. A and B. Yeast on indicator (YOI): Inhibition zone around the yeast colony (A), a halo of reduced growth rate in the indicator lawn around the yeast colony (B). C and D. Detection of cell killing effect by replica-plating: test plate before replica-plating (C) and its replicated copy (D). E and F. Indicator on yeast (IOY): the yeast inhibits the growth of indicator (E), the yeast lawn does not inhibit the growth of indicator (F). Tested yeast is marked with *y*; indicator organism is marked with *i*.

The test plate can be prepared by (a) mixing the indicator organism (cells, spores or conidia) into molten agar medium before pouring it into the Petri dish (e.g. Björnberg and Schnürer, 1993; Hodgson et al., 1995; Goerges et al., 2006; Andrade et al., 2014; Mehlomakulu et al., 2014; de Melo Pereira et al., 2016; Acuña-Fontecilla et al., 2017; Peña and Ganga, 2019; Delali et al., 2021; Iacumin et al., 2017) or by (b) pouring the medium into the dish and flooding it with a suspension of the indicator organism afterwards (e.g. Bilinski et al., 1985; Comitini et al., 2004; Zheng et al., 2004; Sipiczki, 2006; Nally et al., 2015; Núñez et al., 2015; Ruiz-Moyano et al., 2016; Younis et al., 2017; da Cunha et al., 2018; Sipiczki and Selim, 2019; Pawlikowska et al., 2019; Csoma et al., 2020; Hicks et al., 2021; Yang et al., 2021; Staneviciene et al., 2021). If the suspension is dense enough, the cells/hyphae of the indicator will form a continuous lawn on the surface of the medium. A lawn can also be created by pouring a thin layer of molten agar medium supplemented with indicator cells (over-layer) onto the plate (Iacumin et al., 2020).

The yeast that is tested for antagonistic activities is then inoculated onto the plate of the indicator organism. It can be done in two ways: (a) either by filling a suspension of its cells into a well cut in the agar plate (b) or by inoculating it on the surface of the plate. The latter can be done in numerous ways: e.g. by smearing large amount of cells with a loop, dropping an aliquot of dense suspension of cells, placing a filter-paper disc soaked with the cell suspension or a nitrocellulose membrane on which the yeast is inoculated. In the latter case, the inhibition becomes visible in/on the plate after the removal of the membrane (provided the membrane is penetrable for the inhibitor).

When the growth requirements of the yeast differ from those of the indicator organism, a well is cut into the plate inoculated with the indicator organism. The well is then filled with a medium optimal for the yeast. For example when a yeast strain is tested for antibacterial antagonism, the test plate can be LB agar and the medium filled in the well can be a standard yeast-extract-glucose agar. The yeast is then inoculated on the top of the filled well or is mixed into the filling medium prior to being filled into the well. Alternatively, an agar block is placed on the test plate which is cut from a plate on which the yeast grew well (e.g. Roostita et al., 2011; Rajkowska et al., 2012).

If the yeast releases the inhibitor before the indicator organism starts growing, a clear inhibition zone with sharp edge will be seen around its colony upon incubation. However, when the indicator organism grows fast or the antagonist exerts its activity slowly, a turbid zone of poorer growth with blurred edge is formed.

Lack of a visible inhibition zone does not necessarily mean that the yeast has no antagonistic effect on the indicator organism. It can be due to its slow growth or delayed exertion of inhibitory effect. By the time the yeast begins to exert its antagonistic effect, the indicator microorganism overgrows the medium. This delayed effect can be visualised in two ways: (a) by adding methylene blue to the medium (e.g. Bilinski et al., 1985; Ferraz et al., 2016; Chen and Chou, 2017) or (b) by replica-plating the test plate onto a fresh plate. In the former case, a blue ring is formed around the colony because the dying cells of the indicator absorb the dye and turn blue. Living cells also uptake the dye, but the active enzymes within their cytoplasm process (reduce) it into a colourless form. This simple assay is widely used in the identification of killer strains although it is not specific of killer-factor mediated cell killing. Ignoring this fact can lead to the false conclusion that the yeast strain is a killer even if it does not produce a killer factor. In the latter case, a halo of poor or no growth will be visible in the lawn of the indicator around the print of the yeast colony because the indicator cells killed by the yeast colony do not grow (Fig. 1C and D).

3.2. Indicator on yeast (IOY)

This method is the inverse of YOI. Here the test plate is inoculated massively with the yeast, and the indicator is spotted on it (Fig. 1E and F). The antagonistic effect of the yeast is manifested by no or reduced growth of the indicator colony.

As in the case of YOI, the test plate can be prepared in two ways: (a) by mixing the yeast cells into the agar medium before poring it into the Petri dish (e.g. Petersson and Schnürer, 1995; Masoud and Kaltoft, 2006; Bleve et al., 2006; Virgili et al., 2012; Ruiz-Moyano et al., 2016; Piasecka-Jozwiak and Chabłowska, 2017; Peromingo et al., 2019) or (b) by pouring the medium into the dish and flooding it with a suspension of the yeast cells after it has solidified (e.g. MacWilliam, 1959; Patiño-Vera et al., 2005; Zheng et al., 2005; Wang et al., 2008; Mohamed and Saad, 2009; Wang et al., 2010; Hernández-Montiel et al., 2010; Medina-Córdova et al., 2016; Núñez et al., 2015; Hilber-Bodmer et al., 2017; Shen et al., 2019; Meftah et al., 2018; Tryfinopoulou et al., 2020; Fernandez-San Millan et al., 2021; Zou et al., 2021).

The surface of the yeast plate can be inoculated with the indicator organism in numerous ways: e.g. by placing an agar block cut from a colony, or by methods similar to those used for the inoculation of yeasts on the target organism in YOL (smearing large amount of cells or conidia with a loop, dropping dense suspension of cells or conidia or placing a filter-paper disc soaked with the suspension of conidia). If the yeast has antagonistic effect against the indicator, the latter either does not grow or forms a smaller colony than on the yeast-free control plate. The extent of inhibition is usually determined as the percentage of colony diameter decrease compared to the control. Therefore this method can only be used for the detection of antagonistic effect against indicator organisms that form continuously growing colonies (e.g. mycelium) on solid media.

This method can lead to false conclusions because the lower extension rate of the mycelium is not necessarily due to antagonism. It can be caused by extensive nutrient depletion in the plate by the yeast cells. The likelihood of this problem can be reduced by inoculating the plate with a low density suspension of yeast cells that form detached individual colonies. This version is also called binary competition assay (Hilber-Bodmer et al., 2017).

3.3. Grid assay by replica-plating (GARP)

The yeast strains to be tested for antagonistic interactions and the indicator organisms are streaked on agar plates (optimally two or three parallel lines on one plate) and incubated until thick line-shaped cultures are formed. These cultures are then replica-plated onto fresh plates perpendicularly to each other to produce grids of prints in which the strains intersect each other (Fig. 2). If a yeast line intersects a sensitive indicator, it inhibits the growth of the latter in the vicinity of the intersection. If the inhibition is ambiguous, it is recommended to replica-plate a copy of the grid to another (third) plate where the dead cells of the indicator will not propagate. This method is not suitable for testing yeast strains for antagonism with mycelium-forming indicators.

3.4. Cellophane-agar-layer assay (CALA) and filter-agar-layer assay (FALA)

These methods are modified versions of IOY. The yeast cells are not spread directly on the plate but onto a sterile cellophane sheet or a sterile bacteriological filter disc (routinely used for filter-sterilisation) laid on the plate. After several days of incubation the sheet/disc with the yeast culture is removed and the indicator microorganism is inoculated onto the plate either by spreading its cells/conidia or by placing a block cut from a mould colony on the site of the removed yeast culture. The lower growth rate of the indicator organism on this plate relative to that on the control plate (no yeasts were spread on the disc) is interpreted as an evidence of antagonism (e.g. Marsico et al., 2021; Hassan et al., 2021; Hatoum et al., 2013). Although the discs are permeable for compounds in both directions, the reduced growth rate is not necessarily due to an antagonistic substance. Since the organism growing on the disc depletes the medium of most nutrients, the lower growth rate of the indicator organism can simply be due to shortage of basic nutrients rather than to an inhibitor. When cellophane is to be used, it is advisable

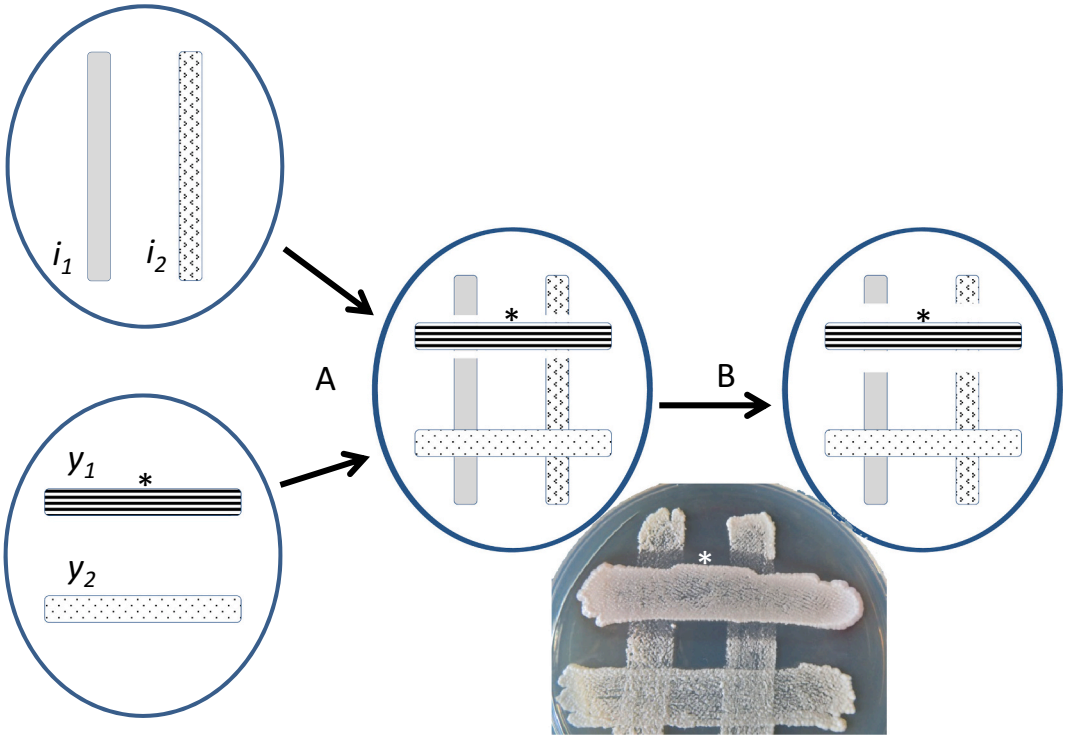

Fig. 2. Grid assay by replica-plating (GARP). A. Creation of a grid by replica-plating of the indicator organisms i_1 and i_2 and the yeasts y_1 and y_2. B. Visualisation of cell killing at the edge of the inhibition zones. Star: antagonistic yeast.

to take into account that different brands are differently penetrable/permeable.

3.5. Agar well assay (AWA)

This method is used for testing the yeast strain for antagonising the germination of conidia in mixed suspensions (e.g. He et al., 2003; Andrade et al., 2014; Chanchaichaovivat et al., 2007; Li et al., 2011; Mari et al., 2012; Hassan et al., 2021; Sabaghian et al., 2021; Lanhuang et al., 2022). Discs are cut and removed from the agar plates to form wells and the wells are half-filled with the suspension of conidia of the indicator fungus. On the test plate, equal volume of yeast suspension is also filled in the well. On the control plate no yeast suspension is added into the well. On the latter plates the conidia germinate and a gradually extending mould colony is formed on the agar medium around the well. On the test plate either no indicator colony is formed (the yeast inhibits the germination of the conidia) or the colony formation is delayed and/or the growth of the colony is slow compared to the control (incomplete antagonism).

3.6. Culture filtrate assay in agar medium (CFAA)

The antagonistic potential of the yeast strain can be examined by testing its cell-free culture medium for an adverse effect on the growth of the indicator organism on/in an agar medium (e.g. da Cunha et al., 2018; Hatoum et al., 2013; Al-Dulaimi et al., 2020). The yeast cells are cultivated in a liquid medium till stationary phase or longer to allow them to release metabolites, including those of antagonistic activity, into the medium. The cells are then separated from the medium by centrifugation and the supernatant is sterilised by filtration. The cell-free filtrate can be tested in two ways. (a) An aliquot is added to molten agar medium which is then poured into a Petri dish. After solidification, an agar block cut from the colony of the indicator organism is placed on the plate. The rate of the extension of the colony growing from the block is compared to that of the colony growing on the control plate (containing no filtrate). (b) The other possibility is to fill the aliquot into a well cut into a plate containing the cells/conidia/spores of the indicator organism. If the filtrate has antagonistic activity, an inhibition zone (either clear or turbid) is formed around the well.

3.7. Competition assay by co-culturing on agar medium (CACA)

With CACA, the yeast can be tested for antagonism against surface-colonising micro-organisms. For example against bacteria that grow on the surface of meat products or cheese (e.g. Goerges et al., 2006). Mixed suspensions containing cells of the yeast and the indicator microorganism in various proportions are prepared and samples are spread onto the agar medium. After incubation at a temperature optimal for the indicator organism (for a period of time depending on the growth rate of the indicator organism; shorter for bacteria longer for fungi), blocks are cut from the medium and the cell lawn is washed from them in fixed volumes of a sterile liquid medium. The resulting suspension is serially diluted and appropriate dilutions are spread onto agar media, on which both organisms can produce colonies. A control is set up with the same starting cell density of the indicator microorganism but without yeasts. If the propagation of the indicator organism is adversely affected by the yeast cells, its colony number per unit volume will be lower in the mixed culture than in the control culture.

3.8. Confrontation assay on agar medium (CFAA)

This method is frequently referred to as “dual plate assay” or “dual culture assay” because two microorganisms are inoculated on the same agar plate. However, this terminology is misleading because two organisms are also cultured together in most other methods. In CFAA, one organism (culture) is the yeast to be tested for antagonistic effect and the other organism (culture) is the indicator (usually a filamentous fungus) that forms a continuously extending colony able to gradually colonise the entire plate when cultivated alone (Fig. 3). The latter grows on the top of the yeast colony if the yeast cells do not inhibit the extension of its

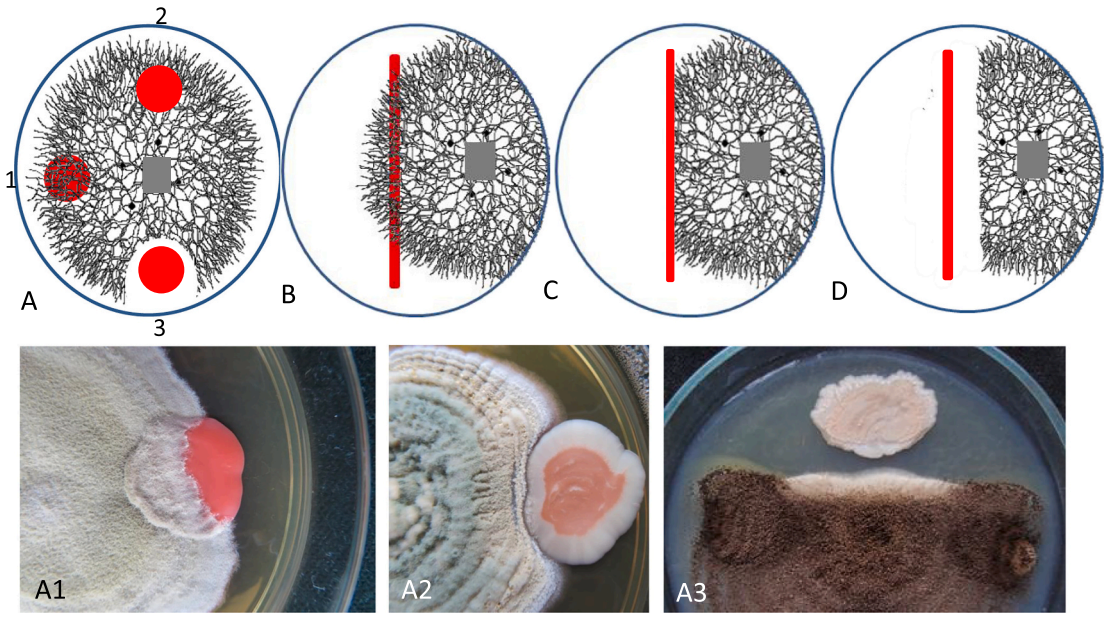

Fig. 3. Confrontation assay on agar medium (CFAA). A–D. Yeast strains (red) can be inoculated as patches or as lines. The mycelial indicator is grey. Modes of interactions: no antagonism (1 in A, A1 and B), contact inhibition (2 in A, A2 and C) and antagonism by released inhibitor (3 in C, A3 and D). Yeasts: *Rhodotorula glucinosa* (A1), *Metschnikowia pulcherrima* (A2) and *Mycosaarcoma aegypticum* (A3). Indicators: *Alternaria alternata* (A1), *Penicillium expansum* (A2) and *Aspergillus niger* (A3).

hyphae (Fig. 3A1). If the yeast is antagonistic against the fungus, the mycelium doesn't grow on the yeast colony. If the yeast cells release an agent that inhibits (either directly or indirectly by competition for a nutrient) the growth of the fungus, it halts the growth of the mycelium at distance from its colony (Fig. 3A3). If the inhibition is only partial, the extension of the mycelium is not stopped, it only becomes thinner. The yeast can also stop the extension of the mycelium by physical contact with the mycelium at the edge of its colony (the fungus does not grow on the top of the yeast colony) (Fig. 3A2). This mode of inhibition is referred to as contact inhibition. Since CFAA is very popular and the literature on its application is immense, only a few arbitrarily selected examples of recent publications will be cited here.

The tests can be carried out in various experimental set-ups. (a) A block of mycelium (or a mycelial disc) cut from the growing part of the mould colony is placed (upside down) in the centre of the plate and loopful amounts of the yeast culture (or cultures) is (are) inoculated as patch(es) at a certain distance around it (e.g. Sipiczki, 2016; Cordero-Bueso et al., 2017; Sipiczki and Selim, 2019) (Fig. 3A). (b) The fungus is inoculated on the plate and the yeast is inoculated as a line on one side (e.g. Raspor et al., 2010) (Fig. 3B–D) or as two lines on both sides of the block (e.g. Shude et al., 2022) or in a triangular format around the block (Mekbib et al., 2011). Instead of using a mycelium block, a sample of conidia can also be applied (e.g. Pereyra et al., 2021). (c) When the yeast is dimorphic and forms a radially growing mould-like culture (e.g. *Aureobasidium*, *Galactomyces/Geotrichum*), its antagonistic effect is usually manifested by halting the extension of the indicator fungal colony when its growing edge encounters the growing edge of the indicator fungus (contact inhibition) (Sipiczki and Hrabovszki, 2023) (Fig. 4A and B). The yeast colony can also reduce the growth rate of the fungus without releasing an inhibitory agent or being in physical contact with it, for example due to the depletion of the medium of a nutrient. However, this effect is not necessarily antagonism because, in many fungi, the colonies of the same strain can also stop each-other's growth (Fig. 4C and D).

3.9. Trap assay (TRAP)

A lawn is prepared from the yeast on an agar plate. The sterile mouth (3–4 cm in diameter) of a chipped-mouth Erlenmeyer flask (or beaker) is gently pressed onto the lawn and then onto a fresh agar plate to create a broken-circle (semicircle) print of the yeast culture. The imprint will not be a full circle; it remains open where the flask mouth is chipped. Then an agar block cut from the mycelium of the indicator microorganism is placed in the centre of the circle or an aliquot of the suspension of its conidia is dropped into the centre (Fig. 5). If the yeast has an antagonistic effect, the mycelium of the indicator microorganism will stop growing at a distance from (or at the edge of) the yeast semicircle. But it can escape from the trap between the ends of the semicircle. The difference between the diameter of the indicator colony in the trapped part and in the escape part shows how efficient the yeast is in growth inhibition.

4. Assays in liquid media (liquid co-culture assays)

4.1. Co-culturing of planktonic cells (CPPC)

This method is used to examine antagonistic interactions between microorganisms propagating by producing planktonic (free-swimming) cells in liquid media. A yeast strain can be tested for antagonism against other yeasts or bacteria in a liquid medium (e.g. Rajkowska et al., 2012; Csoma et al., 2020; Hicks et al., 2021). The medium is inoculated simultaneously both with the yeast to be tested and with the indicator strain (e.g. in 1:1 proportion) and the mixed culture is incubated at continuous agitation to prevent sedimentation of any of the partners. Samples are taken at regular time intervals and the proportion of the partners is determined either by microscopic observation (e.g. yeast cells vs. bacterium cells) or by plating diluted aliquots on agar media to obtain individual colonies. If the partners differ in colony morphology, their proportion can be determined by counting the different colonies. If the partners differ in the utilisation of different nutrients, their proportion can be determined by spreading aliquots on media containing different supplementations. For example if the partners are different yeast species, they can be differentiated on the basis of their different abilities to utilise certain sugars as carbon sources. If one strain assimilates galactose but does not assimilate melibiose and the other strain assimilates melibiose but does not assimilate galactose, their proportion

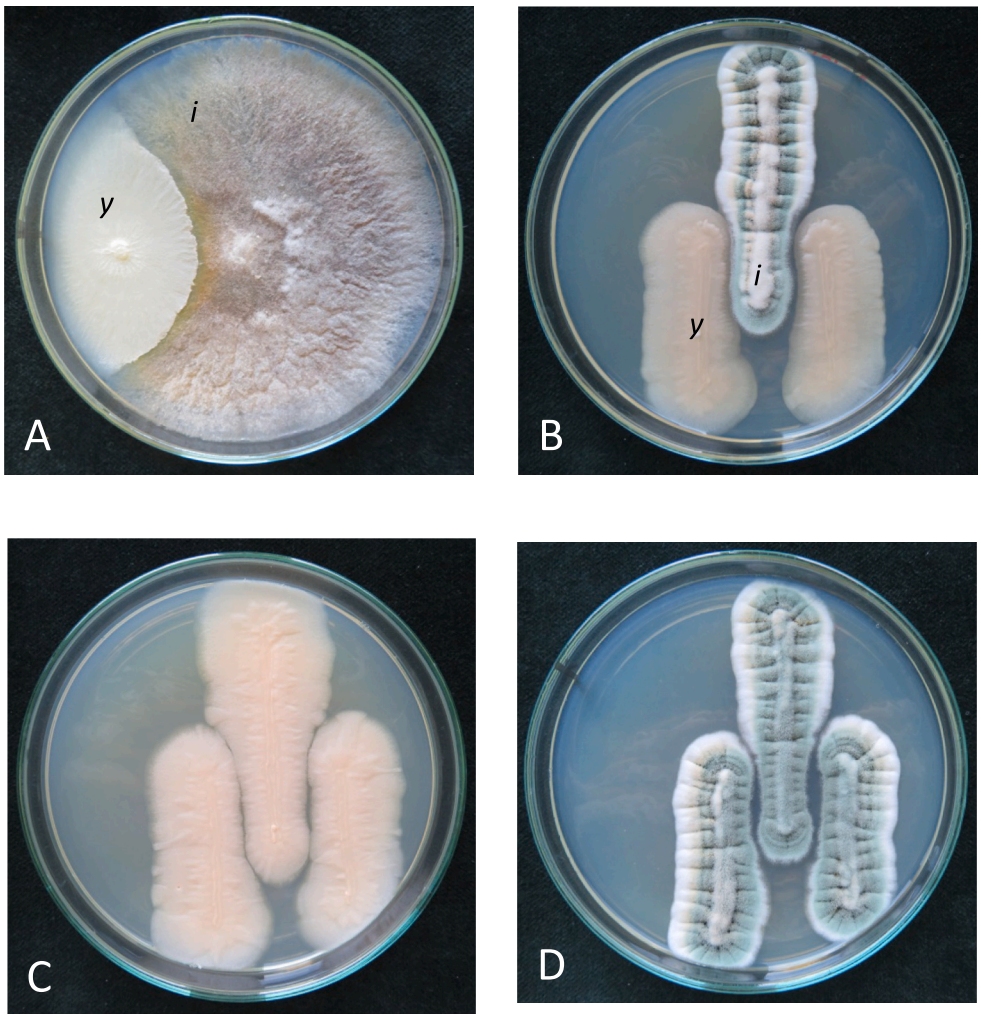

Fig. 4. Confrontation assay on agar medium (CFAA). A. *Galactomyces* (*y*) halts the extension of the *Botrytis* mycelium (*i*). B. *Aureobasidium* (*y*) reduces the growth of *Penicillium* (*i*). C and D. "selfantagonism" of an *Aureobasidium* and a *Penicillium* strain.

in the mixed culture can be determined by spreading equal volumes of samples simultaneously onto a medium containing galactose and onto a medium containing melibiose instead of glucose as carbon source (Csoma et al., 2020). If the indicator organism is a bacterium, the samples are spread on a yeast-specific medium (containing an antibacterial agent) and a bacterium-specific medium (supplemented with antifungal antibiotics) (Rajkowska et al., 2012). However, changes in their proportion do not necessarily mean antagonism because their growth rates can be different even in pure cultures. Therefore, the growth of both strains is also monitored in pure cultures as controls. Co-culturing can be done in larger volumes and in microplate wells.

4.2. Co-culturing of planktonic yeast cells and a hyphal indicator organism (CPHC)

When the indicator organism is a filamentous fungus, its growth cannot be monitored by cell counting. Instead, the change of the size of the mycelium is examined (e.g. Liu and Tsao, 2009; Ghanbari et al., 2019; Ferraz et al., 2021). The liquid medium is inoculated simultaneously with yeast cells and the conidia of the mould. The mixed culture is then incubated on a shaker at least until the yeast culture reaches the stationary phase. If the presence of the yeast cells is not inhibitory to the mould, the conidia germinate and form a mycelial mass visible as swimming pellets (pads, mats) in the medium and a ring on the wall of the flask. If the fungus does not form conidia, an agar block cut from the growing part of the mould colony is placed into the liquid medium. After incubation on a shaker for the proper period of time, the yeast culture is decanted and the size of the mycelium around the block is compared with the size of the mycelium formed in the yeast-free control. If the medium is not agitated, the mould forms colonies on the surface, whose size can be compared with colonies on the control (yeast-free) culture. Tests can also be carried out in microplates but in this case growth can only be evaluated visually.

4.3. Culture filtrate assay in liquid medium (CFAL)

The cell-free filtrate of the yeast culture is tested for antagonism by mixing it with conidia or cells of the indicator microorganism (e.g. Spotti et al., 2009; Núñez et al., 2015). The test can be performed in microplates (small volumes) or in agitated flasks (larger volumes). In the former case the growth of the indicator organism is monitored by measuring optical density. In the latter case the change of the weight of the centrifuged pellet (when the indicator forms planktonic cells) or the size of the mycelium is monitored.

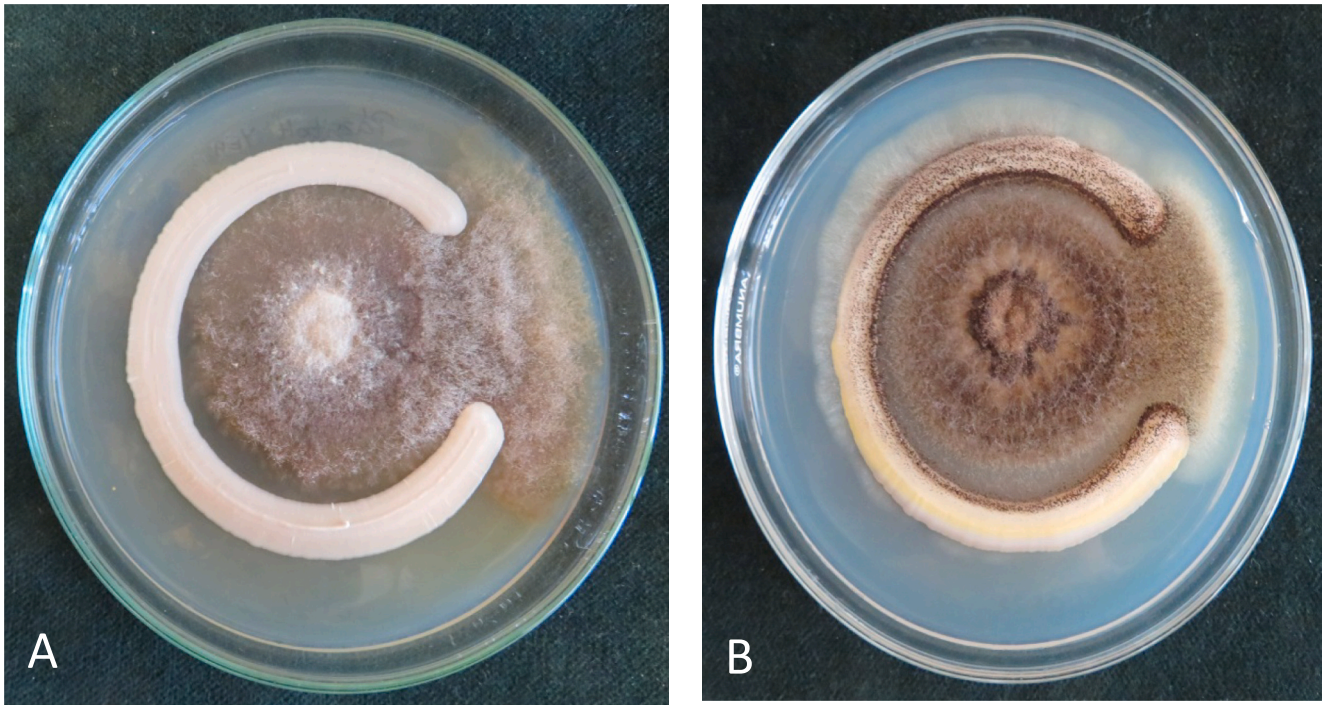

Fig. 5. Trap assay (TRAP). The yeast (*Metschnikowia*) semicircle traps the *Botrytis* mycelium (A), but does not stop the growth of *Aspergillus* (B). Both indicator fungi can escape from the trap between the ends of the yeast semicircle.

5. Assays on natural substrates

5.1. Protection assays on plant material

5.1.1. Protection assays on wounded plant material (PWPM)

In these tests the interactions of the yeast and an indicator are tested in artificially formed wounds on the surface of live plant material (fruits, leaves, tubers, seed, plantlets, etc.). The wounds are artificially infected with both microorganisms. The yeast and the indicator can be applied simultaneously or in different time points to emulate real situations. When the yeast is applied first, its cells can colonise the wound by the time of the application of the indicator (disease prevention test) (Guinebretiere et al., 2000; da Cunha et al., 2018). By applying the pathogen first, the curative power of the yeast is tested (disease curation test) (Platania et al., 2012; da Cunha et al., 2018). The yeast and the indicator can be applied in several ways: by direct injection of aliquots of suspensions of both microorganisms into the wounds, by submersion of the wounded plant material into suspensions of cells (conidia in the case of fungi), by spraying the wounded plant material with suspensions and by combining these procedures.

5.1.1.1. Injection into wounds. The plant surface (e.g. fruit, leaf, tuber) is disinfected by washing with a solution of an antimicrobial agent (e.g. sodium hypochlorite, 70 % ethanol) to inactivate the natural surface microorganisms and then rinsed with sterile water to eliminate the rest of the disinfectant. The disinfected surface is wounded (e.g. with a sterilised needle, awl, cork borer, knife, micropipette tip) and samples (usually 5–20 μl) of the suspensions of cells (and/or conidia) of both microorganisms are pipetted into the wounds. The indicator is a pathogenic organism that can evoke rotting or necrosis in the plant tissues around the wound. The controls are injected with equal volumes of sterile water or only with the suspension of the yeast (to prove that it is not pathogenic) or only with the suspension of the indicator (to monitor the progression of the disease in the absence of the yeast). The radial growth of the disease lesion around the wounds is monitored for a time period depending on the plant type. If the yeast has an antagonistic effect, the lesion appears with delay in its presence around the double-inoculated wounds and/or the diameter of the lesion is smaller. Example references: Piano et al., 1997; Vero et al., 2013; Zhao et al., 2021; Lanhuang et al., 2022; Steglińska et al., 2022.

5.1.1.2. Immersion/dipping. To test the yeast for antagonism, the material to be protected against the pathogenic indicator is disinfected and immersed/dipped into suspensions containing the yeast cells and the cells/conidia of the indicator. The immersion can be carried out by using a mixed suspension or by applying successively a pure suspension of the yeast and a pure suspension of the pathogen (Perez et al., 2019). The controls are dipped in sterile water or only in the yeast suspension or in the suspension of the indicator. The incidence of lesions and the size of the lesions on the double-treated and on the indicator-treated material are monitored and compared. Both intact and wounded plant material can be used (Marsico et al., 2021).

5.1.1.3. Spraying. The material to be protected is sprayed with the suspension of the yeast cells and, after the surface turns dry, with the suspension of the cells or conidia of the indicator. The controls are sprayed with sterile water or only with the yeast suspension or only with the indicator suspension. The incidence of lesions and the size of the lesions on the double-treated and on the indicator-treated material are monitored and compared (Shude et al., 2022; Yan et al., 2022). Nevertheless, it has to be borne in mind that the inhibitory activity of the tested yeast is concentration-dependent (Yan et al., 2022). It is worth mentioning that Shude et al. (2022) found a perfect correlation between the biocontrol efficacy of two epiphytic yeasts against *Fusarium* in in-vitro and in-vivo evaluations.

5.1.1.4. Combination of immersion and injection. The disinfected fruits or tubers are dipped in the yeast suspension. They can be wounded before or after dipping. The wounds are then filled with 5–20 μl of suspensions of cells/conidia of the indicator organism. The wounds of the controls are filled with equal volumes of sterile water. The development of the lesions is monitored on the indicator-inoculated and on

the control material and the incidence of disease and the size of lesions are compared (Cordero-Bueso et al., 2017; Marsico et al., 2021; Nandhini et al., 2021; Steglińska et al., 2022). Alternatively, the indicator is filled first into the injuries, and the infected material is dipped in the yeast suspension.

5.1.1.5. Combination of immersion and spraying. The disinfected fruits (intact and wounded) are dipped in the suspension of the yeast cells as described in the previous paragraph, and after drying, their surface is sprayed with the suspension of the indicator. The percentage of the wounds showing the disease symptoms and the size of the lesions are compared on the sprayed and unsprayed fruits (Zahavi et al., 2000).

5.1.2. Protection assays on plant organs (PPO)

5.1.2.1. Leaf and leaf-disc assays (PPO-L). The antifungal activity of yeasts against pathogens causing plant leaf diseases can be tested in-situ on leaves or in the laboratory on leaf discs. In the former case, suspensions of the yeast cells, the conidia of the pathogen (indicator) and their mixture are pipetted on the under-surface of different leaves of vitroplants. The development of the disease symptoms is monitored on the three sets of leaves and the percentages of leaves showing the symptoms of the disease are compared (Masih and Paul, 2002). In the latter case, the leaves are removed from the plant and surface sterilised (e.g. by immersion in NaOCl solution or 70 % ethanol) followed by three rinses in distilled water. Discs are cut from the surface-sterilised leaves and placed on sterilised moist filter paper in Petri dishes. The leaf discs are inoculated with aliquots of the suspension of yeast cells, indicator conidia and their mixture. The appearance of the disease symptoms and the progression of the lesions are compared in the three sets of discs (Buck, 2002). In both methods, the control leaves remain non-inoculated.

5.1.2.2. Flower assay (PPO-F). Organs of flowers can also be targets of pathogens. To identify yeasts effective against diseases caused by these pathogens, the antagonism tests have to be carried out on flowers. Healthy flowers are placed in sterile glass tabs or plastic boxes with the stems inserted in micro-centrifuge tube racks (or in holes of synthetic foam) over sterile distilled water. To inactivate most of the surface microbiota, the flowers are shortly illuminated with a germicidal lamp. A set of flowers is treated with yeasts by applying aliquots of their suspension on the petals. A subset of the yeast-treated flowers and a set of untreated flowers are then sprayed with the suspension of the conidia of the pathogenic indicator. The control flowers are sprayed with sterile distilled water. The efficiency of the yeast antagonism is shown by the difference between the disease incidences in the two groups of flowers sprayed with the pathogen (Lopes et al., 2015).

5.1.2.3. Tests on plantlets (PPO-P). The test of the yeast for antagonism against the indicator organism is carried out on young plants (seedlings) by surface treatment. Both the yeast and the indicator are applied onto the surface of the seedlings. The seedlings are sprayed with a suspension of the yeast cells and afterwards with a suspension of cells/conidia of the indicator pathogen (Gafni et al., 2015). The infection with the pathogen can also be done by placing agar blocks overgrown by its mycelium on the yeast-treated and untreated (control) plant surfaces (Wachowska and Borowska, 2014).

5.1.2.4. Tests on roots and germinating seed (PPO-R). Yeasts can be tested for antagonism against root-infecting pathogens in hydroponic cultures and in soil.

In the former method, seedlings grown in a liquid culture medium are used (Fernandez-San Millan et al., 2021). To facilitate the entry of the microorganisms in the plant tissues, the tips of their roots can be cut. Then the seedlings are transferred into flasks or tubes containing a sterile culture medium so that the roots are submerged in the medium. In the tubes, the medium is supplemented with yeast cells (test of the yeast for pathogenesis) or cells/conidia of the indicator microorganism (infection with the pathogen) or with their mixture (test of the ability of the yeast to protect the plant against the pathogen). The efficiency of the anti-pathogen activity of the yeast is evaluated by comparing the incidence and/or severity of the disease in the latter two groups of seedlings. Alternatively, the treatments can be carried out in sterilised soil or in an artificial substrate (Lara-Capistran et al., 2020).

Several methods have been described for testing the yeasts for root protection in soil. In one of the methods the antagonistic effect was tested by monitoring the germination of seeds sown into soil pre-treated with yeasts (El-Tarabily, 2004). Pre-treatment was done by mixing soya bean bran colonised by the yeast with dry soil grains. The mixture was then watered to allow the yeast cells to propagate. After several days of incubation the yeast-treated soil was re-mixed with the conidia of the indicator pathogen. Surface-sterilised seeds were sown into this soil and into soil treated only with the pathogen. The percentages of successful seed germination and healthy plants were compared in the two types of soil to enumerate the efficiency of the yeast to protect the plants against the pathogen.

In another method, young plants grown in soil were infected by dipping their roots in a suspension of the indicator pathogen and transferred in pots containing wet autoclaved substrate (soil). The pots were then watered with a yeast suspension or sterile water. The growth of plants and the development of the disease symptoms in yeast-treated and control plants were monitored and compared (Fernandez-San Millan et al., 2021). When interpreting the results, it has to be taken into consideration that the yeast cells decompose in the soil and may also have a fertilising (growth-promoting) effect.

Antagonistic tests were also done by seed pre-treatment. The disinfected grains (e.g. by sodium hypochlorite) were dipped in (or soaked with) a suspension of yeast cells or in a suspension of fungal (indicator) conidia for longer periods of time. After drying, they were dipped (soaked) in the suspension of the other microorganism. The order can be yeast-then-pathogen (Medina-Córdova et al., 2016) or vice versa (Limtong et al., 2020). The double-treated, pathogen-treated, and non-treated seeds were sown in sterilised soil and incubated in greenhouse for a period of time appropriate for the test plant cultivar. The efficiency of the protective power of the yeast treatment was evaluated by comparing the numbers of germinating seeds, the incidence and the severity of the disease in the double-treated and pathogen-treated populations of plantlets. The germination efficiency can also be determined by incubating the seeds on wet sterile filter paper.

5.1.3. Assays on fruit blocks (FBA)

This method was used to test yeasts, which had previously been found antagonistic against post-harvest fungal diseases, for antibacterial antagonism against food-borne pathogens (Leverentz et al., 2006). The yeast suspension was pipetted onto apple tissue plugs stored in sterile, capped glass tubes. Then an equal volume of bacterial (indicator) suspension was filled in the tubes. The control plugs were only treated with bacteria. After various periods of incubation, the growth intensity of the bacteria and the yeasts was compared by plating out diluted samples of the microbial populations of the tubes on bacterial and yeast culture media. If the yeast had antibacterial effect, the growth rate of the bacteria was lower in the mixed tube than in the control tube.

5.2. Assays on cheese (ChA)

Long-lasting dairy products are usually colonised by complex fungal communities that are important quality determinants for certain cheeses but can be detrimental to other cheeses. The propagation of spoilage fungi can be prevented or at least delayed by yeasts that can be co-components of the natural cheese microbiota. Several methods have been elaborated for the identification of yeasts having antagonistic effects against cheese-spoiling fungi. The tests can be carried out on the

surface of non-sterilised slices (blocks) of hard cheese or on plates of sterilised soft cheese or on cheese-agar plates. When hard cheese slices are used, wells are cut into the slices which are then filled individually with a suspension of the yeast cells, a suspension of the conidia of the indicator fungus and a mixed yeast-fungus suspension. If the yeast is antagonistic, fungal colonies are formed with delay (or not at all) around the wells inoculated with the mixed suspension (Liu and Tsao, 2009). When the cheese is soft, it can be autoclaved and then filled in a sterile Petri dish to create a cheese plate (Sipiczki and Hrabovszki, 2023). The yeast and the indicator fungus are then inoculated on the plates in spatial arrangements described in paragraph 3.8. Sterile plates can also be prepared from hard cheese. For this, gratings of the cheese are mixed with components of an agar medium, autoclaved and poured into sterile Petri dishes (van den Tempel and Jakobsen, 2000).

5.3. Assays on dry-cured/fermented meat products (MBA)

Blocks are cut from the ham or sausage and their surface is disinfected e.g. by dipping in ethanol and flaming. The surface-disinfected blocks are placed in sterile Petri dishes and spread with suspended yeast cells. Aliquots of the suspension of the indicator fungal conidia are then spotted on the top of blocks. The control blocks are only inoculated with the fungus. After various periods of incubation, the surface microbiota is washed off, and diluted samples of the suspensions are spread on plates of culture media to compare the numbers of fungal colony-forming units on the two types of blocks (Simoncini et al., 2014). Alternatively, the blocks/slices of the dry-cured/fermented meat product can be sprayed with suspensions of yeasts, conidia and their mixture. After incubation, the extent of colonisation of the slices by the fungus (the mould load) is determined by real-time quantitative PCR using primers targeting a

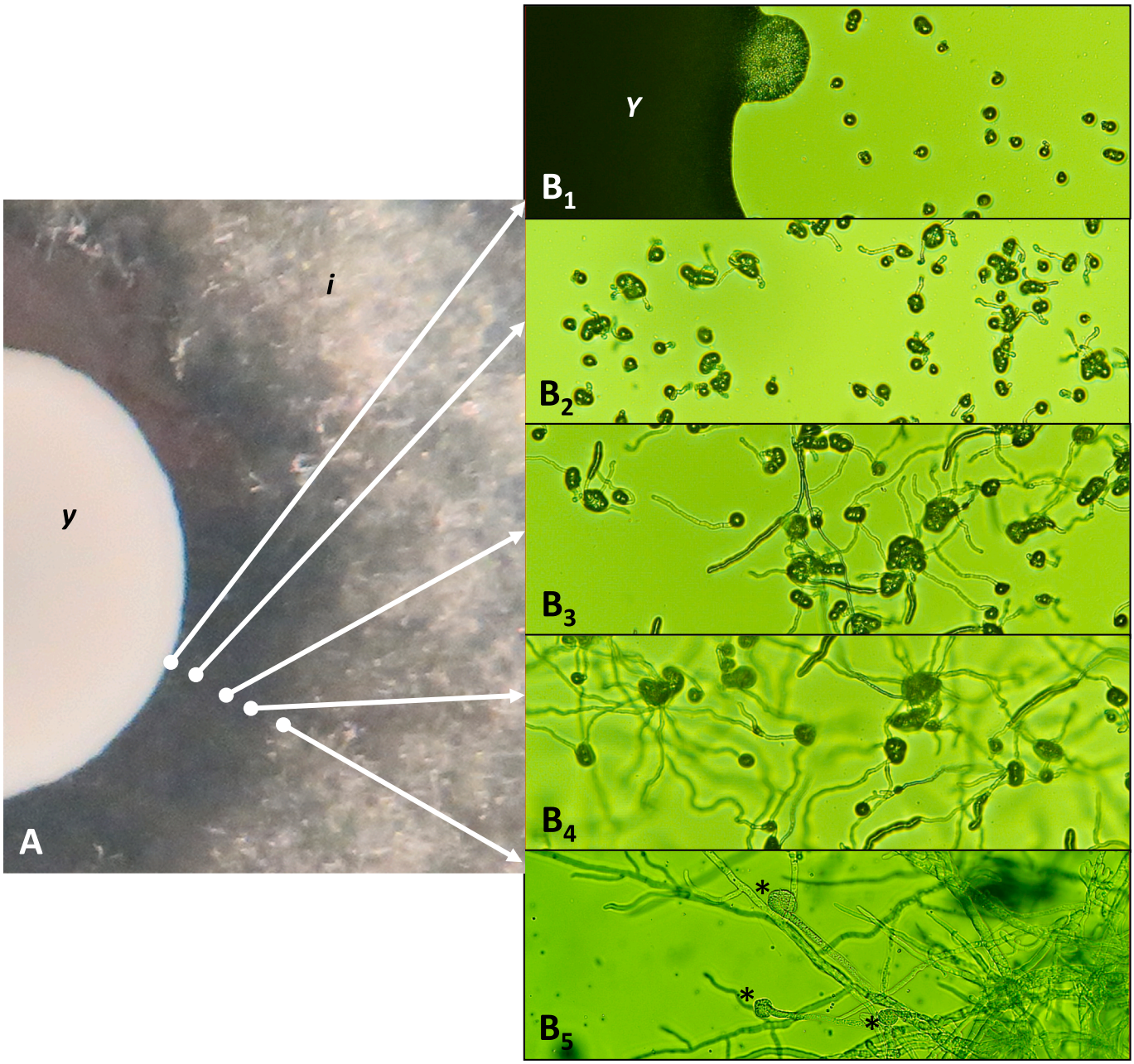

Fig. 6. Microscopic examination of antagonistic effect (agar plate assay). A. Yeast colony (*y*) growing on agar plate spread with conidia of the indicator fungus (*i*). B. Antagonistic effect of the yeast colony on the germination of conidia (B1), the elongation of germ tubes (B2 to B4) and the growth of hyphae (B5). The yeast is *M. pulcherrima*, the indicator is *Botrytis cinerea*. Stars mark lysing hyphal tips.

specific gene of the fungus (Núñez et al., 2015). If the growth of the fungus is adversely affected by the yeast, the RT-PCT test will detect less fungal DNA in the double-treated samples.

6. Microscopic methods

In these methods the effect of the yeast cells is examined by the microscopic observation of the morphological changes of the indicator organisms. Microscopic methods have been mainly applied to the examination of the response of moulds and dimorphic fungi to the presence of yeast cells. Aberrations in the germination of conidia and the growth of hyphae can easily be detected and monitored under the microscope. The tests can be carried out both in liquid media and on the surface of media solidified with agar.

6.1. Spore (conidium) germination assay on agar plate

The suspension of the conidia of the indicator organism is spread out on an agar plate. After drying, the yeast is inoculated in the centre of the plate (either by smearing cells with a loop or by dropping a dense suspension). The plates are incubated at the appropriate temperature and the conidia are examined under a microscope at time intervals (Fig. 6). If the yeast has an antagonistic effect, the conidia do not germinate in the vicinity of its colony, or the germination tubes dye off (Sipiczki, 2006). It is also worth to observe the morphology of the hyphae at the edge of the inhibition zone because the morphology of the hyphal tips can provide clues to the mode of the inhibition of hyphal growth (e.g. tip lysis, Fig. 6B_5). If the yeast cells do not grow fast, the test can be carried out by spreading out the two organisms mixed on the plates (Cabañas et al., 2020). The interactions of the yeast and the indicator fungus can also be examined on plates of a confrontation assay (CFAA). However, in this case the colonies have to be washed off from the plates to make the plates transparent for microscopic observation (Zhang et al., 2010).

6.2. Spore (conidium) germination assay in liquid medium

A suspension of yeast cells and a suspension of the conidia of the indicator fungus are prepared separately in a liquid medium. Parts of the suspensions are mixed in various proportions. All suspensions are then incubated. At intervals, aliquots are taken from the mixed culture and the culture of the conidia for microscopic comparison. If the conidia do not germinate in the mixed culture or their germination is slower than in the pure indicator culture, the yeast is antagonistic. The tests can be performed in larger volumes of the medium (e.g. in agitated Erlenmeyer flasks, test-tubes or centrifugation tubes) (e.g. Pan et al., 2022; Podgórska-Kryszczuk et al., 2022; Zhao et al., 2021; Hassan et al., 2021; Ruiz-Moyano et al., 2016) or in wells of tissue culture plates or microplates (Maluleke et al., 2022; Perez et al., 2019). When the cultures are agitated, the conidia frequently form clumps, which can prevent the accurate determination of the degree of antagonism. This problem can be circumvented by using excavated microscopic slides (Nally et al., 2015) or by incubation of thin layers of the cultures in Petri dishes without agitation.

A specific liquid assay method examines the interaction of physically separated partners. The yeast suspension is filled in a well of a tissue culture plate, and the suspension of the conidia of the indicator fungus is filled in a polystyrene cylinder which is sealed at the bottom with a membrane permeable for the components of the medium but not for microorganisms. The cylinder is placed in the well. After the appropriate period of incubation, the cylinder is removed from the well and the conidia settled on the membrane and their germ tubes are examined microscopically (Janisiewicz et al., 2000; Di Francesco et al., 2017).

7. Tests for antagonism by VOCs (volatile organic compounds)

Over the last years rapidly increasing numbers of studies have reported on the involvement of volatile organic compounds (VOCs) in the antifungal antagonisms of yeasts (for a recent review, see Oufensou et al., 2023). The cells of many yeast species turned out to release compounds in the air that adversely affect the growth of other organisms when both are cultured in the same closed air-space. Several methods have been developed for detecting VOC-mediated antagonisms in yeast cultures. In all laboratory tests, the yeast and the indicator organism are cultured in a closed common airspace but physically separated from each other to prevent interactions between them by means of non-volatile agents (Fig. 7). The airspace is usually tightly closed to prevent VOCs leakage. If the indicator organism grows more slowly in the presence of the yeast colony than in its absence, the yeast is considered antagonistic. However, it is usually overlooked that the slower growth of an aerobic indicator (e.g. many moulds) can also be attributed to the faster exhaustion of oxygen when the space is shared by two organisms than when only the indicator is present (Fig. 7B and C). Another consequence of oxygen depletion is that the yeast switches from respiration to fermentation in which it may produce volatile metabolites that are not produced in real-life bioprotection situations. Co-culturing in closed air-space is not suitable for testing aerobic (e.g. basidiomycetous) yeasts for VOC-mediated antagonism.

7.1. Double-plate assay (DP-VOC)

This method (Fig. 7A) is also called “double-dish system” (DDS) (Tejero et al., 2021) and “mouth-to-mouth method” (Medina-Córdova et al., 2016). The yeast and the indicator microorganism are inoculated on the surface of agar plates in different Petri dishes. The lids of the dishes are removed and the bottom parts of the dishes containing the agar plates are placed on top of each other so that the inoculated surfaces of their plates are turned towards each other (face-to-face). The dishes stacked in this way create a closed air-space in which the volatile compounds can diffuse between the two physically separated cultures. To prevent the released VOCs from leaving the space, the dishes are sealed with layers of Parafilm. The yeast can be inoculated on the plate as a spot smeared by a loop or as a drop of suspension. More intense VOC release can be achieved by spreading yeast cells on the entire surface of the plate. The indicator organism, usually a fungus is inoculated in a spot (e.g. by placing an agar block cut from a plate covered by the mycelium of the fungus) around which a colony can be formed by radial growth. In the control pair of plates the counterpart of the yeast-inoculated plate remains non-inoculated (sterile). The growth of the fungal colonies is monitored in both plate pairs. Faster growth in the control indicates that the yeast cells release volatile compounds that have adverse effect on the growth of the fungus. As high number of studies applied this method, only selected recent references are cited here (e.g. Indratmi et al., 2016; Konsue et al., 2020; Choińska et al., 2020; Marsico et al., 2021; Oztekin and Karbancioglu-Guler, 2021; Zou et al., 2022). To identify the inhibitory compound, samples can be taken from the air-space separating the plates and subjected to an analysis by gas chromatography (e.g. Ruiz-Moyano et al., 2020).

7.2. Split-plate assay (SP-VOC)

VOCs production can also be detected on split plates (e.g. Lopes et al., 2015; da Cunha et al., 2018; Yan et al., 2022) (Fig. 7E). The agar medium is poured into split (two-compartment or three-compartment) Petri dishes. The yeasts and the indicator organism are inoculated into different compartments. Since the airspaces of the compartments are connected under the lid of the dish, the VOCs of the yeast culture can diffuse into the compartment of the indicator colony. No yeast is inoculated in the control plate. The dishes are then sealed with Parafilm. The growth of the indicator colony in the presence and in the absence of a yeast colony is compared.

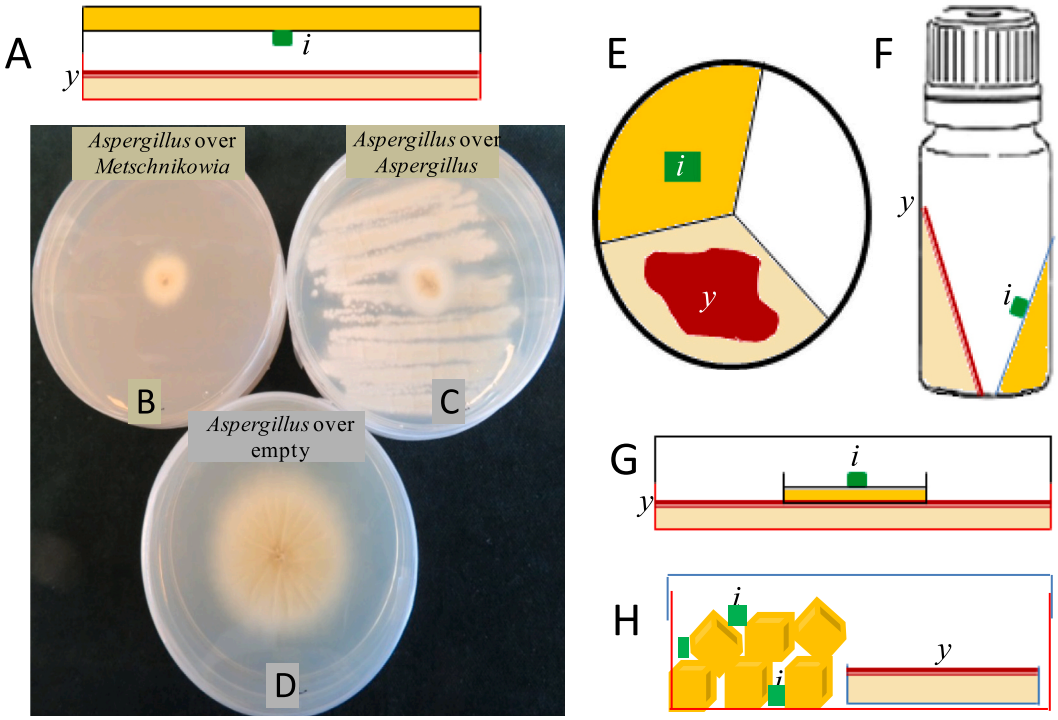

Fig. 7. Tests for antagonism mediated by VOCs (volatile organic compounds). A–D. Double-plate assay (DP-VOC). Reduced growth rate of the indicator in closed air space is not necessarily VOC-mediated antagonism: the indicator colony grows equally poorly over the yeast lawn (B) and over itself (C). E. Split-plate assay (SP-VOC). F. Double-slant-agar (vial) assay (Vial-VOC). G. Plate-on-Plate assay (PP-VOC). H. Assays on fruit blocks (F-VOC). Tested yeast is marked with *y*; indicator organism is marked with *i*.

7.3. Double-slant-agar (vial) assay (Vial-VOC)

Cultures of microorganisms can be maintained on the surface of slanted agar medium (agar slants) in a test-tube or in a capped vial. In this assay a capped vial containing two physically separated agar slants facing towards each other (Fig. 7F) is used to test a yeast culture for the ability to reduce the growth rate of the indicator microorganism through the air. The yeast is inoculated on one side (on one slant agar) and the indicator microorganism is inoculated on the other side (on the other slant agar). In the control vial the yeast is missing. After closing the vials with caps, the growth of the indicator colonies is monitored in both vials. If the presence of the yeast on the slant reduces the growth rate of the indicator organism on the other slant, then its cells are likely to emit inhibitory VOC(s) (Maluleke et al., 2022) or create a low-oxygen atmosphere.

7.4. Plate-on-Plate assay (PP-VOC)

In this method a larger Petri dish and a smaller Petri dish are used (Tongsri and Sangchote, 2009; Zou et al., 2022) (Fig. 7G). Agar plates are prepared in both dishes. The yeast cells are spread on the plate of the larger dish, whereas the indicator organism is inoculated in the centre of the smaller plate. The latter dish is then placed on the surface of the former and the two plates are covered with the lid of the larger dish. Since the smaller plate is not covered with its own lid, the two cultures share the same air-space. The larger dish is then sealed with Parafilm. In the control pair of dishes no yeast cells are spread onto the larger plate. The growth of the indicator is compared in the two pairs of dishes.

7.5. Fruit assay of VOC-mediated antagonism (F-VOC)

Two methods have been developed to test yeasts for VOC-mediated antagonism on fruits. In one of the methods (Huang et al., 2011; Chen et al., 2018) fruits (intact or wounded) are placed in three plastic boxes. In two boxes the fruits are sprayed with the conidia or cells of the indicator microorganism. Simultaneously, agar plates spread with yeast cells are prepared in Petri dishes. The dishes are placed without being covered by their lids in one of the boxes containing sprayed fruits. The other boxes serve as controls. The boxes are then covered to maintain high humidity (Fig. 7H). The fruits can also be infected by immersion in the suspension of the indicator, or by injection aliquots of the suspension into surface wounds. The incidence of symptoms and the development of the disease are compared in the box containing yeast cultures and in the control boxes. In the other method (Ruiz-Moyano et al., 2020) agar plates are prepared in two sterilised plexiglass boxes. Cells of the yeast strain are spread on one plate; the other plate remains non-inoculated. The bottoms of two other boxes are perforated at multiple sites. The fruits are placed into these boxes and sprayed with the indicator pathogen. The fruit-containing boxes are then placed on the top of the boxes containing the agar plates and covered with their lids. Both box pairs are sealed with Parafilm. The development of the disease is compared in the two pairs to see if the presence of the yeast-inoculated plates can inhibit the indicator pathogen.

8. Combinations of methods

None of the assays covered in this review can detect all types of yeast antagonism against other microorganisms. Strains showing antagonistic effects in an assay may prove to be non-antagonistic in a different assay. For example, yeast strains that reduce the growth rate of the colony of a fungus in a “indicator on yeast” (IOY) assay may not inhibit the growth of the fungus in a “yeast on indicator” (YOI) assay or in a CFAA confrontation assay (e.g. Cordero-Bueso et al., 2017; Fernandez-San Millan et al., 2021). Strains proving antagonistic in certain culturing conditions may turn out to be neutral in other conditions. For example when the antagonistic effect is due to fast consumption of a nutrient by the yeast which is essential for the growth of the other microorganism, the result of the assay depends on the composition of the medium (e.g.

Guzzon et al., 2014; Sipiczki, 2016; Maluleke et al., 2022). A strain showing no antagonism in laboratory tests can inhibit fruit rotting due to its ability to form a biofilm on the wounds (Lei et al., 2022). The mycelia of many mould strains stop growing when they get in physical contact with the colonies of the dimorphic *Galactomyces* (*Geotrichum*) and *Aureobasidium* species. But the colonies of these species can also inhibit each other. In the VOC tests the slower growth of the fungal colony is automatically attributed to volatile compounds released by the yeast cells although it can also be due to the depletion of oxygen in the closed airspace by their respiratory metabolism. Because the testing assays are sensitive to the environmental conditions and differ in efficiency and specificity, their combined application is recommended.

9. Conclusions

Because of the increasing demand of consumers for food products protected from harmful microorganisms with biological agents, yeasts have come to the forefront of attention due to the fairly widespread incidence of antimicrobial antagonism among them. However, the yeasts to be tested, the malevolent microorganisms to be combatted and the plants or food products to be protected against them are highly diverse. Therefore a plethora of diverse identification methods have been developed. Unfortunately, there is no universally applicable ("best") method among them and therefore different laboratories often use different tests and assays. Because of the different specificity and efficiency of the methods, the results are frequently not comparable, and yeast strains found active against an indicator organism in one test may not show significant activity against the same organism in another assay. Besides, numerous methods can lead to false positive results if processes not related to antagonism are ignored (e.g. oxygen consumption in VOC tests, different growth rates in liquid media or the effect of general nutrient depletion in the medium in confrontation tests and the IOY tests). To avoid methodological pitfalls, it is advisable to test the yeast strains with two or even more methods. However, it has to be stressed that the laboratory tests covered in this review are only suitable for screening the strains for antagonistic activity. There is no guarantee for correlation between the laboratory results and the suitability of the antagonistic yeast strains for bioprotection. Further tests performed under technological conditions will show which strains can be used as bioprotective agents. These tests are beyond the scope of this review.

CRediT authorship contribution statement

Matthias Sipiczki: this work has one author.

Funding

This work was supported by the National Research, Development and Innovation Office, Hungary [2019-1.1.1-PIACI-KFI-2019-00345; 2020-1.1.2-PIACI-KFI-2020-00130].

Declaration of competing interest

The author declares no conflict of interest.

Data availability

Data will be made available on request.

References

Acuña-Fontecilla, A., Silva-Moreno, E., Ganga, M.A., Godoy, L., 2017. Evaluation of antimicrobial activity from native wine yeast against food industry pathogenic microorganisms. CyTA J. Food 15, 457–465.

Al-Dulaimi, F.K.Y., Al-Tarjuman, J.K., Mulla Abid, F.N., 2020. Inhibitory activity of *Saccharomyces cerevisiae*, *Rhodotorula glutinis* and *Lactobacillus* spp against *Escherichia coli* isolated from children diarrhea infection. Ann. Trop. Public Health 23, 931–934.

Andrade, M.J., Thorsen, L., Rodríguez, A., Córdoba, J.J., Jespersen, L., 2014. Inhibition of ochratoxigenic moulds by *Debaryomyces hansenii* strains for biopreservation of dry-cured meat products. Int. J. Food Microbiol. 170, 70–77.

Bilinski, C.A., Innamorato, G., Stewart, G.G., 1985. Identification and characterization of antimicrobial activity in two yeast genera. Appl. Environ. Microbiol. 50, 1330–1332.

Björnberg, A., Schnürer, J., 1993. Inhibition of the growth of the grain-storage molds in vitro by the yeast *Pichia anomala* (Hansen) Kurtzman. Can. J. Microbiol. 39, 623–628.

Bleve, G., Grieco, F., Cozzi, G., Logrieco, A., Visconti, A., 2006. Isolation of epiphytic yeasts with potential for biocontrol of *Aspergillus* carbonarius and *A. niger* on grape. Int. J. Food Microbiol. 108, 204–209.

Buck, J.W., 2002. In vitro antagonism of *Botrytis cinerea* by phylloplane yeasts. Can. J. Bot. 80, 885–891.

Cabañas, C.M., Hernández, A., Martínez, A., Tejero, P., Vázquez-Hernández, M., Martín, A., Ruiz-Moyano, S., 2020. Control of *Penicillium glabrum* by indigenous antagonistic yeast from vineyards. Foods 9, 1864.

Chanchaichaovivat, A., Pintip, C.R., Bhinyo, P., 2007. Screening and identification of yeast strains from fruits and vegetables: potential for biological control of postharvest chili anthracnose (*Colletotrichum capsici*). Biol. Control 42, 326–335.

Chen, P.H., Chou, J.Y., 2017. Screening and identification of yeasts antagonistic to pathogenic fungi show a narrow optimal pH range for antagonistic activity. Pol. J. Microbiol. 66, 101–106.

Chen, P.H., Chen, R.Y., Chou, J.Y., 2018. Screening and evaluation of yeast antagonists for biological control of *Botrytis cinerea* on strawberry fruits. Mycobiology 46, 33–46.

Choińska, R., Piasecka-Jóźwiak, K., Chabłowska, B., Dumka, J., Łukaszewicz, A., 2020. Biocontrol ability and volatile organic compounds production as a putative mode of action of yeast strains isolated from organic grapes and rye grains. Antonie Van Leeuwenhoek 113, 1135–1146.

Comitini, F., De, I.J., Pepe, L., Mannazzu, I., Ciani, M., 2004. *Pichia anomala* and *Kluyveromyces wickerhamii* killer toxins as new tools against *Dekkera/Brettanomyces* spoilage yeasts. FEMS Microbiol. Lett. 238, 235–240.

Cordero-Bueso, G., Mangieri, N., Maghradze, D., Foschino, R., Valdetara, F., Cantoral, J. M., Vigentini, I., 2017. Wild grape-associated yeasts as promising biocontrol agents against *Vitis vinifera* fungal pathogens. Front. Microbiol. 8, 2025.

Csoma, H., Kallai, Z., Antunovics, Z., Czentye, K., Sipiczki, M., 2020. Vinification without *Saccharomyces*: interacting osmotolerant and "spoilage" yeast communities in fermenting and ageing botrytised high-sugar wines (Tokaj Essence). Microorganisms 9, 19.

da Cunha, T., Ferraz, L.P., Wehr, P.P., Kupper, K.C., 2018. Antifungal activity and action mechanisms of yeasts isolates from citrus against *Penicillium italicum*. Int. J. Food Microbiol. 276, 20–27.

de Melo Pereira, G.V., Beux, M., Pagnoncelli, M.G., Soccol, V.T., Rodrigues, C., Soccol, C. R., 2016. Isolation, selection and evaluation of antagonistic yeasts and lactic acid bacteria against ochratoxigenic fungus *Aspergillus westerdijkiae* on coffee beans. Lett. Appl. Microbiol. 62, 96–101.

Delali, K.I., Chen, O., Wang, W., Yi, L., Deng, L., Zeng, K., 2021. Evaluation of yeast isolates from kimchi with antagonistic activity against green mold in citrus and elucidating the action mechanisms of three yeast: *P. kudriavzevii*, *K. marxianus*, and *Y. lipolytica*. Postharvest Biol. Technol. 176, 111495.

Di Canito, A., Mateo-Vargas, M.A., Mazzieri, M., Cantoral, J., Foschino, R., Cordero-Bueso, G., Vigentini, I., 2021. The role of yeasts as biocontrol agents for pathogenic fungi on postharvest grapes: a review. Foods 2021 (10), 1650.

Di Francesco, A., Ugolini, L., D'Aquino, S., Pagnotta, E., Mari, M., 2017. Biocontrol of *Monilinia laxa* by *Aureobasidium pullulans* strains: insights on competition for nutrients and space. Int. J. Food Microbiol. 248, 32–38.

El-Tarabily, K.A., 2004. Suppression of *Rhizoctonia solani* diseases of sugar beet by antagonistic and plant growth-promoting yeasts. J. Appl. Microbiol. 96, 69–75.

Fernandez-San Millan, A., Larraya, L., Farran, I., Ancin, M., Veramendi, J., 2021. Successful biocontrol of major postharvest and soil-borne plant pathogenic fungi by antagonistic yeasts. Biol. Control 160, 104683.

Ferraz, L.P., Cunha, T.D., da Silva, A.C., Kupper, K.C., 2016. Biocontrol ability and putative mode of action of yeasts against *Geotrichum citri-aurantii* in citrus fruit. Microbiol. Res. 188-189, 72–79.

Ferraz, P., Brandão, R.L., Cássio, F., Lucas, C., 2021. *Moniliophthora perniciosa*, the causal agent of cacao witches' broom disease is killed in vitro by *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* yeasts. Front. Microbiol. 12, 706675.

Gafni, A., Calderon, C.E., Harris, R., Buxdorf, K., Dafa-Berger, A., Zeilinger-Reichert, E., Levy, M., 2015. Biological control of the cucurbit powdery mildew pathogen *Podosphaera xanthii* by means of the epiphytic fungus *Pseudozyma aphidis* and parasitism as a mode of action. Front. Plant Sci. 6, 132.

Ghanbari, R., Rezaie, S., Noorbakhsh, F., Khaniki, G.J., Soleimani, M., Aghaee, E.M., 2019. Biocontrol effect of *Kluyveromyces lactis* onaflatoxin expression and production in *Aspergillus parasiticus*. FEMS Microbiol. Lett. 366, fnz114.

Gianvito, P.D., Englezos, V., Rantsiou, K., Cocolin, L., 2022. Bioprotection strategies in winemaking. Int. J. Food Microbiol. 364, 109532.

Gil-Rodríguez, A.M., Garcia-Gutierrez, E., 2021. Antimicrobial mechanisms and applications of yeasts. Adv. Appl. Microbiol. 114, 37–72.

Goerges, S., Aigner, U., Silakowski, B., Scherer, S., 2006. Inhibition of *Listeria monocytogenes* by food borne-yeasts. Appl. Environ. Microbiol. 72, 313–318.

Guinebretiere, M.H., Nguyen-The, C., Morrison, N., Reich, M., Nicot, P., 2000. Isolation and characterization of antagonists for the biocontrol of the postharvest wound pathogen *Botrytis cinerea* on strawberry fruits. J. Food Protection 3, 386–394.

Guzzon, R., Franciosi, E., Larcher, R., 2014. A new resource from traditional wines: characterisation of the microbiota of "Vino Santo" grapes as a biocontrol agent against *Botrytis cinerea*. Eur. Food Res. Technol. 239, 117–126.

Hassan, H., Mohamed, M.T.M., Yusoff, S.F., Hata, E.M., Tajidin, N.E., 2021. Selecting antagonistic yeast for postharvest biocontrol of *Colletotrichum gloeosporioides* in papaya fruit and possible mechanisms involved. Agronomy 11, 760.
Hatoum, R., Labrie, S., Fliss, I., 2013. Identification and partial characterization of antilisterial compounds produced by dairy yeasts. Probiotics Antimicrob. Proteins 5, 8–17.
He, D., Zheng, X.D., Yin, Y.M., Sun, P., Zhang, H.Y., 2003. Yeast application for controlling apple postharvest diseases associated with *Penicillium expansum*. Bot. Bull. Acad. Sin. 44, 211–216.
Hernández-Montiel, L.G., Ochoa, J.L., Troyo-Diéguez, E., Larralde-Corona, C.P., 2010. Biocontrol of postharvest blue mold (*Penicillium italicum* Wehmer) on Mexican lime by marine and citrus *Debaryomyces hansenii* isolates. Postharvest Biol. Technol. 56, 181–187.
Hernandez-Montiel, L.G., Droby, S., Preciado-Rangel, P., Rivas-García, T., González-Estrada, R.R., Gutiérrez-Martínez, P., Ávila-Quezada, G.D., 2021. A sustainable alternative for postharvest disease management and phytopathogens biocontrol in fruit: antagonistic yeasts. Plants (Basel) 10, 2641.
Hicks, R.H., Moreno-Beltrán, M., Gore-Lloyd, D., Chuck, C.J., Henk, D.A., 2021. The oleaginous yeast *Metschnikowia pulcherrima* displays killer activity against avian-derived pathogenic bacteria. Biology (Basel) 10, 1227.
Hilber-Bodmer, M., Schmid, M., Ahrens, C.H., Freimoser, F.M., 2017. Competition assays and physiological experiments of soil and phyllosphere yeasts identify *Candida subhashii* as a novel antagonist of filamentous fungi. BMC Microbiol. 17, 4.
Hodgson, V.J., Button, D., Walker, G.M., 1995. Anti-*Candida* activity of a novel killer toxin from the yeast *Williopsis mrakii*. Microbiology (Reading) 141, 2003–2012.
Huang, R., Li, G., Zhang, J., Yang, L., Che, H.J., Jiang, D.H., Huang, H.C., 2011. Control of postharvest *Botrytis* fruit rot of strawberry by volatile organic compounds of *Candida intermedia*. Phytopathology 101, 859–869.
Iacumin, L., Manzano, M., Andyanto, D., Comi, G., 2017. Biocontrol of ochratoxigenic moulds (*Aspergillus ochraceus* and *Penicillium nordicum*) by *Debaryomyces hansenii* and *Saccharomycopsis fibuligera* during speck production. Food Microbiol. 62, 188e195.
Iacumin, L., Arnoldi, M., Comi, G., 2020. Effect of a *Debaryomyces hansenii* and *Lactobacillus buchneri* starter culture on *Aspergillus westerdijkiae* Ochratoxin A production and growth during the manufacture of short seasoned dry-cured ham. Microorganisms 8, 1623.
Indratmi, D., Sastrahidayat, I.R., Abadi, A.L., Djauhari, S., 2016. Antagonist effect of volatile organic compounds produced by *Debaryomyces hansenii* on *Colletotrichum gloeosporoides* as anthracnose reason of tropical apples. J. Bio. Env. Sci. 9, 133–140.
Janisiewicz, W.J., Tworkoski, T.J., Sharer, C., 2000. Characterizing the mechanism of biological control of postharvest diseases on fruits with a simple method to study competition for nutrients. Phytopathology 90, 1196–1200.
Konsue, W., Dethoup, T., Limtong, S., 2020. Biological control of fruit rot and anthracnose of postharvest mango by antagonistic yeasts from economic crops leaves. Microorganisms 8, 317.
Lanhuang, B., Yang, Q., Godana, E.A., Zhang, H., 2022. Efficacy of the yeast *Wickerhamomyces anomalus* in biocontrol of gray mold decay of tomatoes and study of the mechanisms involved. Foods 11, 720.
Lara-Capistran, L., Zulueta-Rodriguez, R., Castellanos-Cervantes, T., Reyes-Perez, J.J., Preciado-Rangel, P., Hernandez-Montiel, L.G., 2020. Efficiency of marine bacteria and yeasts on the biocontrol activity of *Pythium ultimum* in ancho-type pepper seedlings. Agronomy 10, 408.
Lei, X., Deng, B., Ruan, C., Deng, L., Zeng, K., 2022. Phenylethanol as a quorum sensing molecule to promote biofilm formation of the antagonistic yeast *Debaryomyces nepalensis* for the control of black spot rot on jujube. Postharvest Biol. Technol. 185, 111788.
Leverentz, B., Conway, W.S., Janisiewicz, W., Abadias, M., Kurtzman, C.P., Camp, M.J., 2006. Biocontrol of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* Serovar poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists. Appl. Environ. Microbiol. 72, 1135–1140.
Li, R., Zhang, H., Liu, W., Zheng, X., 2011. Biocontrol of postharvest gray and blue mold decay of apples with *Rhodotorula mucilaginosa* and possible mechanisms of action. Int. J. Food Microbiol. 146, 151–156.
Li, X., Zeng, S., Wisniewski, M., Droby, S., Yu, L., An, F., Leng, Y., Wang, C., Li, X., He, M., Liao, Q., Liu, J., Wang, Y., Sui, Y., 2022. Current and future trends in the biocontrol of postharvest diseases. Crit. Rev. Food Sci. Nutr. 18, 1–13.
Limtong, S., Into, P., Attarat, P., 2020. Biocontrol of rice seedling rot disease caused by *Curvularia lunata* and *Helminthosporium oryzae* by epiphytic yeasts from plant leaves. Microorganisms 8, 647.
Liu, S.-Q., Tsao, M., 2009. Biocontrol of dairy moulds by antagonistic dairy yeast *Debaryomyces hansenii* in yoghurt and cheese at elevated temperatures. Food Control 20, 852–855.
Lopes, M.R., Klein, M.N., Ferraz, L.P., da Silva, A.C., Kupper, K.C., 2015. *Saccharomyces cerevisiae*: a novel and efficient biological control agent for *Colletotrichum acutatum* during pre-harvest. Microbiol. Res. 175, 193–199.
Ma, Y., Wu, M., Qin, X., Dong, Q., Li, Z., 2023. Antimicrobial function of yeast against pathogenic and spoilage microorganisms via either antagonism or encapsulation: a review. Food Microbiol. 112, 104242.
MacWilliam, I.C., 1959. A survey of the antibiotic powers of yeasts. J. Gen. Microbiol. 21, 410–414.
Maluleke, E., Jolly, N.P., Patterton, H.G., Setati, M.E., 2022. Antifungal activity of non-conventional yeasts against *Botrytis cinerea* and non-*Botrytis* grape bunch rot fungi. Front. Microbiol. 13, 986229.
Mari, M., Martini, C., Spadoni, A., Rouissi, W., Bertolini, P., 2012. Biocontrol of apple postharvest decay by *Aureobasidium pullulans*. Postharvest Biol. Technol. 73, 56–62.
Marsico, A.D., Velenosi, M., Perniola, R., Bergamini, C., Sinonin, S., David-Vaizant, V., Maggiolini, F.A.M., Hervè, A., Cardone, M.F., Ventura, M., 2021. Native vineyard non-*Saccharomyces* yeasts used for biological control of *Botrytis cinerea* in stored table grape. Microorganisms 9, 457.
Masih, E., Paul, B., 2002. Secretion of β-1,3-glucanases by the yeast *Pichia membranifaciens* and its possible role in the biocontrol of *Botrytis cinerea* causing grey mold disease of the grapevine. Curr. Microbiol. 44, 391–395.
Masoud, W., Kaltoft, C.H., 2006. The effects of yeasts involved in the fermentation of *Coffea arabica* in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus*. Int. J. Food Microbiol. 106, 229–234.
Medina-Córdova, N., López-Aguilar, R., Ascencio, F., Castellanos, T., Campa-Córdova, A. I., Angulo, C., 2016. Biocontrol activity of the marine yeast *Debaryomyces hansenii* against phytopathogenic fungi and its ability to inhibit mycotoxins production in maize grain (*Zea mays* L.). Biol. Control 97, 70–79.
Meftah, S., Abid, S., Dias, T., Rodrigues, P., 2018. Effect of dry-sausage starter culture and endogenous yeasts on *Aspergillus westerdijkiae* and *Penicillium nordicum* growth and OTA production. LWT - Food Sci. Technol. 87, 250–258.
Mehlomakulu, N.N., Setati, M.E., Divol, B., 2014. Characterization of novel killer toxins secreted by wine-related non-*Saccharomyces* yeasts and their action on *Brettanomyces* spp. Int. J. Food Microbiol. 188, 83–91.
Mekbib, S.B., Regnier, T.J.C., Lise Korsten, L., 2011. Efficacy and mode of action of yeast antagonists for control of *Penicillium digitatum* in oranges. Trop. Plant Pathol. 36, 233–240.
Mohamed, H., Saad, A., 2009. The biocontrol of postharvest disease (*Botryodiplodia theobromae*) of guava (*Psidium guajava* L.) by the application of yeast strains. Postharvest Biol. Technol. 53, 123–130.
Mukherjee, A., Verma, J.P., Gaurav, A.K., Chouhan, G.K., Patel, J.S., Hesham, A.E., 2020. Yeast a potential bio-agent: future for plant growth and postharvest disease management for sustainable agriculture. Appl. Microbiol. Biotechnol. 104, 1497–1510.
Nally, M.C., Pesce, V.M., Maturano, Y.P., Rodriguez Assaf, L.A., Toro, M.E., Castellanos de Figueroa, L.I., Vazquez, F., 2015. Antifungal modes of action of *Saccharomyces* and other biocontrol yeasts against fungi isolated from sour and grey rots. Int. J. Food Microbiol. 204, 91–100.
Nandhini, M., Harish, S., Aiyanathan, K.E.A., Durgadevi, D., Beaulah, A., 2021. Glycerol-based liquid formulation of the epiphytic yeast *Hanseniaspora guilliermondii* isolate YBB3 with multiple modes of action controls postharvest *Aspergillus* rot in grapes. J. Plant Pathol. 103, 1253–1264.
Núñez, F., Lara, M.S., Peromingo, B., Delgado, J., Sánchez-Montero, L., Andrade, M.J., 2015. Selection and evaluation of *Debaryomyces hansenii* isolates as potential bioprotective agents against toxigenic penicillia in dryfermented sausages. Food Microbiol. 46, 114–120.
Oufensou, S., Ul Hassan, Z., Balmas, V., Jaoua, S., Migheli, Q., 2023. Perfume guns: potential of yeast volatile organic compounds in the biological control of mycotoxin-producing fungi. Toxins 15, 45.
Oztekin, S., Karbancioglu-Guler, F., 2021. Bioprospection of *Metschnikowia* sp. isolates as biocontrol agents against postharvest fungal decays on lemons with their potential modes of action. Postharvest Biol. Technol. 181, 111634.
Pan, H., Zhong, C., Wang, Z., Deng, L., Li, W., Zhao, J., Long, C.-A., Li, L., 2022. Biocontrol ability and action mechanism of *Meyerozyma guilliermondii* 37 on soft rot control of postharvest kiwifruit. Microorganisms 10, 2143.
Patiño-Vera, M., Jiménez, B., Balderas, K., Ortiz, M., Allende, R., Carrillo, A., Galindo, E., 2005. Pilot-scale production and liquid formulation of *Rhodotorula minuta*, a potential biocontrol agent of mango anthracnose. J. Appl. Microbiol. 99, 540–550.
Pawlikowska, E., James, S.A., Breierova, E., Antolak, H., Kregiel, D., 2019. Biocontrol capability of local *Metschnikowia* sp. isolates. Antonie Van Leeuwenhoek 112, 1425–1445.
Peña, R., Ganga, M.A., 2019. Novel antimicrobial peptides produced by *Candida intermedia* LAMAP1790 active against the wine-spoilage yeast *Brettanomyces bruxellensis*. Antonie Van Leeuwenhoek 112, 297–304.
Pereyra, M.M., Díaz, M.A., Soliz-Santander, F.F., Poehlein, A., Meinhardt, F., Daniel, R., Dib, J.R., 2021. Screening methods for isolation of biocontrol epiphytic yeasts against *Penicillium digitatum* in lemons. J. Fungi 7, 166.
Perez, M.F., Díaz, M.A., Pereyra, M.M., Córdoba, J.M., Isas, A.S., Sepúlveda, M., Dib, J. R., 2019. Biocontrol features of *Clavispora lusitaniae* against *Penicillium digitatum* on lemons. Postharvest Biol. Technol. 155, 57–64.
Peromingo, B., Andrade, M.J., Delgado, J., Sánchez-Montero, L., Núñez, F., 2019. Biocontrol of aflatoxigenic *Aspergillus parasiticus* by native *Debaryomyces hansenii* in dry-cured meat products. Food Microbiol. 82, 269–276.
Petersson, S., Schnürer, J., 1995. Biocontrol of mould in high-moisture wheat stored under airtight conditions by *Pichia anomala*, *Pichia guilliermondii* and *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 61, 1027–1032.
Piano, S., Neyrotti, V., Migheli, Q., Gullino, M.L., 1997. Biocontrol capability of *Metschnikowia pulcherrima* against *Botrytis* postharvest rot of apple. Postharvest Biol. Technol. 11, 131–140.
Piasecka-Jozwiak, K., Chabłowska, B., 2017. Anti-mold properties of yeast strains as a biological agent for protection of grain during storage. J. Res. Appl. Agric. Eng. 62, 84–89.
Platania, C., Restuccia, C., Muccilli, S., Cirvilleri, G., 2012. Efficacy of killer yeasts in the biological control of *Penicillium digitatum* on Tarocco orange fruits (*Citrus sinensis*). Food Microbiol. 30, 219–225.
Podgórska-Kryszczuk, I., Solarska, E., Kordowska-Wiater, M., 2022. Biological control of *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium* poae by antagonistic yeasts. Pathogens 11, 86.
Rajkowska, K., Bska, A.K., Rygal'a, A., 2012. Probiotic activity of *Saccharomyces cerevisiae* var. *boulardii* against human pathogens. Food Technol. Biotechnol. 50, 230–236.

Raspor, P., Miklič-Milek, D., Avbelj, M., Čadež, N., 2010. Biocontrol of grey mould disease on grape caused by *Botrytis cinerea* with autochthonous wine yeasts. Food Technol. Biotechnol. 48, 336–343.
Reyes-Bravo, P., Acuña-Fontecilla, A., Rosales, I.M., Godoy, L., 2021. Non-conventional yeasts as biocontrol agents against fungal pathogens related to postharvest diseases. Sydowia 74, 71–78.
Roostita, L.B., Fleet, G.H., Wendry, S.P., Apon, Z.M., Gemilang, L.U., 2011. Determination of yeasts antimicrobial activity in milk and meat products. Adv. J. Food Sci. Technol. 3, 442–445.
Ruiz-Moyano, S., Martín, A., Villalobos, M.C., Calle, A., Serradilla, M.J., Córdoba, M.G., Hernández, A., 2016. Yeasts isolated from figs (*Ficus carica* L.) as biocontrol agents of postharvest fruit diseases. Food Microbiol. 57, 45–53.
Ruiz-Moyano, S., Hernández, A., Galvan, A.I., Córdoba, M.G., Casquete, R., Serradilla, M. J., Martín, A., 2020. Selection and application of antifungal VOCs-producing yeasts as biocontrol agents of grey mould in fruits. Food Microbiol. 92, 103556.
Sabaghian, S., Braschi, G., Vannini, L., Patrignani, F., Samsulrizal, N.H., Lanciotti, R., 2021. Isolation and identification of wild yeast from Malaysian grapevine and evaluation of their potential antimicrobial activity against grapevine fungal pathogens. Microorganisms 9, 2582.
Shen, H., Wei, Y., Wang, X., Xu, C., Shao, X., 2019. The marine yeast *Sporidiobolus pararoseus* ZMY-1 has antagonistic properties against *Botrytis cinerea* in vitro and in strawberry fruit. Postharvest Biol. Technol. 150, 1–8.
Shude, S.P.N., Mbili, N.C., Yobo, K.S., 2022. Epiphytic yeasts as potential antagonists against *Fusarium* head blight of wheat (*Triticum aestivum* L.) caused by *Fusarium graminearum* sensu stricto. J. Saudi Society Agric. Sci. 21, 404–411.
Simoncini, N., Virgili, R., Spadola, G., Battilani, P., 2014. Autochthonous yeasts as potential biocontrol agents in dry-cured meat products. Food Control 46, 160–167.
Sipiczki, M., 2006. *Metschnikowia* strains isolated from botrytized grapes antagonize fungal and bacterial growth by iron depletion. Appl. Environ. Microbiol. 72, 6716–6724.
Sipiczki, M., 2016. Overwintering of vineyard yeasts: survival of interacting yeast communities in grapes mummified on vines. Front. Microbiol. 7, 212.
Sipiczki, M., Hrabovszki, V., 2023. Galactomyces candidus diversity in the complex mycobiota of cow-milk bryndza cheese comprising antagonistic and sensitive strains. Int. J. Food Microbiol. 388, 110088.
Sipiczki, M., Selim, S.A., 2019. Antagonistic yeasts from a salt-lake region in Egypt: identification of a taxonomically distinct group of phylloplane strains related to *Sporisorium*. Antonie Van Leeuwenhoek 112, 523–541.
Spotti, E., Berni, E., Cacchioli, C., Simoncini, N., Quintavalla, S., 2009. Growth and antagonistic activity of *Hyphopichia burtonii* against other fungal species frequently found on meat products during maturation. Ind. delle Conserve 84, 11e22.
Staneviciene, R., Lukša, J., Strazdaite-Zieliene, Ž., Ravoityte, B., Losinska-Siciuniene, R., Mozuraitis, R., Serviene, E., 2021. Mycobiota in the carposphere of sour and sweet cherries and antagonistic features of potential biocontrol yeasts. Microorganisms 9, 1423.
Steglińska, A., Kołtuniak, A., Berłowska, J., Czyżowska, A., Szulc, J., Cieciura-Włoch, W., Okrasa, M., Kręgiel, D., Gutarowska, B., 2022. *Metschnikowia pulcherrima* as a biocontrol agent against potato (*Solanum tuberosum*) pathogens. Agronomy 12, 2546.
Tejero, P., Martín, A., Rodríguez, A., Galván, A.I., Ruiz-Moyano, S., Hernández, A., 2021. In vitro biological control of *Aspergillus flavus* by *Hanseniaspora opuntiae* L479 and *Hanseniaspora uvarum* L793, producers of antifungal volatile organic compounds. Toxins 13, 663.
Tongsri, V., Sangchote, S., 2009. Yeast metabolites inhibit banana anthracnose fungus *Colletotrichum musae*. Asian J. Food. Agro. Industry 2, 807–816.
Tryfinopoulou, P., Chourdaki, A., Nychas, G.E., Panagou, E.Z., 2020. Competitive yeast action against *Aspergillus carbonarius* growth and ochratoxin A production. Int. J. Food Microbiol. 317, 108460.
van den Tempel, T., Jakobsen, M., 2000. The technological characteristics of *Debaryomyces hansenii* and *Yarrowia lipolytica* and their potential as starter cultures for production of Danablu. Int. Dairy J. 10, 263–270.
Vero, S., Garmendia, G., González, M.B., Bentancur, O., Wisniewski, M., 2013. Evaluation of yeasts obtained from Antarctic soil samples as biocontrol agents for the management of postharvest diseases of apple (*Malus* × *domestica*). FEMS Yeast Res. 13, 189–199.
Virgili, R., Simoncini, N., Toscani, T., Leggieri, M.C., Formenti, S., Battilani, P., 2012. Biocontrol of *Penicillium nordicum* growth and ochratoxin a production by native yeasts of dry cured ham. Toxins 4, 68e82.
Wachowska, U., Borowska, J., 2014. Antagonistic yeasts competes for iron with winter wheat stem base pathogens. Gesunde Pflanzen 66, 141–148.
Wang, Y., Bao, Y., Shen, D., Feng, W., Yu, T., Zhang, J., Zheng, X.D., 2008. Biocontrol of *Alternaria alternata* on cherry tomato fruit by useof marine yeast *Rhodosporidium paludigenum* Fell & Tallman. Int. J. Food Microbiol. 123, 234–239.
Wang, Y., Yu, T., Xia, J., Yu, D., Wang, J., Zheng, X., 2010. Biocontrol of postharvest gray mold of cherry tomatoes with the marine yeast *Rhodosporidium paludigenum*. Biol. Control 53, 178–182.
Yan, F., Zhang, D., Ye, X., Wu, Y., Fang, T., 2022. Potential of *Saturnispora diversa* MA as a postharvest biocontrol agent against anthracnose in loquat fruit. Biol. Control 173, 105006.
Yang, H., Wang, L., Li, S., Gao, X., Wu, N., Zhao, Y., Sun, W., 2021. Control of postharvest grey spot rot of loquat fruit with *Metschnikowia pulcherrima* E1 and potential mechanisms of action. Biol. Control 152, 104406.
Younis, G., Awad, A., Dawod, R.E., Yousef, N.E., 2017. Antimicrobial activity of yeasts against some pathogenic bacteria. Vet. World 10, 979–983.
Zahavi, T., Cohen, L., Weiss, B., Schena, L., Daus, A., Kaplunov, T., Zutkhi, J., Ben-Arie, R., Droby, S., 2000. Biological control of *Botrytis*, *Aspergillus* and *Rhizopus* rots on table and wine grapes in Israel. Postharvest Biol. Technol. 20, 115–124.
Zhang, D., Spadaro, D., Garibaldi, A., Gullino, M.L., 2010. Efficacy of the antagonist *Aureobasidium pullulans* PL5 against postharvest pathogens of peach, apple and plum and its modes of action. Biol. Control 54, 172–180.
Zhang, X., Li, B., Zhang, Z., Chen, Y., Tian, S., 2020. Antagonistic yeasts: a promising alternative to chemical fungicides for controlling postharvest decay of fruit. J. Fungi (Basel) 6, 158.
Zhao, L.N., Wang, Y.J., Dhanasekaran, S., Guo, Z.P., Chen, S.J., Zhang, X.Y., Zhang, H.Y., 2021. Efficacy of *Wickerhamomyces anomalus* yeast in the biocontrol of blue mold decay in apples and investigation of the mechanisms involved. BioControl 66, 547–558.
Zheng, X., Zhang, H., Xi, Y., 2004. Effects of *C. laurentii* on biocontrol of postharvest decay of arbutus berries. Bot. Bull. Acad. Sinica 45, 55–60.
Zheng, X.D., Zhang, H.Y., Sun, P., 2005. Biological control of postharvest green mold decay of oranges by *Rhodotorula glutinis*. Eur. Food Res. Technol. 220, 353–357.
Zou, X., Wei, Y., Dai, K., Xu, F., Wang, H., Shao, X., 2021. Yeasts from intertidal zone marine sediment demonstrate antagonistic activities against *Botrytis cinerea in vitro* and in strawberry fruit. Biol. Control 158, 104612.
Zou, X., Wei, Y., Jiang, S., Cao, Z., Xu, F., Wang, H., Zhan, P., Shao, X., 2022. Volatile organic compounds and rapid proliferation of *Candida pseudolambica* W16 are modes of action against gray mold in peach fruit. Postharvest Biol. Technol. 183, 111751.