Plant growth-promoting traits of yeasts isolated from Spanish vineyards: benefits for seedling development

A. Fernandez-San Millan, I. Farran, L. Larraya, M. Ancin, L.M. Arregui and J. Veramendi

Institute for Multidisciplinary Research in Applied Biology (IMAB), Universidad Pública de Navarra, Campus Arrosadía, 31006 Pamplona, Spain

E-mail addresses:
A. Fernández-San Millán: alicia.fernandez@unavarra.es
I. Farran: farran@unavarra.es
L. Larraya: luis.larraya@unavarra.es
M. Ancín: maria.ancin@unavarra.es
L.M. Arregui: arregui@unavarra.es
J. Veramendi: jon@unavarra.es

Corresponding author: Alicia Fernández San Millán (Tel.+34 948 168 014; Fax.+34 948 232 191)

Date of submission: 12th November 2019
Number of tables: 4
Number of figures: 3
Word count: 8,586
Supplementary Data: 2 figures and 3 tables

Abstract

It is known that some microorganisms can enhance plant development. However, the use of yeasts as growth-promoting agents has been poorly investigated. The aim of this study was the characterisation of a collection of 69 yeast strains isolated from Spanish vineyards. Phytobeneficial attributes such as solubilisation of nutrients, synthesis of active biomolecules and cell wall-degrading enzyme production were analysed. Strains that revealed multiple growth-promoting characteristics were identified. The *in vitro* co-culture of *Nicotiana benthamiana* with yeast isolates showed enhancement of plant growth in 10 strains (up to 5-fold higher shoot dry weight in the case of *H. pseudoburtonii* Hp-54), indicating a beneficial direct yeast-plant interaction. In addition, 18 out of the 69 strains increased dry weight and the number of roots per seedling when tobacco seeds were inoculated. Two of these, *Pichia dianae* Pd-2 and *M. guilliermondii* Mg-11, also increased the chlorophyll content. The results in tobacco were mostly reproduced in lettuce with these two strains, which demonstrates that the effect of the yeast-plant interaction is not species-specific. In addition, the yeast collection was evaluated in maize seedlings grown in soil in a phytotron. Three isolates (*Debaryomyces hansenii* Dh-67, *Lachancea thermotolerans* Lt-69 and *Saccharomyces cerevisiae* Sc-6) promoted seedling development (increases of 10 % in dry weight and chlorophyll content). In conclusion, our data confirm that several yeast strains can promote plant growth and could be considered for the development of biological fertiliser treatments.

Key words: Plant growth-promoter, yeast, vineyard, biofertiliser, maize, lettuce

1. Introduction

Worldwide agricultural food production needs to be boosted in order to feed a growing population. Sustainable and cost-effective crop farming is a global challenge that has drawn increasing attention among scientists, policy makers and industry (Seufert et al., 2012; Wezel et al., 2014). Although efforts have been made to mitigate declining mineral nutrient reserves, the over-use of mineral fertilisers remains a serious problem (Kahiluoto et al., 2014). Hence, the production of biofertilisers based on microorganisms is gaining increasing attention owing to the negative impacts of chemical-based fertilisers and the growing awareness of the association between microorganisms and plants (Berg et al., 2016; Uroz et al., 2019). Furthermore, as the use of biofertilisers is mandatory in organic farming and the implementation of regulatory policies by various governments is increasing, this market is experiencing strong growth (more than 1.8 billion US$ in 2018, growing at a compound annual growth rate of around 14.3% during 2011-2018). Europe, followed by North America, is the largest market for biofertilisers and together account for more than 50% of global revenue (Biofertilizer Market Report 2019; https://www.researchandmarkets.com/reports/4775814/biofertilizer-market-global-industry-trends). The current situation has motivated scientists to investigate microorganisms that exhibit plant growth-promoting (PGP) traits and explore the numerous mutualistic interactions between plant roots and the microbiome.

In addition to the microorganisms present in the soil, several microbial species, such as filamentous fungi, yeasts, bacteria, algae or protozoa can be isolated from surfaces and inner parts of the plant (Berendsen et al., 2012; Lindow and Brandl, 2003). Plant-associated microorganisms fulfil different functions in plant development, such as stimulating plant growth or increasing resistance to biotic and abiotic stresses (Berendsen et al., 2012; Lindow and Brandl, 2003). PGP microorganisms facilitate plant growth through different mechanisms: nitrogen fixation, production of plant hormones such as indole-3-acetic acid (IAA), enhanced nutrient uptake, solubilisation of inorganic minerals, iron chelation, ammonia (NH_3) production or direct competition with other pathogenic microorganisms (Arora et al., 2013; El-Tarabily and Sivasithamparam, 2006). To date, rhizobacteria are the most studied PGP microbes (Gouda et al., 2018) and research has resulted in multifunctional formulations for commercial agriculture (Backer et al., 2018; García-Fraile et al., 2015). However, usage of PGP rhizobacteria remains controversial due to the variability in their performance, which is probably subject to environmental factors that affect their growth and proliferation in the plant (Backer et al., 2018; Gouda et al., 2018).

Yeasts are unicellular fungi that occur naturally in soil and plants, but in a lower proportion compared to bacteria and filamentous fungi (Yurkov, 2018). The role

played by yeasts in agricultural ecosystems is not completely understood and research on these microorganisms as PGP agents is scarce (Amprayn et al., 2012; Barbieri and Galli, 1993; Botha, 2011; de Souza et al., 2019; Fu et al., 2016). However, a diversity of studies indicates that plant growth may be directly or indirectly enhanced by yeasts, being described as potential biofertilisers. The promoting effect of yeasts could be due to the active substances produced (phytohormones, amino acids, vitamins or NH_3), solubilisation of inorganic phosphate or zinc, iron capture through siderophores and restriction of pathogen colonisation (Amprayn et al., 2012; Cloete et al., 2009; de Souza et al., 2019; El-Tarabily and Sivasithamparam, 2006; Freimoser et al., 2019; Nassar et al., 2005). For example, some species of *Candida*, *Rhodotorula*, *Saccharomyces*, *Geotrichum* and *Williopsis* are able to nitrify ammonium to nitrate (Al-Falih, 2006). *Candida* spp., *Hanseniaspora uvarum*, *Meyerozyma caribbica*, *Saccharomyces cerevisiae* or *Torulaspora* spp. among others, have been described as producing IAA and some of them also synthesise siderophores, catalase, NH_3 and cell wall-degrading enzymes (de Souza et al., 2019; Freimoser et al., 2019; Fu et al., 2016; Nutaratat et al., 2014; Sun et al., 2014).

Although most studies on yeast growth-enhancing capacity to date have been conducted *in vitro*, the effectiveness of field applications has also been demonstrated. Nakayan et al. (2013) reported that *Meyerozyma guilliermondii* CC1 increased the seed vigour index in maize and Chinese cabbage, and applications combined with a half dose of chemical fertiliser significantly improved the dry weight and nutrient uptake of maize and lettuce under greenhouse conditions. Amprayn et al. (2012) showed that *Candida tropicalis* CtHY inoculated on rice seedlings rapidly colonised the roots, increasing plant dry weight up to 35% compared to non-inoculated control seedlings. These results validated the inclusion of this strain in the commercial biofertiliser product BioGro (Hien et al., 2014).

The soil and plant ecosystem of vineyards (*Vitis vinifera* L.) offers a complex environment that is the source of a vastly diverse pool of filamentous fungi, yeast and bacteria. These play a coordinated role in impacting on the sanitary state of grapes and can have a direct influence on the winemaking process and, therefore, on wine quality (Setati et al., 2012). In this context, the relevance of isolation and characterisation of local microbial strains as a valuable source of biodiversity and agronomic potential must be emphasised.

The objective of this study was the characterisation of local yeast strains of Spanish vineyards and their screening for the presence of PGP traits. The ultimate goal of this research would be to find indigenous adapted yeast strains that could have growth-promoting effects for future use, not only in local vineyards but also in other crops of agronomic interest. As a first approach, an evaluation of the effects of isolated yeasts

on the development of seedlings of several species was made, both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Yeast maintenance

A collection of 69 yeast strains (Table 1) isolated from grapes collected in Spanish vineyards by the Lev2050 company (https://lev2050.com/) was preserved at -80 ºC. For solid culture, yeasts were plated onto YMA medium containing (g L^{-1}) malt extract 3.0, yeast extract 3.0, peptone 4.0, dextrose 10.0 and bacto agar 20.0 at pH 6.8. For liquid culture YPD broth was used, containing (g L^{-1}) yeast extract 10.0, peptone 20.0 and dextrose 20.0 at pH 6.8. Flasks were incubated on a rotary shaker at 150 rpm and 28 ºC.

2.2. Solubilisation of nutrients: phosphate and zinc oxide solubilisation assay

All yeast isolates were examined for phosphate solubilisation of tri-calcium diphosphate. Petri dishes were used with 20 mL of modified Pikovskaya medium (based on Hashem and Metwally, 2014) containing (g L^{-1}) glucose 10.0, $Ca_3(PO4)_2$ 5.0, $(NH_4)_2SO_4$ 0.5, KCl 0.2, $MgSO_4.7H_2O$ 0.1, KCl 0.2 and yeast extract 0.5. The pH was adjusted to 6.8 before autoclaving. In each agar plate 5 μl of 10^8 CFU mL^{-1} yeast cultures (two days at 28 ºC in YPD medium at 150 rpm) were transferred as triplicate spots and incubated at 28 ºC for seven days. Positive results were observed as lighter-colour halos around the colonies. Solubilisation efficiency (SE) was calculated as (Colony diameter + Halo zone diameter)/Colony diameter.

In a similar way, to evaluate the zinc solubilising ability, yeasts were plated onto the same media as the phosphate solubilisation assay but using ZnO 1.0 g L^{-1} instead of $Ca_3(PO_4)_2$ and incubated at 28ºC for five days. As described by Fu et al., 2016, the clearing zone around the colonies was measured and SE calculated.

2.3. Production of biomolecules

2.3.1. In vitro *screening for IAA production*

Yeast inoculum was prepared on YPD broth amended with or without 0.1% (w/v) L-tryptophan (L-Trp) at pH 6.8 (Fu et al., 2016). Culture flasks were incubated in the dark on an orbital shaker at 150 rpm and 28 ºC for seven days. Because it is known

that IAA production amounts vary during the culture period (Oliveira et al., 2019), 1 mL samples were collected at three, five and seven days of culture for quantitative colorimetric analysis of IAA. After 5 min centrifugation at 7,000 *g*, the supernatants (100 μL) were transferred into 96-well microplates with each well receiving 100 μL of Salkowsky reagent (49 mL 35% perchloric acid and 1 mL 0.5 M $FeCl_3.6H_2O$; Gordon and Weber, 1951) and incubated at room temperature while protected from light for 30 min for colour development. Absorbance was measured in triplicate at 540 nm wavelength. A calibration curve (0-100 μg mL^{-1}) was established in the same microplate using commercial IAA for calculation of concentration. IAA levels were expressed as μg mg^{-1} of dry yeast (yeasts were dried in an oven at 65 ºC until constant weight). The maximum level among seven days was chosen for each strain.

2.3.2. Siderophore production assay

A modified chrome azurol S (CAS) assay (Milagres et al., 1999) was used to test the ability of yeast isolates to produce siderophores in solid medium. YMA plates were prepared and half of the solidified medium was cut and replaced with CAS blue agar. The yeast isolates were inoculated in triplicate (5 μl of a 10^8 CFU mL^{-1} culture) onto YMA half way towards the border with the CAS blue agar and plates were incubated in the dark at 28°C for ten days. The presence of siderophores was determined by an advancing colour change in the CAS blue agar starting from the borderline between the two halves.

2.3.3. Catalase test

Five μL of a 10^8 CFU mL^{-1} yeast culture were transferred as spots by triplicate onto YMA plates and grown at 28 ºC for three days. Fifty μL of 3% hydrogen peroxide were added directly over each colony to determine oxygen production (based on Fu et al., 2016). The development of oxygen bubbles was indicative of a positive result. A visual comparison was made for all yeast strains.

2.3.4. NH_3 production

Production of NH_3 by the yeast isolates was tested in peptone water containing (g L^{-1}) peptone 10.0 and NaCl 5.0. The pH was adjusted to 6.8 before autoclaving. Yeast inoculum (100 μL of 10^8 CFU mL^{-1} culture) of each strain was transferred by triplicate to a tube containing 5 mL of peptone water and incubated on a rotary shaker at 28 °C for five days. One mL aliquots (adjusted at 10^5 CFU mL^{-1}) were centrifuged at 10,000 *g* for 5 min and 100 μL of Nessler´s reagent was added to the supernatant. The development of a yellow-to-brown colour was indicative of a positive result for NH_3

production (Cappuccino and Sherman, 2002). A visual comparison was made for all yeast strains.

2.4. Extracellular lytic enzymes activity

Five μL of a 10^8 CFU mL^{-1} yeast culture were plated onto different media in triplicate. Chitinolytic and β-1,3-glucanase activity were determined by a clear zone on modified YPD medium containing (g L^{-1}) yeast extract 10.0, peptone 20.0, glucose 2.0, bacto agar 15.0 and colloidal chitin 5.0 or yeast β-glucan 2.0 at pH 5.5. The plates were incubated for three days at 28 ºC. Colonies showing a clear halo by Congo Red staining (Teather and Wood, 1982) were identified as positive.

Extracellular production of proteases was assayed on YMA plates supplemented with 3% skimmed milk powder (based on Cattelan et al., 1999). After five days at 28 ºC, a clear zone around the yeast colony demonstrated protease degradation activity (DA), which was calculated as (Colony diameter + Halo zone diameter)/Colony diameter.

Cellulase activity was screened in plates with carboxymethylcellulose as substrate containing (g L^{-1}) KH_2PO_4 0.5, $MgSO_4.7H_2O$ 0.1, NaCl 0.2, $CaCl_2.2H_2O$ 0.2, $FeCl_3.6H_2O$ 0.01, carboxymethylcellulose 4.0, and bacto-agar 12.0 at pH 5.5. The plates were incubated for seven days at 28 ºC. Cellulase activity was identified as positive when a clear halo appeared around the colony after staining with Lugol's iodine solution and DA was calculated (Kasana et al., 2008).

Pectinase activity was determined in citrus pectin agar medium (slightly modified from Merín et al., 2011) containing (g L^{-1}) peptone 20.0, yeast extract 10.0, citrus pectin 10.0, and bacto agar 20.0 at pH 5.5. The plates were incubated at 28 ºC for five days. The colonies were rinsed off with distilled water before staining with Lugol's iodine solution. Colonies showing a clear halo were identified as positive and DA was calculated.

2.5. Assessment of growth promotion of Nicotiana benthamiana *seedlings in co-culture with yeasts under* in vitro *conditions*

Nicotiana benthamiana seeds were surface sterilised using 1% (v/v) sodium hypochlorite solution with 0.1% (v/v) Tween 20 for 30 min and washed five times with sterilised water. The seeds were sown on quarter-strength Murashige and Skoog (MS) mineral salts and vitamins (Murashige and Skoog, 1962) supplemented with 1% (w/v) sucrose (pH 5.7) and solidified with 0.5% (w/v) agar. After seven days of culture at 21 ºC and 16 h light at 70 μmol m^{-2} s^{-1}, four seedlings per plate and six plates per

strain were co-cultured with yeasts in 8.5 cm Petri dishes containing the same modified MS medium gelified with 1.5% (w/v) agar. Seedlings were placed on the upper half of each Petri dish and the bottom half was inoculated with a pipette by streaking 30 μL of a 10^8 CFU mL^{-1} yeast culture (Fu et al., 2016). Plates were placed vertically in a growth chamber for an additional three weeks. Negative control treatments without yeasts were performed in parallel. The dry weight of aerial and root parts of the plants were quantified after drying plant material to a constant weight in a ventilated oven at 60 ºC. Samples were pooled due to low weight of individual seedlings. The experiment was performed twice.

2.6. Seed bioassay

Nicotiana tabacum (cv. Petite Havana) and *Lactuca sativa* (cv. Batavia) seeds were surface sterilised with 1% sodium hypochlorite-0.1% (v/v) Tween 20 solution for 30 min and washed five times with sterile water. Twenty seeds were spread out on one sheet of filter paper (moistened with 2 mL of sterilised tap water) inside a water-agar Petri plate (Amprayn et al., 2012; Verma et al., 2019) and two plates were seeded per yeast strain. The experiment was performed twice (tobacco) or three times (lettuce). The inoculum (10^8 CFU mL^{-1}) of the different yeast strains was taken from a two-day yeast YPD culture suspension (28 ºC and 150 rpm). Prior to inoculation, 2 mL of the yeast cultures were pelleted by centrifugation (10,000 *g* 10 min), washed twice, resuspended in 1 mL of sterile water and poured onto the plate over the seeds. For negative controls, seeds received 1 mL of water. Seeds were incubated at 28 ºC and 16 h light at 200 μmol m^{-2} s^{-1} for 15 days. Germination was considered to have occurred when the radicles were >2 mm. The germination percentage was recorded every 24 h for five days. Percentage and precocity of seed germination, dry weight, primary root length and numbers of secondary roots (> 0.5 cm) were measured. Chlorophylls were extracted with acetone. Samples (0.1 g leaf material with 8 mL 80% acetone) were homogenised and centrifuged at 18,000 *g* for 10 min at 4 ºC. The amount of chlorophyll a and b was measured spectrophotometrically and calculated according to Lichtenthaler (1987). Samples for dry weight and chlorophyll content were pooled due to low weight of individual seedlings. To assess the survival of the yeasts on the roots, at the end of the cultivation period some seedlings were placed directly on top of the YMA medium. The yeast growth on the plate after two days at 28 ºC was observed.

2.7. Assaying promotion of in vitro *maize growth by yeasts*

A phytotron experiment was carried out with the maize cv. Julliet. Seeds were surface sterilised with 70% ethanol, washed once with sterile water followed by application of 1% sodium hypochlorite-0.1% (v/v) Tween 20 solution for 30 min and washed again five times with sterile water. Seeds were pre-germinated in a closed paper tray onto sterile moist paper towels at 28 ºC in the dark for three days to obtain uniform seedlings. Visual selection was made to ensure homogeneity among germinated seedlings (roots were about 15 mm and hypocotyl 10 mm long). One seedling was placed per glass tube (15 x 2.5 cm) filled with 6 g of sterilised peat:perlite (3:1) and covered with another 2 g of substrate. Twenty to 40 replicates per yeast were assayed. The experiment was repeated twice.

Yeast inoculum was prepared on YPD broth at pH 6.8 and incubated in the dark on an orbital shaker at 150 rpm and 28 ºC for two days. Cultures were collected by centrifugation for 5 min at 3,000 *g* and pellets were resuspended in the same volume of sterile water. The concentration of the inoculum was measured and adjusted to 10^8 CFU mL^{-1}. Each maize seedling received 3 mL of yeast solution after watering with 13 mL of sterile water. Control seedlings received an additional 3 mL of water instead of yeast. Plants were placed in a phytotron at 28 ºC, 16 h light and 200 μmol m^{-2} s^{-1} for 15 days. Plants were watered with 13 mL of sterile water every five days. At the end of the 15-day culture period chlorophyll was measured in the central part of the 3rd leaf (SPAD-502 Plus, Konica-Minolta). The length of the most expanded leaf and the fresh/dry weight of the shoots or roots after washing off the substrate were used to measure the effects of yeasts on plant growth. To assess yeast survival on the roots at the end of the cultivation period, 1 cm root fragments were taken, washed three times with distilled water and placed directly on YMA medium. Live yeasts were able to grow on the plate after two days at 28 ºC.

2.8. Statistical analysis

The numerical data are presented as the arithmetic mean and standard deviation. The significance of differences among the treatments was statistically evaluated by t-tests and one-way ANOVA with Tukey's pairwise comparison as *post hoc* test for multiple comparisons ($p<0.05$). Homogeneity of variance was calculated by Levene's test. Pearson's correlation test was performed to measure the strength of associations. All calculations were performed with SPSS.24 software.

3. Results

A subset of 69 strains from a collection of yeasts naturally occurring in Spanish vineyard environments was selected (Table 1), representing a broad taxonomic diversity from a collection that originated from grape samples. Sixty-six isolates were classified as Ascomycota and three as Basidiomycota (*Rhodotorula* spp.), grouped into 13 genera and 30 species.

3.1. Analysis of plant growth-promoting traits

3.1.1. Solubilisation of nutrients: phosphate and zinc solubilisation capacity

Phosphorous in soil may be chemically fixed with other metal cations and its availability is usually limited. In this study, 33 out of 69 yeast strains exhibited *in vitro* tri-calcium diphosphate solubilising ability (Table 1), revealing different diameters of the clear solubilisation zones (Sup. Fig.S1). The strongest solubilising efficiency (SE) was recorded in *Lachancea thermotolerans* (5 strains out of 7 with SE> 2 and the maximum, strain Lt-47, with SE= 2.56). All *Saccharomyces* spp. and *Hanseniaspora uvarum* strains exhibited phosphate solubilisation ability. No strains of the genera *Debaryomyces*, *Issatchenkia*, *Meyerozyma*, *Rhodotorula* and *Torulaspora* showed any solubilisation capacity.

Nevertheless, all isolates exhibited ZnO solubilising activity to some extent, with *Candida pimensis* Cpi-27 being the strain with the maximum capacity (SE=6.25), followed by *C. apicola* Ca-40 (SE=5.83).

3.1.2. Production of biomolecules: IAA, siderophores, catalase and NH_3

IAA production

IAA production by yeasts was tested with or without L-Trp as a biochemical precursor to compare synthesis in a Trp-dependent or independent pathway (Table 1). IAA production was generally higher in the presence of 0.1% L-Trp, ranging from 0.13 to 27.83 μg mg^{-1} DW produced by *Meyerozyma guilliermondii* Mg-62, which was the highest IAA producer (Sup. Table S1). Other high IAA-producers were *C. zemplinina* strains Cz-37, 41 and 68, *C. pimensis* Cpi-27, *L. lanzarotensis* Ll-21 and 43, and *R. mucilaginosa* Rm-8. Some of these strains increased the IAA production capacity by up to 57-fold (Ll-21) in the presence of L-Trp in the culture media. Three isolates produced significantly higher IAA levels (*C. oleophila* Co-13, *L. thermotolerans* Lt-60 and *Saccharomyces cerevisiae* Sc-44) and 41 isolates produced lower IAA content in the absence of exogenous L-Trp. The maximum amount of IAA produced without L-Trp was 11.12 μg mg^{-1} DW by *L. thermotolerans* Lt-60 (Sup. Table S1), which surprisingly produced 9 times more IAA in the absence of L-Trp than when it was present. Without L-Trp, two isolates did not produce any measurable IAA (*H. uvarum*

Hu-19 and *L. thermotolerans* Lt-7). Only one strain, *L. thermotolerans* Lt-7, produced no measurable IAA under any growing condition. The level of IAA produced within a given species was strain-dependent, as can be observed in *C. zemplinina* or *M. guilliermondii* isolates, for example.

Siderophores

Siderophores are high-affinity iron-chelating compounds secreted by many microorganisms. CAS medium agar plates were used to detect the presence of siderophores when the blue colour turns to orange (Sup. Fig. S1). Fifty-three isolates showed positive results for siderophore production (Table 1) and all *Rhodotorula* spp. and *Metschnikowia pulcherrima* strains exhibited the highest producing capacity.

Catalase

Catalase is an enzyme that catalyses the decomposition of hydrogen peroxide to water and oxygen, being important in microorganisms for resistance to damage (Freimoser et al., 2019; Kaushal et al., 2018). In this study it was produced by all yeast isolates tested (Table 1 and Sup. Fig. S1). The highest oxygen production was observed in *H. pseudoburtonii* Hp-54, *C. oleophila* Co-65, all strains of *Pichia fermentans*, and two out of the three strains of *I. terricola* (It-34 and 56). However, other genera like *Debaryomyces, Lachancea, Meyerozyma, Saccharomyces* and *Torulaspora* showed low oxygen production.

NH_3

In this study 39 strains exhibited NH_3-producing abilities (Table 1 and Sup. Fig. S1), with *L. lanzarotiensis* Ll-21, *M. caribbica* Mc-52, *R. mucilaginosa* Rm-8 and all *M. guilliermondii* strains having the highest production. Some variability was observed in the rest of the strains even though they belonged to the same species.

3.1.3. Cell wall-degrading enzyme production

Yeasts were investigated for their ability to synthesise some fungal cell wall-degrading hydrolytic enzymes (Table 1 and Sup. Fig. S1). In fungi, the cell wall consists basically of chitin, β-1,3-glucan and some proteins (Latgé, 2007), therefore extracellular chitinase, β-1,3-glucanase and protease activities were detected by clearing zones in agar plates in the presence of appropriate substrates. All isolates of *C. lusitaniae*, *Debaryomyces* spp., *M. pulcherrima*, *Meyerozyma* spp. and *Rhodotorula* spp. were highly active. In contrast, none of the strains of *C. apicola, H. uvarum, L. lanzarotensis* and *P. fermentans* produced detectable chitinase or β-1,3-glucanase activity.

Protease activity was also species-dependent, with *R. glutinis* Rg-10 and *M. pulcherrima* Mp-23 having the highest activity. However, *Meyerozyma* spp. and most of the *Lachancea* spp. did not show protease activity.

Cellulase activity was measured because the cell walls of some important plant pathogens (Oomycetes) contain cellulose (Clavaud et al., 2009). Cellulase activity was present in 24 isolates. *C. apicola* Ca-40 and *L. thermotolerans* Lt-7 exhibited the strongest activity.

Pectinase activity was also studied in all isolated strains because it can be related to the plant biomass degradation ability. Pectinases comprise several enzymes that promote the natural degradation of pectins, which are polysaccharides present in plant cell walls (Merín et al., 2015). Sixty-three strains had some kind of pectinolytic activity in solid medium containing citrus pectin, but only four of them had a DA> 1.5 (*R. glutinis* Rg-10, *L. lanzarotensis* Ll-21, *L. thermotolerans* Lt-7 and Lt-29), which is considered a very good level of pectinase production (Kabir and Tasmim, 2019).

430 **Table 1.** Plant growth-promoting traits of yeasts isolated from grapes of *Vitis vinifera*.

431

Strains		Solubilisation efficiency of minerals		IAA production		Biomolecule production			Cell wall-degrading enzyme production					*In vitro* plant tests	
		TCP (SE)	ZnO (SE)	w/o L-Trp	with 0,1% L-Trp	Siderophores	Catalase test	NH_3	Chitinase	β-1,3-Glucanase	Protease (DA)	Cellulase (DA)	Pectinase (DA)	*Nicotiana benthamiana* co-culture	Tobacco seedling promotion
Ca-12	*Candida apicola*	1.13	3.13	1.00 (± 0.55)	0.97 (± 0.03)	-	+	-	-	-	1.10	-	1.14	N	-
Ca-40		1.24	5.83	0.38 (± 0.02)	1.80 (± 0.08) *	-	+	-	-	-	1.18	2.56	-	<C	-
Ci-3	*Candida intermedia*	-	2.13	2.62 (± 0.15)	3.40 (± 0.26)	++	+++	++	+++	++++	-	-	-	N	-
Cl-14	*Candida lusitaniae*	-	1.88	1.11 (± 0.03)	3.92 (± 0.07)*	+	+	++	+++	++++	-	-	1.11	N	-
Cl-28		-	1.86	0.68 (± 0.11)	1.64 (± 0.04) *	+	+	++	+++	++++	-	-	1.06	<C	-
Cl-66		-	1.71	3.76 (± 0.10)	4.54 (± 0.05) *	+	+	++	++++	++++	-	-	1.06	N	-
Co-13	*Candida oleophila*	-	1.67	0.91 (± 0.03) *	-	+	++	-	+++	++	-	-	1.22	=C	-
Co-65		-	2.56	2.27 (± 0.34)	1.73 (± 0.09)	++	++++	+	+	+++	1.68	1.20	-	=C	-
Cp-18	*Candida parapsilosis*	-	2.33	0.57 (± 0.01)	0.77 (± 0.14)	++	+	++	++	++	-	-	1.12	>C,SR	-
Cpi-27	*Candida pimensis*	-	6.25	5.10 (± 0.15)	10.88 (± 0.25) *	+	+	-	+++	++++	1.80	1.20	1.13	N	-
Cr-26	*Candida railenensis*	-	1.50	1.21 (± 0.01)	2.26 (± 0.02) *	+	+++	+	+++	++	-	-	1.11	<C	-
Cv-15	*Candida vanderwaltii*	-	2.50	0.08 (± 0.03)	3.61 (± 0.06) *	+	+	+	+	+	1.17	-	1.13	>C	-
Cz-37	*Candida zemplinina*	1.44	3.50	0.94 (± 0.10)	20.92 (± 5.13) *	+	+	-	-	-	1.13	-	1.08	N	+
Cz-41		1.14	1.83	5.83 (± 0.49)	14.79 (± 0.57) *	+	+	-	-	-	-	-	1.08	N	-
Cz-63		-	2.50	0.84 (± 0.12)	1.71 (± 0.86)	+	+	+	++	+	-	1.25	1.40	>C	+
Cz-68		1.25	2.67	6.34 (± 0.39)	12.61 (± 2.02) *	++	++	-	-	-	-	-	1.08	N	-
Dh-17	*Debaryomyces hansenii*	-	2.22	0.46 (± 0.02)	1.12 (± 0.04) *	++	+	+	+++	+++	1.18	-	1.18	>C,SR	-
Dh-49		-	2.75	1.75 (± 0.22)	2.95 (± 0.04) *	-	+	-	+++	++	-	1.38	1.47	<C	+
Dh-67		-	1.75	0.97 (± 0.04)	1.18 (± 0.04)	++	+	++	+++	++++	1.10	-	1.12	N	-
Dn-33	*Debaryomyces nepalensis*	-	2.25	1.53 (± 0.65)	1.35 (± 0.07)	++	+	+	++++	++++	-	-	1.11	<C	-
Hu-19	*Hanseniaspora uvarum*	1.53	2.40	-	2.23 (± 0.13) *	++	++	+	-	-	1.19	-	-	>C	+
Hu-24		1.38	2.33	1.16 (± 0.01)	5.55 (± 0.24) *	+	+	-	-	-	1.71	-	1.40	>C	+
Hu-25		1.32	2.17	0.25 (± 0.03)	3.82 (± 0.04) *	+	++	-	-	-	1.19	-	1.13	=C	+
Hu-48		1.17	1.80	1.77 (± 0.08)	4.83 (± 0.14) *	++	+	-	-	-	1.09	1.20	1.13	>C	-
Hp-54	*Hyphopichia pseudoburtonii*	-	1.75	2.62 (± 0.05)	5.89 (± 0.14) *	++	++++	+	++++	++++	1.60	-	1.10	>C	-
It-34	*Issatchenkia terricola*	-	2.08	0.86 (± 0.02)	2.01 (± 0.10) *	+	++++	-	+	+	1.16	-	1.14	>C	-
It-36		-	2.20	1.31 (± 0.09)	3.16 (± 0.05) *	+	++	-	+	+	1.20	-	1.27	>C	-
It-56		-	2.00	1.75 (± 0.47)	2.89 (± 0.08)	+	++++	-	++	+	1.09	-	1.43	>C	+
Ll-21	*Lachancea lanzarotensis*	-	2.33	0.18 (± 0.01)	10.36 (± 0.08) *	++	+	+++	-	-	-	1.75	1.50	N	-
Ll-43		-	2.00	1.65 (± 0.82)	15.14 (± 0.46) *	-	++	-	-	-	-	1.38	1.17	<C	-
Lt-7	*Lachancea thermotolerans*	1.67	2.00	-	-	+	+	+	++	+	-	2.25	1.57	<C	-
Lt-29		1.90	2.60	0.68 (± 0.23)	1.40 (± 0.32)	+	+	+	+	+	-	2.00	1.64	<C	-
Lt-42		2.30	2.20	0.62 (± 0.03)	1.04 (± 0.16)	+	+	+	+++	+	-	-	1.13	N	-
Lt-47		2.56	2.00	0.77 (± 0.08)	1.25 (± 0.3)	+	+	++	+++	+++	-	1.13	1.29	<C	+

Lt-60	*Lachancea thermotolerans*	2.48	2.50	11.12 (± 0.10) *	1.93 (± 0.03)	+	+	+	+++	++	-	1.50	1.31	<C	-
Lt-61		2.52	2.67	0.11 (± 0.04)	0.18 (± 0.03)	+	+	++	+++	+	1.16	-	1.21	<C	-
Lt-69		2.22	2.17	0.12 (± 0.01)	0.45 (± 0.16)	-	+	++	+++	+	-	1.50	1.29	<C	-
Mp-16	*Metschnikowia pulcherrima*	1.43	2.25	1.92 (± 0.97)	2.73 (± 0.15)	+++	++	++	++++	++++	1.46	-	1.13	>C,SR	-
Mp-22		1.22	1.89	1.86 (± 1.06)	3.68 (± 0.15)	+++	++	++	++++	++++	-	1.10	1.13	<C	-
Mp-23		1.26	1.67	0.89 (± 0.03)	4.64 (± 0.11) *	+++	++	+	++++	++++	2.13	1.25	1.13	<C	-
Mp-30		1.38	2.22	2.46 (± 0.18)	4.09 (± 0.14) *	+++	++	+	++++	++++	1.78	-	1.08	<C	-
Mp-35		-	2.43	2.47 (± 0.30)	3.74 (± 0.13)*	+++	++	++	++++	++	1.95	1.25	1.07	>C,SR	+
Mp-50		1.20	1.75	1.95 (± 0.1 0)	5.38 (± 0.14) *	+++	++	++	++++	++++	1.98	-	1.07	<C	+
Mc-52	*Meyerozyma caribbica*	-	2.13	2.08 (± 0.06)	4.11 (± 0.14) *	-	+	+++	++++	++++	-	-	1.06	<C	-
Mc-57		-	2.50	1.06 (± 0.04)	2.22 (± 0.03) *	++	+	++	++++	++++	-	-	1.13	N	-
Mg-11	*Meyerozyma guilliermondii*	-	2.44	0.45 (± 0.03)	0.37 (± 0.02)	-	+	+++	+++	+++	-	1.10	1.07	N	+
Mg-46		-	1.88	2.41 (± 0.36)	4.92 (± 0.14) *	-	+	+++	+++	+++	-	-	1.13	N	-
Mg-62		-	2.75	4.54 (± 0.07)	27.83 (± 1.32) *	-	+	+++	+++	++++	-	-	1.12	<C	+
Pd-2	*Pichia dianae*	1.25	4.71	0.68 (± 0.06)	2.37 (± 0.10) *	+	++	-	-	+	1.18	-	1.36	N	+
Pf-31	*Pichia fermentans*	-	2.00	0.66 (± 0.24)	1.32 (± 0.29)	+	++++	-	-	-	1.43	-	1.11	>C	-
Pf-51		-	1.80	1.18 (± 0.22)	2.92 (± 0.46)	+	++++	-	-	-	1.39	-	1.12	>C	-
Pf-70		1.25	1.91	1.91 (± 0.49)	0.84 (± 0.25)	-	++++	-	-	-	1.66	-	-	=C	-
Pm-55	*Pichia membranifaciens*	-	4.29	0.58 (± 0.05)	1.79 (± 0.06) *	+	++++	-	-	-	1.87	1.75	1.43	<C	+
Rd-1	*Rhodotorula dairenensis*	-	2.83	0.58 (± 0.03)	1.60 (± 0.11) *	+++	+	+	+++	+++	1.72	-	1.13	>C	+
Rg-10	*Rhodotorula glutinis*	-	2.00	5.02 (± 1.12)	6.19 (± 0.48)	+++	++	++	+++	+++	2.50	-	1.86	=C	-
Rm-8	*Rhodotorula mucilaginosa*	-	1.54	2.22 (± 0.39)	15.64 (± 1.41) *	+++	++	+++	+++	+++	1.97	-	1.13	<C	+
Sb-20	*Saccharomyces bayanus*	1.41	2.75	0.14 (± 0.02)	4.82 (± 0.08) *	-	+	-	+	+	1.31	-	1.33	<C	-
Sc-4	*Saccharomyces cerevisiae*	1.43	3.00	0.24 (± 0.03)	0.60 (± 0.04) *	+	+	-	++	++	1.13	1.50	1.43	N	-
Sc-6		1.28	3.38	0.35 (± 0.02)	2.76 (± 0.12) *	+	+	-	++	+	1.13	1.13	1.13	N	+
Sc-39		1.50	4.38	0.94 (± 0.15)	3.65 (± 0.09) *	-	+	-	-	-	1.21	-	-	N	+
Sc-44		1.17	1.86	1.91 (± 0.21) *	0.13 (± 0.02)	-	+	+	-	-	-	-	1.23	<C	-
Sc-45		1.28	3.13	1.14 (± 0.06)	2.52 (± 0.06) *	-	+	-	+	+	1.38	1.50	1.23	<C	-
Sc-64		1.29	4.00	1.61 (± 0.17)	1.93 (± 0.08)	+	+	+	+	+	1.19	-	1.13	N	+
Su-5	*Saccharomyces uvarum*	1.33	3.43	0.41 (± 0.06)	6.91 (± 0.07) *	+	+	-	++	+	1.18	1.25	1.43	N	+
Td-38	*Torulaspora delbrueckii*	-	2.25	0.32 (± 0.2)	0.35 (± 0.02)	-	+	-	+	-	1.31	-	1.13	N	-
Td-53		-	2.22	1.69 (± 0.07)	2.04 (± 0.46)	+	+	-	-	-	-	-	1.43	N	-
Td-58		-	2.50	0.46 (± 0.13)	0.62 (± 0.17)	+	+	-	-	-	1.31	-	1.17	N	-
Wa-32	*Wickerhamomyces anomalus*	1.43	2.00	3.30 (± 0.22)	6.91 (± 0.35) *	-	++	++	+++	+++	1.95	1.50	1.31	>C	-
Wa-59		1.30	2.22	0.73 (± 0.04)	1.77 (± 0.05) *	+	+	-	+	+	1.11	1.25	1.13	N	-

SE (Solubilisation efficiency)= (diameter of colonies and halo zone)/ diameter of colonies; - activity not detected. TCP: tri-calcium diphosphate, ZnO: zinc oxide; IAA: indole-3- acetic acid production (μg mg^{-1} dry weight) expressed as mean of three replicates ±SD, asterisks indicate statistically different IAA values at $P<0.05$ between treatments with or w/o L-Trp according to t-test; L-Trp: L-tryptophan; + slightly positive result to ++++ very strong positive result; DA: degradation activity= (diameter of colonies and halo zone)/ diameter of colonies. Solubilisation efficiency of minerals, biomolecule and cell-wall degrading enzyme production are represented as the mean of three plate replicates. *Nicotiana benthamiana* co-culture <C / >C / =C: less / more / equal growth of seedlings compared to control plants. SR: Short roots; N: no growth detected; Tobacco seedling promotion: - not detected, + growth promotion detected.

3.2. Assessment of growth promotion of Nicotiana benthamiana *seedlings in co-culture with yeasts under* in vitro *conditions*

Yeasts were evaluated *in vitro* for their plant growth-promoting ability using a co-culture system with *Nicotiana benthamiana* (Fu et al., 2016). Seedlings were placed on the upper half of the plate and the lower half of the plate was inoculated with yeast. Remarkable differences in the shoot and root growth were observed compared with controls after three weeks of co-cultivation. It was possible to differentiate five groups of phenotypic responses (shown in Table 1 and Fig.1): (i) no growth and yellowing or necrosis of seedlings (i.e. Cpi-27); (ii) similar growth as control without yeasts; (iii) stronger growth of shoot and root than control plants (i.e. Hp-54, Pa-32, Rd-1); (iv) weaker growth of shoot and root than control plants; (v) stronger growth of shoot but shorter branched roots than control plants (i.e. Mp-16).

Shoot growth was significantly enhanced when seedlings were co-cultivated with 12 of the 69 yeasts tested, reaching levels of dry weight 5-fold higher than control plants in the case of *H. pseudoburtonii* Hp-54 (Fig. 2). In addition, 10 yeasts also significantly increased the root dry weight (Fig. 2), and as much as 20-fold in co-culture with Hp-54 and *R. dairensis* Rd-1 compared to the control plants. Co-cultivation with these yeasts also stimulated the development of lateral roots and root hairs (Fig. 1A-B). However, strain Mp-16 promoted greater shoot development with inhibition of primary root elongation, compared to the control plants (Figs. 1A and 2). Fifty-two yeast strains did not promote any visible effect, prevented seedling development or even promoted necrosis (Table 1). The plant growth enhancement observed seems to have some relationship to the species only in the case of *H. uvarum*, *I. terricola* and *P. fermentans*, in which all or most of the strains were found to have a positive effect on growth. Other species such as *L. thermotolerans*, *M. guilliermondii*, *S. cerevisiae* and *Torulaspora delbruekii* were clearly detrimental to seedling growth in this system.

3.3. Promotion of in vitro *seed germination by yeasts*

An experiment was conducted to evaluate the effects of the 69 yeast strains on the germination process. *Nicotiana tabacum* was the species chosen due to its homogeneity in the germination and phenotype of the seedlings. For this purpose, seeds were inoculated with yeasts on filter paper in a water-agar plate for 15 days (Fig. 3A). The percentage of germination and germination

time were not affected by inoculation with yeasts (data not shown). However, it was observed that some yeast strains significantly promoted the vigour of the tobacco plantlets relative to the control after 15 days of inoculation (Table 2): up to 5.7-fold greater dry weight with *M. guilliermondii* Mg-62 and twice the number of roots with several strains. In addition, five of these strains increased the root length, although only seedlings inoculated with *P. dianae* Pd-2 or *M. guilliermondii* Mg-11 increased the chlorophyll content of leaves. Surprisingly, Mg-11 was the only strain that induced shorter roots than in control plants. However, it could be noted that the roots developed more hairs than the control plants (Fig. 3C). For all strains tested it was verified that yeasts were still alive in the plates at the time of data acquisition.

Table 2. Effects of inoculation of *Nicotiana tabacum* seeds with selected plant growth-promoting yeasts.

Yeast strain	Dry weight (μg/plant)	Nº roots/plant	Root length (cm)	Chlorophyll a+b (mg g^{-1} FW)
Mg-62	979.7 ± 63.1 *	3.0 ± 0.01 *	1. 5 ± 0.01	0.32 ± 0.02
Dh-49	874.9 ± 225.0 *	3.9 ± 0.13 *	1.6 ± 0.08	0.28 ± 0.01
Su-5	858.3 ± 41.7 *	3.3 ± 0.14 *	1.7 ± 0.07	0.23 ± 0.01
Pd-2	832.4 ± 92.5 *	3.8 ± 0.15 *	1.9 ± 0.06 *	0.44 ± 0.01 *
Mp-50	830.0 ± 50.0 *	3.0 ± 0.01 *	1.5 ± 0.01	0.31 ± 0.02
Mg-11	811.1 ± 33.3 *	3.8 ± 0.08 *	1.0 ± 0.01 *	0.52 ± 0.02 *
Sc-39	787.5 ± 20.0 *	3.3 ± 0.15 *	1.7 ± 0.08	0.27 ± 0.02
Mp-35	750.0 ± 70.0 *	3.0 ± 0.00 *	1.5 ± 0.01	0.35 ± 0.05
Lt-47	625.0 ± 125.0 *	3.7 ± 0.13 *	1.5 ± 0.01	0.33 ± 0.01
Sc-6	607.1 ± 35.7 *	3.2 ± 0.11 *	1.5 ± 0.05	0.26 ± 0.04
Hu-25	577.7 ± 44.4 *	3.5 ± 0.13 *	1.8 ± 0.06 *	0.27 ± 0.03
Hu-19	534.9 ± 15.0 *	2.9 ± 0.07 *	1.4 ± 0.04	0.30 ± 0.02
Sc-64	492.8 ± 64.3 *	3.0 ± 0.01 *	2.1 ± 0.06 *	0.33 ± 0.02
Rm-8	476.1 ± 9.5 *	2.9 ± 0.08 *	2.0 ± 0.09 *	0.30 ± 0.01
Cz-37	473.3 ± 93.3 *	4.0 ± 0.01 *	2.2 ± 0.08 *	0.36 ± 0.01
Pm-55	472.2 ± 27.8 *	3.0 ± 0.01 *	1.6 ± 0.05	0.32 ± 0.02
It-56	468.7 ± 31.3 *	2.8 ± 0.11 *	1.6 ± 0.04	0.29 ± 0.01
Cz-63	425.7 ± 45.7 *	2.7 ± 0.11 *	1.6 ± 0.05	0.31 ± 0.01
Rd-1	392.8 ± 78.6 *	2.2 ± 0.11	1.4 ± 0.05	0.31 ± 0.03
Hu-24	344.4 ± 122.2 *	2.1 ± 0.08	1.7 ± 0.06	0.23 ± 0.03
Control	173.2 ± 10.6	2.0 ± 0.05	1.6 ± 0.04	0.25 ± 0.03

Data shown correspond to yeast inoculations that resulted in significantly greater dry weights per tobacco plant after 15 days growing. Roots longer than 0.5 cm were considered for measurements. Root length value corresponds to the longest root. Data are presented as the means ± SD (n=4-20). The experiment was performed twice. Asterisks indicate statistically different values in relation to control within each column at $P<0.05$ according to t-test.

Because of their interesting effects on seedling vigour and root development, the Pd-2 and Mg-11 yeasts were chosen to demonstrate that these yeast-plant interaction effects are not species-specific. The experiment with tobacco was reproduced in lettuce (Fig.3). Results after 15 days of growth are shown in Table 3. As in the tobacco seedlings, strain Pd-2 significantly increased the shoot and root dry weight and the number of roots per plant (Fig. 3B). However, chlorophyll content and root length did not change with respect to the control. Yeast strain Mg-11 also increased the number of roots and the presence of root hairs (Table 3 and Fig. 3B-C), however, the length of roots

was significantly shorter than control as observed in the tobacco experiment. Chlorophyll content, as with the Pd-2 strain, remained unchanged.

To verify that the beneficial effects on plant growth were due to live yeast cells, the same amounts of dead yeasts were co-cultured with lettuce seeds. None of the measured parameters differed significantly from the control plants (Table 3), corroborating that the positive effects on seedling development were induced by live yeast cells. The same experiment with dead yeasts was also performed with tobacco seeds and the results were equivalent to those observed in lettuce (Sup. Table S2).

Table 3. Effects of inoculation of *Lactuca sativa* seeds with selected plant growth-promoting yeasts.

Yeast strain	Leaf dry weight (mg/plant)	Roots dry weight (mg/plant)	Nº roots/plant	Root length (cm)	Chlorophyll a+b (mg g^{-1} FW)
Pd-2	1.70 ± 0.06 a	1.25 ± 0.02 a	6.98 ± 0.22 a	6.24 ± 0.12 a	0.26 ± 0.02 a
Pd-2 (80ºC)	1.50 ± 0.10 b	1.16 ± 0.08 b	4.75 ± 0.01 c	6.69 ± 0.56 a	0.24 ± 0.01 a
Mg-11	1.57 ± 0.07 ab	0.83 ± 0.05 b	5.81 ± 0.25 b	4.94 ± 0.23 b	0.25 ± 0.03 a
Mg-11 (80ºC)	1.26 ± 0.09 b	1.15 ± 0.15 b	4.81 ± 0.31 c	6.75 ± 0.13 a	0.26 ± 0.01 a
Control	1.32 ± 0.09 b	0.96 ± 0.04 b	4.56 ± 0.29 c	6.05 ± 0.38 a	0.25 ± 0.01 a

Sterile lettuce seeds were treated with live or dead (30 min 80ºC) yeasts and cultivated for 15 days. Roots longer than 0.5 cm were considered for measurements. Root length value corresponds to the longest root. Data are presented as the means ± SD (n= 6-15). The experiment was performed three times. Values with the same letter within each column do not differ significantly (P<0.05) according to Tukey's test.

3.4. In vivo *selection of plant growth-promoting yeasts*

An experiment was designed to investigate the growth-promoting effect of the 69-yeast collection *in vivo* under phytotron conditions. Maize seeds were used for this purpose due to their precocity and homogeneity in germination. Yeasts were added to three-day-old pre-germinated maize seedlings in glass tubes with substrate. After 15 days the growth was evaluated by measuring the height, chlorophyll content and the fresh weight of the aerial parts (Supplementary Fig. 2). Three strains were found to have positive effects on all growth parameters (*S. cerevisiae* Sc-6, *Debaryomyces hansenii* Dh-67 and *L. thermotolerans* Lt-69) and only one (*D. hansenii* Dh-17) had negative effects (Sup. Table S3) in comparison with the control. The same experiment was repeated with the three selected growth-promoting yeasts measuring the dry weight of the aerial parts and roots. As negative controls, the growth of maize seedlings was evaluated with water or with the same concentration of dead yeasts (Table 4).

Table 4. Beneficial effects of selected plant growth-promoting yeasts on *Zea mays* seedlings grown under phytotron conditions.

Yeast strain	Leaf dry weight (mg/plant)	Shoot length (cm)	Chlorophyll (SPAD)
Sc-6	163.56 ± 3.53 a	50.11 ± 0.50 a	39.11 ± 0.19 b
Sc-6 (80ºC)	145.83 ± 3.63 b	48.08 ± 0.67 a	35.02 ± 0.38 c
Dh-67	162.03 ± 3.10 a	49.06 ± 0.46 a	40.12 ± 0.19 a
Dh-67 (80ºC)	146.89 ± 3.92 b	47.29 ± 0.67 a	35.19 ± 0.45 c
Lt-69	163.12 ± 4.09 a	49.92 ± 0.56 a	40.63 ± 0.21 a
Lt-69 (80ºC)	147.50 ± 3.43 b	46.77 ± 0.68 a	35.15 ± 0.47 c
Control	147.68 ± 2.85 b	48.19 ± 0.48 a	35.80 ± 0.25 c

Maize seedlings were treated after 3 days of germination with live or dead (30 min 80ºC) yeasts and cultivated for a further 15 days. Data are presented as the means ± SD (n= 40). The experiment was performed twice. Values with the same letter within each column do not differ significantly ($P<0.05$) according to Tukey's test.

The presence of the three live yeast strains increased the values of dry weight and chlorophyll content by around 10% after 15 days of inoculation, but no significant differences were observed in the shoot length. The root weight was neither altered (data not shown). Parameters measured in plants treated with the same amount of dead yeasts did not differ from the control plants (Table 4), corroborating again the beneficial effects for seedling development of live yeast cells. For the three strains studied it was verified that the yeasts were alive in the substrate at the moment of data acquisition.

4. Discussion

The rising growth of organic farming coupled with awareness of the hazards associated with some chemical fertilisers and a stricter regulatory scenario in several countries has required many farmers to implement biofertilisers instead of their chemical counterparts. This new context is expected to boost the demand for biofertilisers over the next decade. In addition, the development of indigenous phytobeneficial products that suit local markets may enhance effectiveness and preserve local environments.

Plants are associated with microorganisms, which have the ability to improve plant growth and stress tolerance, promote nutrition and antagonise pathogens. The integration of beneficial plant-microbe interactions may represent a promising sustainable solution to improve agricultural production (Timmusk et al., 2017). Currently, the composition of most biofertilisers is based on rhizobacteria, but sometimes the results with these species are inconsistent in the field (Backer et al., 2018). In contrast, the use of yeast plant-enhancement agents has been under-exploited, despite their potential to produce bioactive compounds and evidence for enhancing growth, i.e., in rice

(Amprayn et al., 2012; Verma et al., 2019), sugar beet (El-Tarabily, 2004) or maize (Gollner et al., 2006; Nakayan et al., 2013; Nassar et al., 2005; Sarabia et al., 2017). Yeast strains originating from plants in the natural environment are well-adapted and resistant to various factors, such as temperature and pH levels, as well as osmotic and oxidative stresses (Pawlikowska et al., 2019; Sui et al., 2015). Following our review of the literature, it was clear that no data on Spanish crop-associated PGP yeasts was available.

In the present work a collection of 69 yeast strains from 13 genera isolated from grapes in Spanish vineyards (Table 1) was studied. Sixty-six isolates were classified as Ascomycota, which are generally more frequent and abundant in agricultural soils, orchards and grasslands (Yurkov, 2018), and three as Basidiomycota (*Rhodotorula* spp.). Grape berries harbour a wide range of microbes originating from the vineyard environment, in particular yeast of the genera *Candida*, *Hansenula, Hanseniaspora, Kluyveromyces, Metschnikowia, Pichia, Rhodotorula* and *Saccharomyces* (Renouf et al., 2005). Many of them are recognised for their role in the must fermentation process that shapes wine quality (Mezzasalma et al., 2017).

4.1. Plant growth-promoting traits of grape-isolated yeasts

It is known that yeasts can release molecules such as phytohormones, organic acids, siderophores, lytic enzymes or even volatile compounds, which may impact directly or indirectly on plant growth (Botha, 2011; de Souza et al., 2019; El-Tarabily and Sivasithamparam, 2006; Freimoser et al., 2019; Fu et al., 2016; Nassar et al., 2005; Sun et al., 2014). Therefore, we have taken an integrated approach, testing all isolates for a variety of PGP attributes.

Phosphorus solubilising capacity is one of the most valued characteristics in the microorganisms used in biofertilisation since a large portion of the phosphorus present in the soil is in the form of insoluble phosphates and cannot be utilised by the plants easily (Sharma et al., 2013). Phosphate applied as a chemical fertiliser is rapidly immobilised, becoming unavailable to plants. To ameliorate phosphorus deficiency, large amounts of fertiliser are used, which can lead to environmental degradation and increased crop production costs (Sharma et al., 2013). Several phosphate-solubilising microorganisms present in the soil are capable of solubilising inorganic phosphorus by releasing organic acids that promote acidification, secretion of extracellular phosphatases, chelation and exchange reactions (Alori et al., 2017). Among them, some yeast have been studied (Gizaw et al., 2017;

Narsian et al., 2010). In the current study, 33 out of 69 yeast strains exhibited *in vitro* tri-calcium diphosphate solubilising ability (Table 1), the strongest solubilising activity being recorded by *L. thermotolerans*, and in strain Lt-47 in particular. This is the first time that this high capacity has been described for *L. thermotolerans*. Nakayan et al. (2013) observed that soil applications of a yeast strain (*M. guilliermondii*) with a solubilising efficiency similar to Lt-47, reduced the application of chemical fertilisers without affecting the optimal productivity of maize.

On the other hand, zinc is a plant micronutrient that is involved in many physiological functions and its inadequate supply reduces crop yields. Zinc deficiency is the most widespread micronutrient deficiency problem (Hafeez, 2013). All the isolates reported here have shown some ZnO solubilising capacity, with *C. pimensis* Cpi-27 and *C. apicola* Ca-40 having the most outstanding efficiency.

Maximum solubilisation ratios described for P and Zn in the strains studied are higher than those reported in other yeast strains (Fu et al., 2016; Nutaratat et al., 2014). These data bring to light the interesting characteristics of some strains in this collection.

IAA is a major phytohormone used by plants to regulate growth and it is involved in several physiological processes including plant cell elongation and division, germination, vascular development and root growth (Luo et al., 2018). Of particular interest for the study of PGP microorganisms is the influence of IAA on root length and lateral root production. Inoculation with IAA-producing microorganisms such as rhizobacteria (Dobbelaere et al., 1999) or yeasts (Fu et al., 2016; Sun et al., 2014), is known to enhance the formation of lateral roots and root hairs, thus promoting increased access to soil-based nutrients. The reports available to date indicate that IAA synthesis is a frequent trait among yeasts (Fu et al., 2016; Limtong and Koowadjanakul, 2012; Sun et al., 2014;). The data suggest that yeast may have multiple pathways for IAA synthesis, one of which is not dependent on Trp (Rao et al., 2010), and this is an important point as Trp availability may influence yeast IAA production (Fu et al., 2016; Ignatova et al., 2015). To confirm the existence of a Trp-independent pathway in the isolated yeasts, IAA production was analysed in cultures with and without Trp. Assessment of the 69 yeast strains showed that IAA was present in the supernatant of liquid culture of 67 of them in the absence of L-Trp. Therefore, our results corroborate the existence of a general tryptophan-independent IAA biosynthesis pathway. In the presence of L-Trp,

IAA production was enhanced several-fold in 41 strains, supporting the concept that the Trp-dependent pathway is the most efficient in the majority of the studied strains. The IAA quantification indicated that IAA-producing capability varies significantly in different strains of the same species (Sup. Table S1), therefore the trait is prominently strain-dependent. Such results are consistent with previous studies in yeasts (Fu et al., 2016; Streletskii et al., 2016; Sun et al., 2014) and other microorganisms (Ruanpanun et al., 2010).

The production of NH_3 is a desirable PGP activity because it plays an important role in plant growth promotion via nitrogen availability (Marques et al., 2010). NH_3 is a molecule produced and widely utilised by yeasts, probably as a signal mediator for communication between colonies (Palkova et al., 1997). Among the 39 strains that showed positive results, the genus *Meyerozyma* should be highlighted as the highest NH_3 producer.

Siderophores are small compounds secreted by many microorganisms with high-affinity for ferric iron, and these compounds support plant growth via iron chelating (Johnson et al., 2002) and indirectly inhibit plant pathogenic fungi by scavenging available iron from the environment (Ahmed and Holmström, 2014). This primary competition strategy was demonstrated in the yeast *M. pulcherrima*, which produces the red pigment pulcherrimin in the presence of iron and in response to a range of fungal pathogens such as *Botrytis cinerea*, *Alternaria alternata* and *Penicillium expansum* (Saravanakumar et al., 2008). Accordingly, all *M. pulcherrima* strains in our collection assimilated ferric iron efficiently from the surrounding substrate (Table 1). *Rhodotorula* spp. strains were also found to be strong siderophore producers. Rhodotorulic acid has been identified as a siderophore produced by yeasts belonging to this genus (Atkin et al., 1970).

The presence of catalase in microorganisms is useful for cell protection against oxidative damage caused by reactive oxygen species, conferring resistance to environmental, mechanical and chemical damages (Freimoser et al., 2019; Kaushal et al., 2018). Therefore, PGP yeast with catalase activity will have a greater likelihood of surviving in the rhizosphere, promoting plant growth indirectly (Martins and English, 2014). Our results indicated that catalase was produced by all the yeast isolates tested (consistent with Fu et al., 2016 and Nutaratat et al., 2014) and the production of oxygen in the presence of hydrogen peroxide was species dependent. All *P. fermentans*

689 isolates along with Co-65, Hp-54, and the It-34 and 56 strains showed high
690 catalase activity.
691
692 One of the mechanisms whereby PGP yeasts facilitate plant growth is through
693 direct competition with other pathogenic microorganisms, with the production
694 of fungal cell wall-degrading enzymes being an important part of this strategy
695 (de Souza et al., 2019; Freimoser et al., 2019; Fu et al., 2016; Nutaratat et al.,
696 2014). Hence, β-1,3-glucanase, protease, chitinase and cellulase activities
697 were studied. Eight strains were positive for all the enzymes tested (Co-65,
698 Cp-18, Mp-23 and 35, Wa-32 and 59 and Sc-4 and 6). All these species have
699 been reported previously for their biocontrol abilities. For example, *C.*
700 *oleophila*, which is the basis of the commercial products Aspire® and Nexy®
701 that target postharvest spoilage of stored fruits, is capable of producing and
702 secreting cell wall-degrading enzymes (Bar-Shimon et al., 2004; Carmona-
703 Hernandez et al., 2019). Exo-β-1,3 glucanases and chitinases have been also
704 shown to contribute to the mechanism of action of *W. anomalus* strain K
705 (reviewed in Muccilli and Restuccia, 2015), *M. pulcherrima* strain MACH1
706 (Saravanakumar et al., 2009) and *S. cerevisiae* (Abdel-Kareem et al., 2019;
707 Lima et al., 2013). Fallah et al. (2016) demonstrated the fungal antagonistic
708 effect of *C. parapsilosis* on the growth of *Fusarium* species, however its
709 mechanism of action is still unclear.
710 Pectin degrading enzymes can help to unlock the structures of plant cell walls
711 as a new resource of nutrients for plant growth. Most of the yeast isolates
712 analysed in our study showed pectinolytic activity, which can be explained by
713 the fact that there is a high incidence of pectinolytic species in nutrient-poor
714 environments, such as the surface of grapes (Merín et al., 2015), which is the
715 native environment of this yeast collection. It seems that pectinolytic yeasts
716 play an ecological role on the grape surface because they can utilise pectin
717 from cell walls by releasing intracellular sugars to the surface (Merín et al.,
718 2015). Some species have been reported to produce pectinases in the wine
719 ecosystem and include *Lachancea spp., Candida spp.* or *Metschnikowia spp.*
720 (Fernández et al., 2000). *R. glutinis* Rg-10 was the highest pectinase-producer
721 in our collection. Yeasts belonging to these genera have been described
722 previously as pectinolytic microorganisms (Taskin, 2013).
723
724 The knowledge of the particular characteristics of each strain opens up new
725 research possibilities to take advantage of their potential, either alone or in
726 biofertilising preparations that include several strains with different abilities.

Consortia of various organisms with different benefits for crops can be integrated to combine different capabilities into one product with several yield-promoting effects (García-Fraile et al., 2015), as in the case of the commercial biofertiliser BioGro (Hien et al., 2014).

4.2. Growth promotion of plants under in vitro *and* in vivo *conditions*

As a first approach to understand the effects of yeast interactions with plant growth and development, isolated strains were evaluated *in vitro* for their growth-promoting ability using an agar plate co-culture system with *Nicotiana benthamiana* seedlings (Fu et al., 2016). The aim of this study was to verify whether this method could be used to correlate the PGP traits observed in the activity tests (Table 1) with enhancing effects in conditions *in vitro* and later *in vivo*. Ten strains significantly enhanced seedling development, both at the aerial and root levels (Fig. 2), with an up to 5-fold increase in shoot dry weight by *H. pseudoburtonii* Hp-54. This strain and *R. dairensis* Rd-1 also induced a dramatic 20-fold increase in the root biomass dry weight compared to the control plants. In parallel, it was observed that the root system architecture (abundance of root hairs and lateral roots) was substantially changed by the presence of some of these yeasts (Fig. 1). However, these results did not have any correlation with the IAA levels (Table 1) produced by the strains in liquid culture (Pearson's correlation coefficient for leaf and root dry weight 0.5 and 0.2, respectively). Up to now, the plate co-culture method had only been used to associate plant growth and alteration of the root system architecture with a very small number (7) of yeast strains that produced varying IAA amounts in liquid culture (Fu et al., 2016; Sun et al., 2014; Tapia-Vázquez et al., 2020). These authors observed various effects on *Nicotiana benthamiana*, *A. thaliana* and tomato, such as vigorous development, increasing lateral root formation or inhibition of primary root elongation, and attributed them to the high levels of yeast IAA production. We hypothesise that, in our study, lack of correlation between seedling growth and the IAA levels previously measured might be due to differences in IAA production in both culture systems: one optimised for yeast growth (YPD medium) and the other for plant development (MS medium). It has been shown that yeast IAA production capacity depends on the sources of nutrients, pH and environmental growing conditions (Scarcella et al., 2017; Sun et al., 2014). Casarrubia et al. (2016) used *A. thaliana* transgenic reporter lines that carry the promoter of an auxin-responsive gene fused with the GUS reporter gene;

765 this approach would be a method to verify *in situ* the possible effects of IAA
766 accumulation in the plant tissues.
767 It is difficult to elucidate which PGP mechanism is attributed to the best
768 performer strains since this growth system, although simple, is a very confined
769 environment. The positive effect of certain diffusible molecules secreted into
770 the culture medium can also be shielded by the negative effect of others. In
771 addition, the presence of volatile organic compounds (VOCs; Freimoser et al.,
772 2019) or even a nonspecific mechanism, such as CO_2 emissions produced by
773 yeasts, may have important repercussions, as reported before for other fungi
774 (Casarrubia et al., 2016). It is noteworthy that several isolates had negative
775 effects on growth (i.e. *C. pimensis* Cpi-27, Fig. 1A), even to the point of being
776 lethal to the seedlings. This is the first study in which such dramatic effects
777 were observed on the seedling growth in yeast co-cultivation under *in vitro*
778 conditions. Indeed, this co-culture system could be very interesting for
779 studying metabolites and VOCs that influence shoot growth and root
780 morphology. Because *in vitro* studies in PGP yeasts have been limited, a
781 broad field of research could be opened up to decipher the molecular origin of
782 the interesting growth-promoting effects observed with some of the yeasts in
783 this collection.
784
785 To further evaluate the yeast growth-promoting effects, two inoculation
786 experiments were proposed at the germination stage. In the first one (Table
787 2), *in vitro* yeast inoculation of tobacco seeds promoted a significant increase
788 in the seedling dry weight (5.7-fold higher by *M. guilliermondii* Mg-62) in the
789 presence of 20 strains, while precocity and germination percentage were not
790 affected. In addition, important effects on root architecture were also observed,
791 as increasing root numbers (2-fold), enlargement of primary roots (37%) or
792 even development of hairy short roots with *M. guilliermondii* Mg-11 (Fig. 3).
793 This strain, along with *P. dianae* Pd-2, was also able to enhance chlorophyll
794 content. In addition, it was demonstrated that the PGP effects observed in
795 tobacco by the strain with the best performance in all attributes measured, Pd-
796 2, could be reproduced in lettuce and this enhancement only was possible
797 when the yeast inoculum was alive (Table 3). Root shortening and hairy
798 phenotype was also reproducible in lettuce with the strain Mg-11 (Fig. 3),
799 indicating that these effects are not plant species-specific. Nakayan et al.
800 (2013) also observed seedling vigour enhancement *in vitro* in maize and
801 Chinese cabbage in an inoculation experiment with *M. guilliermondii* strain
802 CC1, while the germination percentage was not altered significantly. Similarly,

in other *in vitro* assays with *C. tropicalis* and *S. cerevisiae* with rice seeds, vigour index was significantly higher compared to controls, although there was no change in the germination percentage (Amprayn et al., 2012; Verma et al., 2019). However, Tapia-Vazquez et al. (2020) observed a slight germination precocity when tomato seeds were inoculated with several psychrophilic yeasts. In our study, seedling vigour enhancement seemed to be strain dependent to some extent, although in some species several or most of the strains had promoter capacity (*C. zemplinina*, *H. uvarum, M. guilliermondii* or *S. cerevisiae*). Since there is variability among strains in terms of nutrient solubilisation, production of IAA, siderophores or ammonium, it is also possible that growth-promoting factors other than those studied may also have had a role in seedling vigour enhancement.

In order to assess the promoter effect of yeasts on seed germination in a controlled environment that more closely matched in-field conditions, another experiment was undertaken with plants growing in soil. The species chosen was maize because it has very rapid development and its size facilitates screening in peat containers and phytotrons. It was observed that only three strains (*D. hansenii* Dh-67, *L. thermotolerans* Lt-69 and *S. cerevisiae* Sc-6) were associated with significant increases in fresh weight and chlorophyll content relative to control plants (Sup. Table S3). Inoculation with these three strains also increased leaf dry weight (Table 4) but not root dry weight. It was also proven in this experiment that these strains survived in the rhizosphere at the end of the cultivation period but that the growth-promoting effect disappeared if the seeds were inoculated with dead yeast. Out of three isolates that increased the vigour of *in vivo* maize seedlings, only strain Sc-6 showed vigour enhancement of tobacco seedlings *in vitro*. In view of these results, it can be observed that there is a great variability in the behaviour of the yeasts depending on the growing conditions. As the environmental factors get closer to field conditions, the number of strains with growth-promoter potential decreases. Nakayan et al. (2013) and Amprayn et al. (2012) also observed differences in yeast performance among plant species and between *in vitro* and greenhouse or field conditions. The chemical composition of root exudates, which can serve as a potential nutrition source for rhizospheric microbes, varies among plant species according to the nutrient availability of the plants (Carvalhais et al., 2011). This fact along with other changes in environmental conditions that it is known to affect yeast performance, such as water and nutrient availability (Deak, 2006), could explain differences in yeast growth-promoting effects.

From an agronomic point of view, application of beneficial microorganisms to the rhizosphere or seeds is an efficient mechanism for microbial inoculation of soil where they will be well positioned to colonise seedling roots (O'Callaghan, 2016). Recently, microbial seed coating has been considered as an inexpensive and efficient technology for the delivery of inocula, since it contributes to the production of coated seeds toward meeting ecological safety and efficacy standards (Ma, 2019). However, despite the clear laboratory demonstration of the ability of a wide range of beneficial microorganisms to improve crop performance, there are still very few commercially available microbial seed inoculants due to the variability and inconsistency of results between laboratory, greenhouse and field studies (O'Callaghan, 2016). The reason for the discrepancies lies in the incomplete understanding of relationships between plants, microorganisms and environmental conditions (Malusá et al., 2012) and the complexity of factors affecting microbial performance. Together with the genotype and biochemical characteristics of the introduced strains, the survival and colonisation of microbes in the rhizosphere are determined by several environmental factors, such as soil pH, nutrient and moisture content, plant genotype and physiological state or the presence of other microbial species (Naik et al., 2019).

5. Conclusions

Our results show that isolated yeast strains from grapes in Spanish vineyards have physiological features *in vitro* that are compatible with plant growth promotion. Valuable results have been obtained because this is the first time that such a broad yeast collection has been assayed in parallel for *in vitro* and *in vivo* PGP traits. The isolates exhibited multifarious traits like phosphate and zinc solubilisation or production of IAA, siderophores, NH_3, catalase and different hydrolytic enzymes showing the great potential of these strains. Out of 69 isolates, 11 promising yeast strains were selected on the basis of *in vitro* co-culture PGP assays with *Nicotiana benthamiana* seedlings. This system gives partial information concerning yeast-plant interactions as no correlation with *in vivo* condition effects were observed, probably due to some limitations derived from constraints on the confined environment. Twenty isolates showed significant activity *in vitro* in the seedling vigour experiments with tobacco seeds. Two of the most promising strains (*P. dianae* Pd-2 and *M. guilliermondii* Mg-11) also demonstrated their growth enhancing ability in lettuce. This

behaviour pattern varied when the plant species was changed to maize and *in vivo* conditions were tested instead. Three isolates enhanced shoot dry weight and chlorophyll content, of which only *S. cerevisiae* Sc-6 is common to the species that increased tobacco seedling vigour, making it a good candidate to continue with field experiments. The positive results observed in our study with a broad range of yeast species opens a new door for future agronomic developments in this field.

Supplementary data

Supplementary Figure S1: *In vitro* measurements of plant growth-promoting activities in isolated yeasts.

Supplementary Figure S2: Development of maize seedlings with selected plant growth-promoting yeasts under phytotron controlled conditions.

Supplementary Table S1: IAA production in yeasts grown in liquid culture with or without 0.1% L-Trp.

Supplementary Table S2: Effects of inoculation of *Nicotiana tabacum* seeds with selected plant growth-promoting yeasts.

Supplementary Table S3: Growth-promoting effects of yeasts in maize seedling development planted in soil under phytotron conditions.

Acknowledgements

We are grateful to David García (Lev2050, Aizoain, Navarra, Spain) for the collection of yeasts. This work was financed by Departamento de Desarrollo Económico from the Gobierno de Navarra (Spain): grants 0011-1365-2018-000120 and 0011-1411-2019-000009.

Bibliography

Abdel-Kareem, M.M., Rasmey, A.M., Zohri, A.A., 2019. The action mechanism and biocontrol potentiality of novel isolates of Saccharomyces cerevisiae against the aflatoxigenic Aspergillus flavus. Lett. Appl. Microbiol. 68, 104–111. https://doi.org/10.1111/lam.13105

Ahmed, E., Holmström, S.J.M., 2014. Siderophores in environmental research: Roles and applications. Microb. Biotechnol. 7, 196–208. https://doi.org/10.1111/1751-7915.12117

Al-Falih, A.M., 2006. Nitrogen transformation in vitro by some soil yeasts. Saudi J. Biol. Sci. 13, 135–140.

914 Alori, E.T., Glick, B.R., Babalola, O.O., 2017. Microbial phosphorus
915 solubilization and its potential for use in sustainable agriculture. Front.
916 Microbiol. 8, 1–8. https://doi.org/10.3389/fmicb.2017.00971
917 Amprayn, K.O., Rose, M.T., Kecskés, M., Pereg, L., Nguyen, H.T., Kennedy,
918 I.R., 2012. Plant growth promoting characteristics of soil yeast (Candida
919 tropicalis HY) and its effectiveness for promoting rice growth. Appl. Soil
920 Ecol. 61, 295–299. https://doi.org/10.1016/j.apsoil.2011.11.009
921 Arora, N.K., Tewari, S., Singh, R., 2013. Multifaceted Plant-Associated
922 Microbes and their Mechanisms Diminish the Concept of Direct and
923 Indirect PGPRs, in: Kumar Arora Naveen (Ed.), Plant Microbe Symbiosis:
924 Fundamentals and Advances. Springer International Publishing, pp. 412–
925 447. https://doi.org/10.1007/978-81-322-1287-4
926 Atkin, C.L., Neilands, J.B., Phaff, H.J., 1970. Rhodotorulic acid from species
927 of Leucosporidium, Rhodosporidium, Rhodotorula, Sporidiobolus, and
928 Sporobolomyces, and a new alanine-containing ferrichrome from
929 Cryptococcus melibiosum. J. Bacteriol. 103, 722–733.
930 Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci,
931 E., Subramanian, S., Smith, D.L., 2018. Plant growth-promoting
932 rhizobacteria: Context, mechanisms of action, and roadmap to
933 commercialization of biostimulants for sustainable agriculture. Front.
934 Plant Sci. 871, 1–17. https://doi.org/10.3389/fpls.2018.01473
935 Bar-Shimon, M., Yehuda, H., Cohen, L., Weiss, B., Kobeshnikov, A., Daus, A.,
936 Goldway, M., Wisniewski, M., Droby, S., 2004. Characterization of
937 extracellular lytic enzymes produced by the yeast biocontrol agent
938 Candida oleophila. Curr. Genet. 45, 140–148.
939 https://doi.org/10.1007/s00294-003-0471-7
940 Barbieri, P., Galli, E., 1993. Effect on wheat root development of inoculation
941 with an Azospirillum brasilense mutant with altered indole-3-acetic acid
942 production. Res. Microbiol. 144, 69–75. https://doi.org/10.1016/0923-
943 2508(93)90216-O
944 Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere
945 microbiome and plant health. Trends Plant Sci. 17, 478–486.
946 https://doi.org/10.1016/j.tplants.2012.04.001
947 Berg, G., Rybakova, D., Grube, M., Köberl, M., 2016. The plant microbiome
948 explored: Implications for experimental botany. J. Exp. Bot. 67, 995–
949 1002. https://doi.org/10.1093/jxb/erv466
950 Botha, A., 2011. The importance and ecology of yeasts in soil. Soil Biol.
951 Biochem. 43, 1–8. https://doi.org/10.1016/j.soilbio.2010.10.001

Cappuccino, J., Sherman, N., 2002. Microbiology: a laboratory manual, 6th ed. Pearson Education, Inc. Benjamin Cumming CA.

Carmona-Hernandez, S., Reyes-Pérez, J.J., Chiquito-Contreras, R.G., Rincon-Enriquez, G., Cerdan-Cabrera, C.R., Hernandez-Montiel, L.G., 2019. Biocontrol of postharvest fruit fungal diseases by bacterial antagonists: A review. Agronomy 9. https://doi.org/10.3390/agronomy9030121

Carvalhais, L.C., Dennis, P.G., Fedoseyenko, D., Hajirezaei, M.R., Borriss, R., Von Wirén, N., 2011. Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. J. Plant Nutr. Soil Sci. 174, 3–11. https://doi.org/10.1002/jpln.201000085

Casarrubia, S., Sapienza, S., Fritz, H., Daghino, S., Rosenkranz, M., Schnitzler, J.P., Martin, F., Perotto, S., Martino, E., 2016. Ecologically different fungi affect arabidopsis development: Contribution of soluble and volatile compounds. PLoS One 11, 1–23. https://doi.org/10.1371/journal.pone.0168236

Cattelan, A.J., Hartel, P.G., Fuhrmann, J.J., 1999. Screening for Plant Growth–Promoting Rhizobacteria to Promote Early Soybean Growth. Soil Sci. Soc. Am. J. 63, 1670–1680. https://doi.org/10.2136/sssaj1999.6361670x

Clavaud, C., Aimanianda, V., Latge, J.P., 2009. Organization of fungal, oomycete and lichen (1,3)-β-glucans, in: Chemistry, Biochemistry, and Biology of 1-3 Beta Glucans and Related Polysaccharides. https://doi.org/10.1016/B978-0-12-373971-1.00011-X

Cloete, K.J., Valentine, A.J., Stander, M.A., Blomerus, L.M., Botha, A., 2009. Evidence of symbiosis between the soil yeast cryptococcus laurentii and a sclerophyllous medicinal shrub, agathosma betulina (berg.) pillans. Microb. Ecol. 57, 624–632. https://doi.org/10.1007/s00248-008-9457-9

de Souza, C., Christofoleti-Furlan, R.M., de Souza Miranda Muynarsk, E., Vinicius de Melo Pereira, G., Lucas, D.L., Basso, L.C., 2019. Biotechnological Applications of Nonconventional Yeasts, in: Yeasts in Biotechnology. pp. 1–27. https://doi.org/http://dx.doi.org/10.5772/57353

Deak, T., 2006. Environmental factors influencing yeasts, in: Rosa, C.A., Gabor, P. (Eds.), The Yeast Handbook: Biodiversity and Ecophysiology of Yeasts. Springer International Publishing, pp. 155–174.

Dobbelaere, S., Croonenborghs, A., Thys, A., Vande Broek, A., Vanderleyden, J., 1999. Phytostimulatory effect of Azospirillum brasilense wild type and

mutant strains altered in IAA production on wheat. Plant Soil 212, 155–164.
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. https://doi.org/10.1046/j.1365-2672.2003.02043.x
El-Tarabily, K.A., Sivasithamparam, K., 2006. Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Mycoscience 47, 25–35. https://doi.org/10.1007/s10267-005-0268-2
Fallah, B., Zaini, F., Daei Ghazvini, R., Kachuei, R., Kordbacheh, P., Safara, M., Mahmoudi, S., 2016. The antagonistic effects of Candida parapsilosis on the growth of Fusarium species and fumonisin production. Curr. Med. Mycol. 2, 1–6. https://doi.org/10.18869/acadpub.cmm.2.1.1
Fernández, M., Úbeda, J.F., Briones, A.I., 2000. Typing of non-Saccharomyces yeasts with enzymatic activities of interest in wine-making. Int. J. Food Microbiol. 59, 29–36. https://doi.org/10.1016/S0168-1605(00)00283-X
Freimoser, F.M., Paula, M., Mejia, R., Tilocca, B., Migheli, Q., 2019. Biocontrol yeasts : mechanisms and applications. World J. Microbiol. Biotechnol. 4, 1–19. https://doi.org/10.1007/s11274-019-2728-4
Fu, S.F., Sun, P.F., Lu, H.Y., Wei, J.Y., Xiao, H.S., Fang, W.T., Cheng, B.Y., Chou, J.Y., 2016. Plant growth-promoting traits of yeasts isolated from the phyllosphere and rhizosphere of Drosera spatulata Lab. Fungal Biol. 120, 433–448. https://doi.org/10.1016/j.funbio.2015.12.006
García-Fraile, P., Menéndez, E., Rivas, R., 2015. Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioeng. 2, 183–205. https://doi.org/10.3934/bioeng.2015.3.183
Gizaw, B., Tsegay, Z., Genene, T., Aynalem, E., Wassie, M., Abatneh, E., 2017. Phosphate Solubilizing Fungi Isolated and Characterized from Teff. J. Fertil. Pestic. 8. https://doi.org/10.4172/2471-2728.1000180
Gollner, M.J., Püschel, D., Rydlová, J., Vosátka, M., 2006. Effect of inoculation with soil yeasts on mycorrhizal symbiosis of maize. Pedobiologia (Jena). 50, 341–345. https://doi.org/10.1016/j.pedobi.2006.06.002
Gordon, S.A., Weber, R.P., 1951. Colorimetric estimation of indole acetic acid. Plant Physiol. 26, 192–195. https://doi.org/10.1104/pp.26.1.192
Gouda, S., Kerry, R.G., Das, G., Paramithiotis, S., Shin, H.S., Patra, J.K., 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol. Res. 206, 131–140.

https://doi.org/10.1016/j.micres.2017.08.016
Hafeez, B., 2013. Role of Zinc in Plant Nutrition- A Review. Am. J. Exp. Agric. 3, 374–391. https://doi.org/10.9734/ajea/2013/2746
Hashem, M., Metwally, A.K., 2014. Effect of Combined Inoculation of Rhizobium with Soil yeasts on Nodulation, Groth and Yield of Common Bean (Phaseolus vulgaris L.) under Field Condition. J. Plant Nutr. Fertil. Technol. 4, 1–10.
Hien, N.T., van Toan, P., Choudhury, A.T.M.A., Rose, M.T., Roughley, R.J., Kennedy, I.R., 2014. Field Application Strategies for the Inoculant Biofertilizer Biogro Supplementing Fertilizer Nitrogen Application in Rice Production. J. Plant Nutr. 37, 1837–1858. https://doi.org/10.1080/01904167.2014.911320
Ignatova, L. V., Brazhnikova, Y. V., Berzhanova, R.Z., Mukasheva, T.D., 2015. Plant growth-promoting and antifungal activity of yeasts from dark chestnut soil. Microbiol. Res. 175, 78–83. https://doi.org/10.1016/j.micres.2015.03.008
Johnson, G. V., Lopez, A., La Valle Foster, N., 2002. Reduction and transport of Fe from siderophores. Plant Soil 241, 27–33. https://doi.org/10.1023/A:1016007708926
Kabir, M.S., Tasmim, T., 2019. Isolation of Pectinase Producing Bacteria from the Rhizosphere of Andrographis paniculata Nees and 16S rRNA Gene Sequence Comparison of Some Potential Strains. Adv. Microbiol. 09, 1–13. https://doi.org/10.4236/aim.2019.91001
Kahiluoto, H., Kuisma, M., Kuokkanen, A., Mikkilä, M., Linnanen, L., 2014. Taking planetary nutrient boundaries seriously: Can we feed the people? Glob. Food Sec. 3, 16–21. https://doi.org/10.1016/j.gfs.2013.11.002
Kasana, R.C., Salwan, R., Dhar, H., Dutt, S., Gulati, A., 2008. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. Curr. Microbiol. 57, 503–507. https://doi.org/10.1007/s00284-008-9276-8
Kaushal, J., Mehandia, S., Singh, G., Raina, A., Arya, S.K., 2018. Catalase enzyme: Application in bioremediation and food industry. Biocatal. Agric. Biotechnol. 16, 192–199. https://doi.org/10.1016/j.bcab.2018.07.035
Latgé, J.P., 2007. The cell wall: A carbohydrate armour for the fungal cell. Mol. Microbiol. 66, 279–290. https://doi.org/10.1111/j.1365-2958.2007.05872.x
Lichtenthaler, H.K., 1987. Chlorophylls Carotenoids. Chlorophylls Carotenoids Pigment. Photosynth. Biomembr. 148, 350–382.

Lima, J.R., Gondim, D.M.F., Oliveira, J.T.A., Oliveira, F.S.A., Gonçalves, L.R.B., Viana, F.M.P., 2013. Use of killer yeast in the management of postharvest papaya anthracnose. Postharvest Biol. Technol. 83, 58–64. https://doi.org/10.1016/j.postharvbio.2013.03.014

Limtong, S., Koowadjanakul, N., 2012. Yeasts from phylloplane and their capability to produce indole-3-acetic acid. World J. Microbiol. Biotechnol. 28, 3323–3335. https://doi.org/10.1007/s11274-012-1144-9

Lindow, S.E., Brandl, M.T., 2003. Microbiology of the Phyllosphere. Appl. Environ. Microbiol. 69, 1875–1883. https://doi.org/10.1128/AEM.69.4.1875

Luo, J., Zhou, J.J., Zhang, J.Z., 2018. Aux/IAA gene family in plants: Molecular structure, regulation, and function. Int. J. Mol. Sci. 19, 1–17. https://doi.org/10.3390/ijms19010259

Ma, Y., 2019. Seed coating with beneficial microorganisms for precision agriculture. Biotechnol. Adv. 37, 107423. https://doi.org/10.1016/j.biotechadv.2019.107423

Malusá, E., Sas-Paszt, L., Ciesielska, J., 2012. Technologies for beneficial microorganisms inocula used as biofertilizers. Sci. World J. 2012. https://doi.org/10.1100/2012/491206

Marques, A.P.G.C., Pires, C., Moreira, H., Rangel, A.O.S.S., Castro, P.M.L., 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using Zea mays as indicator plant. Soil Biol. Biochem. 42, 1229–1235. https://doi.org/10.1016/j.soilbio.2010.04.014

Martins, D., English, A.M., 2014. Catalase activity is stimulated by H2O2 in rich culture medium and is required for H2O2 resistance and adaptation in yeast. Redox Biol. 2, 308–313. https://doi.org/10.1016/j.redox.2013.12.019

Merín, M.G., Martín, M.C., Rantsiou, K., Cocolin, L., De Ambrosini, V.I.M., 2015. Characterization of pectinase activity for enology from yeasts occurring in Argentine bonarda grape. Brazilian J. Microbiol. 46, 815–823. https://doi.org/10.1590/S1517-838246320140160

Merín, M.G., Mendoza, L.M., Farías, M.E., Morata de Ambrosini, V.I., 2011. Isolation and selection of yeasts from wine grape ecosystem secreting cold-active pectinolytic activity. Int. J. Food Microbiol. 147, 144–148. https://doi.org/10.1016/j.ijfoodmicro.2011.04.004

Mezzasalma, V., Sandionigi, A., Bruni, I., Bruno, A., Lovicu, G., Casiraghi, M., Labra, M., 2017. Grape microbiome as a reliable and persistent signature of field origin and environmental conditions in Cannonau wine production.

PLoS One 12, 1–20. https://doi.org/10.1371/journal.pone.0184615
Milagres, A.M.F., Machuca, A., Napoleão, D., 1999. Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. J. Microbiol. Methods 37, 1–6. https://doi.org/10.1016/S0167-7012(99)00028-7
Muccilli, S., Restuccia, C., 2015. Bioprotective Role of Yeasts. Microorganisms 3, 588–611. https://doi.org/10.3390/microorganisms3040588
Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 473–497.
Naik, K., Mishra, S., Srichandan, H., Singh, P.K., Sarangi, P.K., 2019. Plant growth promoting microbes: Potential link to sustainable agriculture and environment. Biocatal. Agric. Biotechnol. 21, 101326. https://doi.org/10.1016/j.bcab.2019.101326
Nakayan, P., Hameed, A., Singh, S., Young, L. Sen, Hung, M.H., Young, C.C., 2013. Phosphate-solubilizing soil yeast Meyerozyma guilliermondii CC1 improves maize (Zea mays L.) productivity and minimizes requisite chemical fertilization. Plant Soil 373, 301–315. https://doi.org/10.1007/s11104-013-1792-z
Narsian, V., Ahmed, A.A., Patel, H.H., 2010. Rock phosphate dissolution by specific yeast. Indian J. Microbiol. 50, 57–62. https://doi.org/10.1007/s12088-009-0019-8
Nassar, A.H., El-Tarabily, K.A., Sivasithamparam, K., 2005. Promotion of plant growth by an auxin-producing isolate of the yeast Williopsis saturnus endophytic in maize (Zea mays L.) roots. Biol. Fertil. Soils 42, 97–108. https://doi.org/10.1007/s00374-005-0008-y
Nutaratat, P., Srisuk, N., Arunrattiyakorn, P., Limtong, S., 2014. Plant growth-promoting traits of epiphytic and endophytic yeasts isolated from rice and sugar cane leaves in Thailand. Fungal Biol. 118, 683–694. https://doi.org/10.1016/j.funbio.2014.04.010
O'Callaghan, M., 2016. Microbial inoculation of seed for improved crop performance: issues and opportunities. Appl. Microbiol. Biotechnol. 100, 5729–5746. https://doi.org/10.1007/s00253-016-7590-9
Oliveira, B. de, Rodolfo, B.J., Luana, G.S., Marcia, M.R.-M., 2019. Rhizosphere yeast Torulaspora globosa with plant growth promotion traits and improvement of the development of tomato seedlings under greenhouse conditions. African J. Agric. Res. 14, 935–942. https://doi.org/10.5897/ajar2019.13950

1142 Palkova, Z., Janderova, B., Gabriel, J., Zikanova, B., Pospisek, M., Forstova,
1143 J., 1997. Ammonia mediates communication between yeast colonies.
1144 Nature 390, 532–536. https://doi.org/10.1038/37398
1145 Pawlikowska, E., James, S.A., Breierova, E., Antolak, H., Kregiel, D., 2019.
1146 Biocontrol capability of local Metschnikowia sp. isolates. Antonie van
1147 Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 0123456789, 1425–1445.
1148 https://doi.org/10.1007/s10482-019-01272-w
1149 Rao, R.P., Hunter, A., Kashpur, O., Normanly, J., 2010. Aberrant synthesis of
1150 indole-3-acetic acid in Saccharomyces cerevisiae triggers morphogenic
1151 transition, a virulence trait of pathogenic fungi. Genetics 185, 211–220.
1152 https://doi.org/10.1534/genetics.109.112854
1153 Renouf, V., Claisse, O., Lonvaud-funel, A., 2005. Numeration, identification
1154 and understanding of yeast and bacteria ecosystem on the grape berry.
1155 Aust J Grape Wine Res 11, 316–327.
1156 Ruanpanun, P., Tangchitsomkid, N., Hyde, K.D., Lumyong, S., 2010.
1157 Actinomycetes and fungi isolated from plant-parasitic nematode infested
1158 soils: Screening of the effective biocontrol potential, indole-3-acetic acid
1159 and siderophore production. World J. Microbiol. Biotechnol. 26, 1569–
1160 1578. https://doi.org/10.1007/s11274-010-0332-8
1161 Sarabia, M., Cornejo, P., Azcón, R., Carreón-Abud, Y., Larsen, J., 2017.
1162 Mineral phosphorus fertilization modulates interactions between maize,
1163 rhizosphere yeasts and arbuscular mycorrhizal fungi. Rhizosphere 4, 89–
1164 93. https://doi.org/10.1016/j.rhisph.2017.09.001
1165 Saravanakumar, D., Ciavorella, A., Spadaro, D., Garibaldi, A., Gullino, M.L.,
1166 2008. Metschnikowia pulcherrima strain MACH1 outcompetes Botrytis
1167 cinerea, Alternaria alternata and Penicillium expansum in apples through
1168 iron depletion. Postharvest Biol. Technol. 49, 121–128.
1169 https://doi.org/10.1016/j.postharvbio.2007.11.006
1170 Saravanakumar, D., Spadaro, D., Garibaldi, A., Gullino, M.L., 2009. Detection
1171 of enzymatic activity and partial sequence of a chitinase gene in
1172 Metschnikowia pulcherrima strain MACH1 used as post-harvest
1173 biocontrol agent. Eur. J. Plant Pathol. 123, 183–193.
1174 https://doi.org/10.1007/s10658-008-9355-5
1175 Scarcella, A.S., Junior, R.B., Bastos, R.G., Magri, M.M.R., 2017. Temperature,
1176 ph and carbon source, affect drastically indole acetic acid production of
1177 plant growth promoting yeasts. Brazilian J. Chem. Eng. 34, 429–438.
1178 https://doi.org/10.1590/0104-6632.20170342s20150541
1179 Setati, M.E., Jacobson, D., Andong, U.C., Bauer, F., 2012. The Vineyard Yeast

Microbiome, a Mixed Model Microbial Map. PLoS One 7. https://doi.org/10.1371/journal.pone.0052609

Seufert, V., Ramankutty, N., Foley, J.A., 2012. Comparing the yields of organic and conventional agriculture. Nature 485, 229–232. https://doi.org/10.1038/nature11069

Sharma, S., Sayyed, R., Trivedi, M., Gobi, T., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2, 1–14. https://doi.org/https://doi.org/10.1186/2193-1801-2-587

Streletskii, R.A., Kachalkin, A. V., Glushakova, A.M., Demin, V. V., Chernov, I.Y., 2016. Quantitative determination of indole-3-acetic acid in yeasts using high performance liquid chromatography—tandem mass spectrometry. Microbiol. (Russian Fed. 85, 727–736. https://doi.org/10.1134/S0026261716060187

Sui, Y., Wisniewski, M., Droby, S., Liu, J., 2015. Responses of yeast biocontrol agents to environmental stress. Appl. Environ. Microbiol. 81, 2968–2975. https://doi.org/10.1128/AEM.04203-14

Sun, P.F., Fang, W.T., Shin, L.Y., Wei, J.Y., Fu, S.F., Chou, J.Y., 2014. Indole-3-acetic acid-producing yeasts in the phyllosphere of the carnivorous plant Drosera indica L. PLoS One 9, 1–22. https://doi.org/10.1371/journal.pone.0114196

Tapia-Vázquez, I., Sánchez-Cruz, R., Arroyo-Domínguez, M., Lira-Ruan, V., Sánchez-Reyes, A., del Rayo Sánchez-Carbente, M., Padilla-Chacón, D., Batista-García, R.A., Folch-Mallol, J.L., 2020. Isolation and characterization of psychrophilic and psychrotolerant plant-growth promoting microorganisms from a high-altitude volcano crater in Mexico. Microbiol. Res. 232, 126394. https://doi.org/10.1016/j.micres.2019.126394

Taskin, M., 2013. Co-production of tannase and pectinase by free and immobilized cells of the yeast Rhodotorula glutinis MP-10 isolated from tannin-rich persimmon (Diospyros kaki L.) fruits. Bioprocess Biosyst. Eng. 36, 165–172. https://doi.org/10.1007/s00449-012-0771-8

Teather, R.M., Wood, P.J., 1982. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl. Environ. Microbiol. 43, 777–780.

Timmusk, S., Behers, L., Muthoni, J., Muraya, A., Aronsson, A.C., 2017. Perspectives and challenges of microbial application for crop improvement. Front. Plant Sci. 8, 1–10.

https://doi.org/10.3389/fpls.2017.00049
Uroz, S., Courty, P.E., Oger, P., 2019. Plant Symbionts Are Engineers of the Plant-Associated Microbiome. Trends Plant Sci. 1–12. https://doi.org/10.1016/j.tplants.2019.06.008
Verma, S., Verma, P.K., Chakrabarty, D., 2019. Arsenic Bio-volatilization by Engineered Yeast Promotes Rice Growth and Reduces Arsenic Accumulation in Grains. Int. J. Environ. Res. 13, 475–485. https://doi.org/10.1007/s41742-019-00188-7
Wezel, A., Casagrande, M., Celette, F., Vian, J.F., Ferrer, A., Peigné, J., 2014. Agroecological practices for sustainable agriculture. A review. Agron. Sustain. Dev. 34, 1–20. https://doi.org/10.1007/s13593-013-0180-7
Yurkov, A.M., 2018. Yeasts of the soil – obscure but precious. Yeast 35, 369–378. https://doi.org/10.1002/yea.3310

Figures

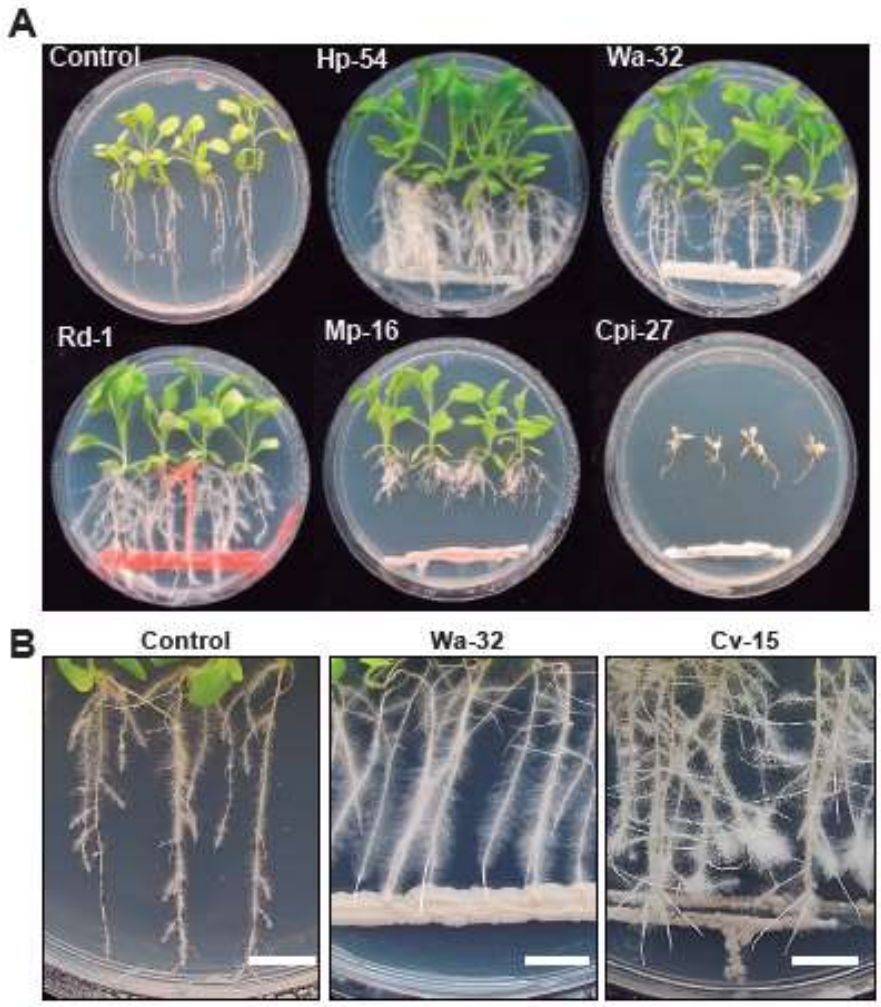


Fig. 1. Effects of plant growth-promoting yeasts on growth and development of *Nicotiana benthamiana* seedlings. **(A)** *N. benthamiana* seedlings (7 days old) were grown on quarter-strength MS agar plates inoculated with yeasts at the opposite ends. The plates were placed vertically and seedlings were co-cultured with yeasts for a further 21 days. Plate samples without yeast served as the control. Several plates are shown as examples of different phenotypes: Hp-54, Wa-32 and Rd-1 enhanced shoot and root weight while Mp-16 only enhanced shoot growth. Cpi-27 is an example of a yeast strain that was deleterious in co-culture with *N. benthamiana* seedlings. **(B)** Detail of *N. benthamiana* roots developed in the presence of yeasts Wa-32 and Cv-15. Greater branching and the presence of longer root hairs can be observed in comparison to the control seedlings roots. Scale bars indicate 1 cm.

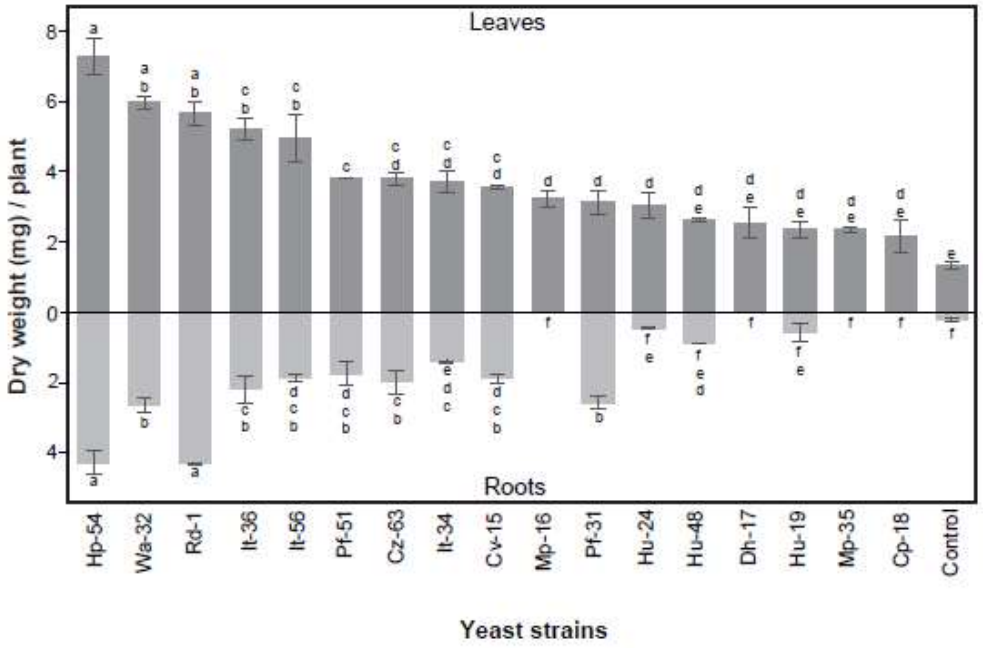


Fig. 2. Effects of plant growth-promoting yeasts on dry weight of leaves and roots of *Nicotiana benthamiana* seedlings after 21 days of *in vitro* co-culture. Data shown are presented as the means ± SD (n=8) and correspond to yeast strains associated with significantly higher shoot dry weight than the control. The experiment was performed twice. Values with the same letter do not differ significantly ($P<0.05$) according to Tukey's test.

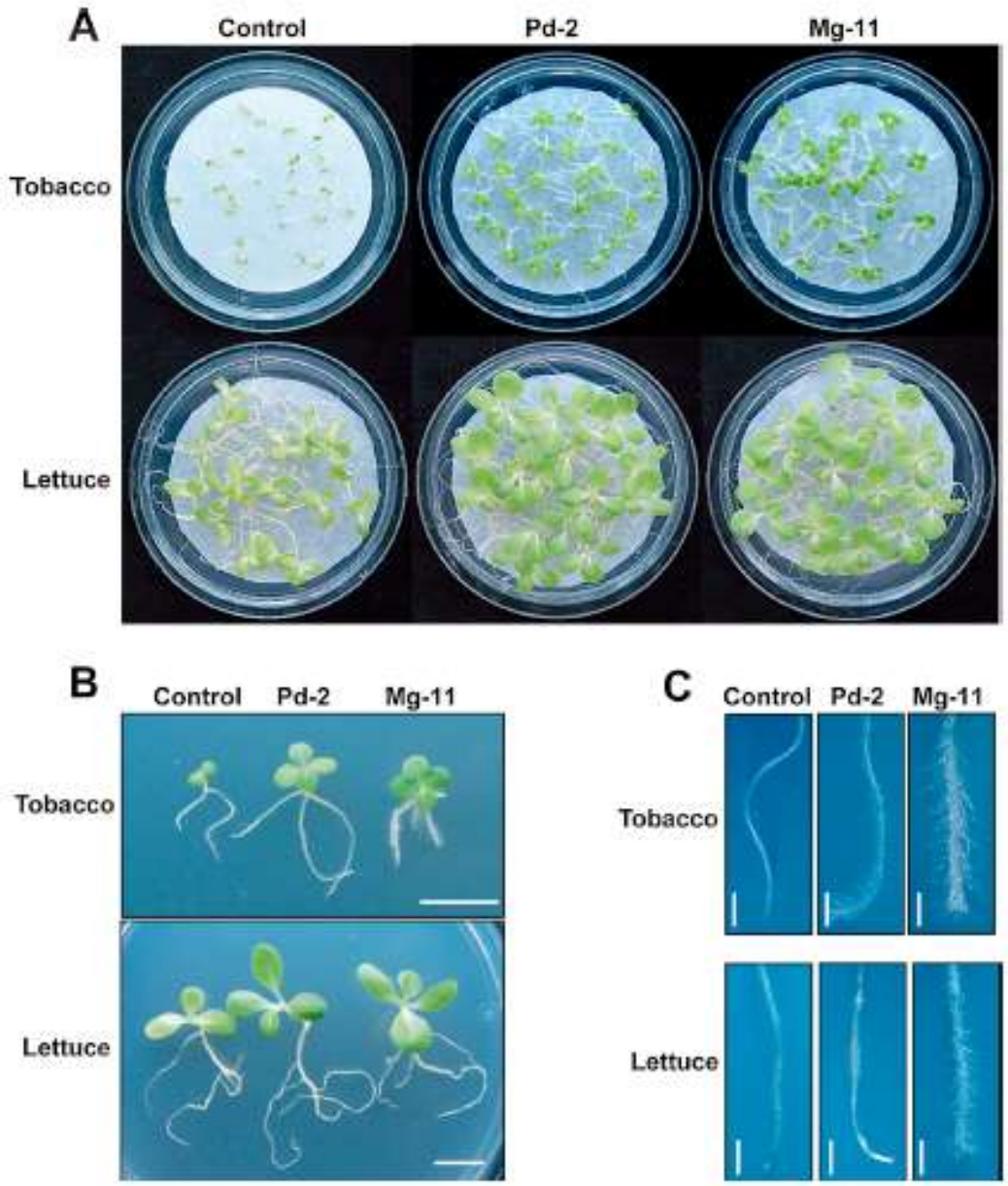


Fig. 3. Effects of plant growth-promoting yeasts on seedling development after 15 days of *in vitro* co-culture. **(A)** Surface sterilised tobacco and lettuce seeds were germinated on filter paper in water-agar plates in the presence of 1 mL of 10^8 CFU mL^{-1} of yeast (Pd-2 or Mg-11) or water as control. **(B)** Yeast inoculated seedlings showed greater shoot and root development than control plants. Scale bars indicate 1 cm. **(C)** Detail of tobacco and lettuce seedling root tips where a large number of root hairs can be observed in the presence of yeast strain Mg-11. Scale bars indicate 1 mm.

Supplementary tables

Supplementary Table 1. IAA production in yeasts grown in liquid culture with or without 0.1% L-Trp. Means of relevant IAA producers (>2.5 μg mg^{-1} dry weight) yeasts were analysed using one-way analysis of variance (ANOVA) and the differences were compared using Tukey's multiple comparison test. Values with the same letter no not differ significantly (P<0.05).

With L-Trp

Yeast strain	a	b	c	d	e	f	g
Mg-62	27,83						
Cz-37	20,92	20,92					
Rm-8		15,64	15,64				
Ll-43		15,14	15,14	15,14			
Cz-41		14,79	14,79	14,79	14,79		
Cz-68		12,61	12,61	12,61	12,61	12,61	
Cpi-27		10,88	10,88	10,88	10,88	10,88	10,88
Ll-21		10,36	10,36	10,36	10,36	10,36	10,36
Wa-32			6,91	6,91	6,91	6,91	6,91
Su-5			6,91	6,91	6,91	6,91	6,91
Rg-10			6,19	6,19	6,19	6,19	6,19
Hp-54			5,89	5,89	5,89	5,89	5,89
Hu-24			5,55	5,55	5,55	5,55	5,55
Mp-50			5,38	5,38	5,38	5,38	5,38
Mg-46			4,92	4,92	4,92	4,92	4,92
Hu-48			4,83	4,83	4,83	4,83	4,83
Sb-20			4,82	4,82	4,82	4,82	4,82
Mp-23			4,64	4,64	4,64	4,64	4,64
Cl-66			4,54	4,54	4,54	4,54	4,54
Mc-52				4,11	4,11	4,11	4,11
Mp-30				4,09	4,09	4,09	4,09
Cl-14				3,92	3,92	3,92	3,92
Hu-25				3,82	3,82	3,82	3,82
Mp-35					3,74	3,74	3,74
Mp-22					3,68	3,68	3,68
Sc-39					3,65	3,65	3,65
Cv-15					3,61	3,61	3,61
Ci-3						3,40	3,40
It-36						3,16	3,16
Dh-49						2,95	2,95
Pf-51						2,92	2,92
It-56						2,89	2,89
Sc-6						2,76	2,76
Mp-16						2,73	2,73
Sc-45						2,52	2,52

W/O L-Trp

Yeast strain	a	b	c	d	e	f	g	h
Lt-60	11,12							
Cz-68		6,34						
Cz-41		5,83	5,83					
Cpi-27		5,10	5,10	5,10				
Rg-10		5,02	5,02	5,02				
Mg-62		4,54	4,54	4,54	4,54			
Cl-66			3,76	3,76	3,76	3,76		
Wa-32				3,30	3,30	3,30	3,30	
Hp-54					2,62	2,62	2,62	2,62
Ci-3					2,62	2,62	2,62	2,62

Supplementary Table 2. Effects of inoculation of *Nicotiana tabacum* seeds with selected plant growth-promoting yeasts.

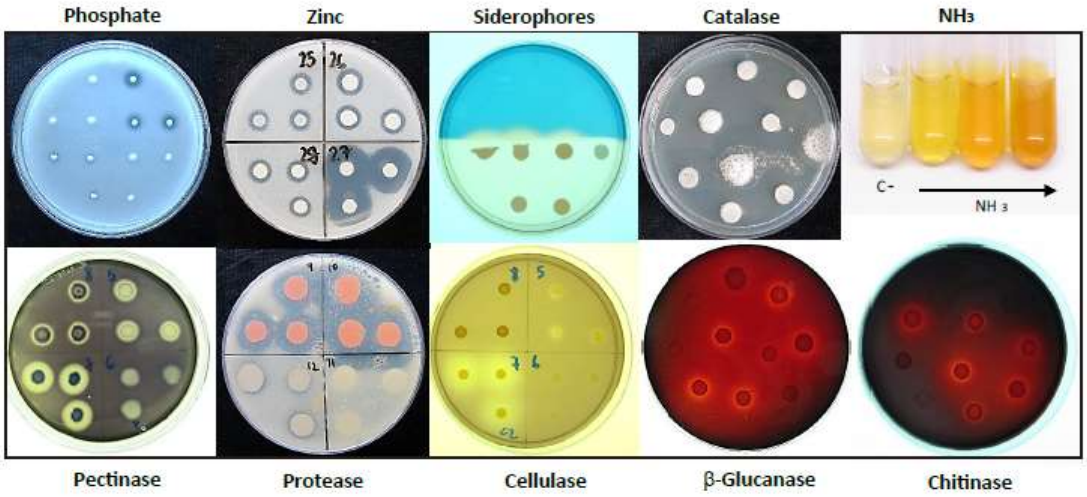
Yeast strain	Dry weight (μg/plant)	Nº roots/plant	Root length (cm)	Chlorophyll a+b (mg g^{-1} FW)
Pd-2	446.2 ± 47.8 a	3.2 ± 0.05 a	1.3 ± 0.02 a	0.30 ± 0.01 b
Pd-2 (80ºC)	95.6 ± 11.2 b	1.5 ± 0.07 b	0.8 ± 0.06 b	0.18 ± 0.01 c
Mg-11	390.0 ± 17.9 a	3.2 ± 0.04 a	0.5 ± 0.01 c	0.47 ± 0.03 a
Mg-11 (80ºC)	100.2 ± 16.3 b	1.3 ± 0.06 b	0.7 ± 0.02 b	0.19 ± 0.05 c
Control	97.0 ± 14.5 b	1.3 ± 0.09 b	0.7 ± 0.03 b	0.17 ± 0.02 c

Sterile tobacco seeds were treated with live or dead (30 min 80ºC) yeasts and cultivated for 10 days. Roots longer than 0.5 cm were considered for measurements. Root length value corresponds to the longest root. Data are presented as the means ± SD (n= 4-30). The experiment was performed twice. Values with the same letter within each column do not differ significantly ($P<0.05$) according to Tukey's test.

Supplementary Table 3. Growth-promoting effects of yeasts in maize seedling development planted in soil under phytotron conditions.

	Leaf Fresh Weight (%)	Shoot Height (%)	Chlorophyll-SPAD (%)
Control	100,00 ± 0,87	100,00 ± 1,55	100,00 ± 2,84
Ca-12	104,35 ± 3,04	99,23 ± 2,68	112,81 ± 2,16 *
Ca-40	115,00 ± 1,50	103,94 ± 1,39	102,61 ± 3,94
Ci-3	100,00 ± 3,04	99,81 ± 2,01	112,50 ± 1,81 *
Cl-14	100,00 ± 4,35	100,57 ± 3,89	105,94 ± 3,22
Cl-28	110,00 ± 5,00	102,07 ± 1,91	107,49 ± 6,38
Cl-66	104,76 ± 3,81	103,38 ± 2,83	98,17 ± 1,94
Co-13	95,65 ± 2,17	97,89 ± 2,68	109,38± 2,03 *
Co-65	104,76 ± 4,76	110,55 ± 2,87 *	99,21 ± 3,14
Cp-18	82,61 ± 8,70	86,21 ± 6,36	100,00 ± 2,59
Cpi-27	110,00 ± 3,00	102,49 ± 1,00	97,72 ± 5,44
Cr-26	110,00 ± 3,00	98,76 ± 1,87	100,98± 3,42
Cv-15	95,65 ± 3,91	99,43 ± 2,57	98,13 ± 2,56
Cz-37	100,00 ± 4,50	96,27 ± 2,76	102,93 ± 4,14
Cz-41	100,00 ± 4,44	100,23 ± 2,00	96,06 ± 4,87
Cz-63	104,765 ± 2,38	106,75 ± 3,23	98,17± 2,54
Cz-68	100,00 ± 4,29	96,62 ± 3,25	97,12 ± 2,25
Dh-17	86,96 ± 4,35 *	86,59 ± 4,29 *	89,69 ± 3,81
Dh-49	111,11 ± 3,33	104,99 ± 2,18	101,08 ± 3,37
Dh-67	109,52 ± 2,38 *	108,23 ± 2,66 *	112,07 ± 1,42 *
Dn-33	100,00 ± 6,00	95,23 ± 3,90	107,49± 5,96
Hu-19	95,65 ± 5,22	99,43 ± 4,27	98,75 ± 2,16
Hu-24	110,00 ± 4,50	102,07 ± 2,16	96,74 ± 2,93
Hu-25	100,00 ± 5,00	98,13 ± 2,97	102,61 ± 4,07
Hu-48	111,11 ± 2,22	104,08 ± 1,36	106,09 ± 4,77
Hp-54	100,00 ± 2,78	99,32 ± 1,81	92,47 ± 5,84
It-34	100,00 ± 5,50	100,41 ± 1,78	111,07 ± 3,16 *
It-36	105,00 ± 6,00	100,41 ± 2,87	98,70± 3,91
It-56	100,00 ± 6,11	95,69 ± 4,81	98,21 ± 5,27
Ll-21	110,00 ± 3,50	101,45 ± 1,37	103,58 ± 3,88
Ll-43	94,44 ± 4,44	95,24 ± 1,97	113,98 ± 2,62
Lt-7	91,30 ± 4,35	93,49 ± 3,18	107,50 ± 2,34
Lt-29	105,00 ± 4,00	97,30 ± 2,49	99,02 ± 3,97
Lt-42	88,89 ± 3,89	97,73 ± 2,09	104,30 ± 4,70
Lt-47	105,55 ± 3,33	104,76 ± 1,00	96,42 ± 4,41
Lt-60	100,00 ± 3,89	102,50 ± 7,39	95,34 ± 5,99
Lt-61	100,00 ± 5,24	106,33 ± 3,02	99,21 ± 2,49
Lt-69	109,52 ± 2,86 *	104,22 ± 3,29 *	114,33 ± 3,02 *
Mp-16	95,65 ± 6,09	98,47 ± 4,90	106,56 ± 2,19
Mp-22	115,00 ± 3,50	103,11 ± 1,47	100,98 ± 3,26
Mp-23	110,00 ± 2,00	103,11 ± 0,93	95,77 ± 3,88
Mp-30	110,00 ± 3,00	103,32 ± 1,83	105,21 ± 2,61
Mp-35	110,00 ± 4,50	102,07 ± 1,29	92,18 ± 3,29
Mp-50	100,00 ± 3,33	103,40 ± 2,15	111,11 ± 3,15
Mc-52	94,44 ± 3,89	100,68 ± 2,72	93,19 ± 4,30
Mc-57	105,56 ± 3,33	100,68 ± 2,20	104,66 ± 3,262
Mg-11	100,00 ± 3,04	97,13 ± 2,59	109,38 ± 3,34
Mg-46	100,00 ± 3,89	101,36 ± 2,13	100,72 ± 3,98
Mg-62	100,00 ± 4,76	101,48 ± 2,17	92,15 ± 2,51 *
Pd-2	95,65 ± 6,52	96,36 ± 5,86	105,94 ± 3,50
Pf-31	110,00 ± 2,50	100,41 ± 1,60	109,45 ± 2,61 *
Pf-51	100,00 ± 2,22	102,95 ± 2,20	100,72 ± 6,02
Pf-70	104,76 ± 3,33	107,81 ± 1,94 *	97,38 ± 2,36
Pm-55	100,00 ± 4,44	96,37 ± 2,90	104,30 ± 3,84
Rd-1	86,96 ± 5,22 *	88,51 ± 5,32	105,63 ± 4,28
Rg-10	95,65 ± 4,78	96,55 ± 3,62	108,75 ± 2,75
Rm-8	100,00 ± 3,91	99,43 ± 3,18	107,50 ± 2,56
Sb-20	91,30 ± 4,78	96,55 ± 3,70	107,19 ± 2,91
Sc-4	95,65 ± 2,61	99,81 ± 2,87	111,88 ± 1,31 *
Sc-6	107,94 ± 2,25 *	105,94 ± 1,65 *	109,25 ± 1,88 *
Sc-39	115,00 ± 3,50 *	102,497 ± 1,00	103,58 ± 1,92
Sc-44	100,00 ± 5,00	101,13 ± 1,47	108,96 ± 4,66
Sc-45	100,00 ± 4,44	99,77 ± 2,24	96,77 ± 4,84
Sc-64	104,76 ± 3,33	110,34 ± 2,19 *	98,69 ± 2,59
Su-5	100,00 ± 3,91	97,51 ± 3,43	110,31 ± 2,69 *
Td-38	105,00 ± 4,50	99,17 ± 1,27	103,58 ± 2,74
Td-53	105,56 ± 3,89	103,85 ± 2,31	105,73 ± 2,69
Td-58	100,00 ± 3,89	99,55 ± 2,12	102,87 ± 5,41
Wa-32	110,00 ± 4,50	101,87 ± 1,72	109,45± 4,04
Wa-59	105,56 ± 5,00	103,17 ± 2,22	97,85 ± 6,74

Maize seedling data after 15 days of yeast inoculation presented as percentages, with control=100%. Data are presented as the means ± SD (n=20). Asterisks indicate statistically different values in relation to control within each column at $P<0.05$ according to t-test.



Supplementary Figure 1: In vitro measurements of plant growth-promoting activities in isolated yeasts.