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Research Paper

Detection of plant growth enhancing features in psychrotolerant yeasts from Patagonia (Argentina)

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This study explores the biotechnological potential for plant production of twelve psychrotolerant yeasts strains from Northwest-Patagonia. These strains were isolated from different substrates associated with *Nothofagus* sp. in native forests and *Vaccinium* sp. in a commercial plantation. Yeasts characterization was performed using *in vitro* assays to evaluate the production of auxin-like compounds and siderophores, ability to solubilize inorganic phosphate and to reduce common plant pathogen growth. Strain YF8.3 identified as *Aureobasidium pullullans* was the main producer of auxin-like and siderophores compounds. Phosphate solubilization was a characteristic observed by strains L8.12 and CRUB1775 identified as *Holtermaniella takashimae* and *Candida maritima*, respectively. Different yeast strains were able to inhibit the growth of *Verticillium dahliae* PPRI5569 and *Pythium aphanidermatum* PPRI 9009, but they all failed to inhibit the growth of *Fusarium oxysporum* PPRI5457. The present study, suggests that yeasts present in different environments in Northwestern-Patagonian have physiological *in vitro* features which may influence plant growth. These results are promising for the developing of biological products based on Patagonian yeasts for plant production in cold-temperate regions.

Keywords: Auxin / Siderophores / Phosphate solubilization / Phytopathogens inhibition

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Introduction

As a result of the high cost (economic, social acceptance, and environmental) of using agrochemicals to enhance plant growth and to control plant-diseases, there is an increasing interest in finding substitutes for these products [1]. Biological products represent an alternative which may lead to a more effective and safer (for consumers and the environment) production of plant related products. Soil and plant related microbial communities have a great potential as resources for plant production, as plant growth promoters (PGP) or biological control (biocontrol) agents [1, 2]. Some of the most studied PGP features are related to the production of plant growth regulators (i.e., phytohormones) or the contribution of the microorganism to the improvement of nutrient uptake by plants. The production of phytohormones has been recognized as an important factor in direct plant growth promoting abilities of rhizosphere microorganisms. Phytohormones, such as auxins, have been associated with enhanced root growth which is a desirable characteristic for improved water and nutrient uptake by the plants [3]. The production of compounds such as siderophores that ligate Fe and other metals from soil [4], or the production of weak organic acids which enhance solubilization of complexed inorganic P [5], increase the availability of these nutrients for plants and could be important for plant production in limiting environments. Some microorganisms are able to reduce pathogen growth by competing for nutrients and/or producing inhibitory substances.

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These microorganisms are considered as potential biocontrol agents [6].

The selection of microorganisms for plant production should consider environmental characteristics of the region (precipitation, temperature, and surrounding vegetation) to secure or improve production. As temperature is one of the main factors driving microbial activity, the use of microorganisms' adapted or tolerant to low temperatures could be an attractive strategy in cold-temperate regions. Northwest-Patagonia is a cold-temperate region having a mean annual temperature of 10 °C, winter temperatures are often below 0 °C with precipitation in the form of snow [7, 8]. In this region, indigenous soil microbial communities could be considered psychrotolerant, as they are periodically exposed to low temperatures [9]. Psychrotolerance is the ability to grow at 20–30 °C while being able to tolerate and grow at or close to 0 °C [10, 11]. These features allow psychrotolerant yeasts to grow during the plant production season (spring–summer) and endure the cold season. This could be important for transplanting of seedlings in the field and for direct field application in cold environments. All the above makes soil yeast communities in Northwest-Patagonia of great interest for the development of biotechnological resources for plant production in cold-temperate environments.

For several years, extensive surveys on soil and plant related substrates were performed on native forests and plant production sites in Northwest-Patagonia [8, 12, 13]. These surveys have provided a great number of yeast isolates, with a large number of possible new species. Yeast communities in these forests soils are characterized by the dominance of *basidiomycetous* species and the uneven distribution of isolates with few taxa accounting for a high percentage of isolates, while most of the taxa are scarcely represented [8, 13]. Soil yeasts influence soil aggregation, contribute to nutrient cycles and interact with the vegetation [14, 15]. Some authors reported the successful use of yeasts as plant growth promoters and biocontrol agents [2, 15–20]. The present study, aims to explore the biotechnological potential of 12 psychrotolerant yeast strains for plant production in cold-temperate environments using several *in vitro* tests.

Materials and methods

Strain origin

Twelve yeast strains isolated from different surveys undertaken in the Northwest-Patagonian region were used for the present study. These surveys included samples from soils and dry fruits associated with *Nothofagus* native forests in Lanin National Park and Nahuel Huapi National Park (Neuquén and Río Negro province, respectively), and *Vaccinium* spp. from commercial plantations in El Hoyo (Chubut province). The *blueberry* production fields in Northwestern-Patagonian region are usually located nearby *Nothofagus* forest. Yeast strains were selected to include different isolation environments (native forests or commercial plantation) and habitats (bulk soil, rhizosphere, ectomycorrhizosphere, and dry fruits), and to include several genera from the Phyla Basidiomycota and Ascomycota [13].

All strains were isolated at 20 °C on solid MYP medium (% w/vol, malt extract 0.7, yeast extract 0.05, peptone 0.25, agar 1.5), supplemented with Rose Bengal 25 $\mu g\,ml^{-1}$ and chloramphenicol 200 $\mu g\,ml^{-1}$ [21]. Pure cultures were cryo-preserved (−80 °C) using MYP liquid medium with 12% glycerol. Yeast strains were included in the CRUB Yeast Collection (CRUB: Yeast Collection of Centro Regional Universitario Bariloche) and author's personal collections. Yeast strains were identified using a polyphasic approach that combined morphological, physiological, and molecular features [8, 12]. For DNA sequence analysis, the D1/D2 domain at the 5′ end of the large subunit (LSU) *rRNA* gene was amplified using primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′). Sequencing of amplicons was carried out by MACROGEN Inc. using primer NL-1. Comparisons with sequences from GenBank database (http://www.ncbi.nlm. nih.gov/) were carried out using BLASTn search algorithm. The GenBank/EMBL/DDBJ accession numbers for the D1/D2 large-subunit rDNA sequences obtained strains are listed in Table 1.

All strains were *in vitro* tested for plant growth promoting characteristics and biocontrol abilities. Incubation temperature was selected based on environmental condition and data from other yeast diversity studies in the region [13, 22]. All tests were performed at 20 °C, which is a temperature compatible with plant production in Andean-Patagonian region.

Plant growth promotion characteristics

Auxin-like compound detection was performed by Salkowski method [23] and quantitative assessment was performed on DEV-tryptophan broth (Merck). All cultures were incubated for 7 days at 20 °C, in triplicate. Cultures were, then, centrifuged at 10,000 rpm for 15 min to recover the supernatant. The supernatants (100 μL) were transferred into 96 wells microplate with each well receiving 100 μL of Salkowski reagent (12 g/L $FeCl_3$, in 7.9 M H_2SO_4) and incubated at room temperature protected from light for 30 min.

Table 1. Identification and origin of yeast strains evaluated in the present study.

Identification	Strain number	Genbank accesion	Substrate	Asociated plant	Location
Basidiomycota					
Cystofilobasidiales					
Guehomyces pullulans[†]	CRUB1772	KU693287	Rhizosphere	*Nothofagus pumilio*	NHNP
Filobasidiales					
Cryptococcus cylindricus	CRUB1771	KU693286	Bulk-soil	*Nothofagus antarctica*	LNP
Holtermanniales					
Holtermanniella sp	L5.6	KU728170	Rhizosphere	*Vaccinium corymbosum* var. *Brigitta*	BF
Holtermanniella takashimae	L8.12	KU728172	Rhizosphere	*Vaccinium corymbosum* var. *Brigitta*	BF
Tremellales					
Asterotremella albida	T9.1	KU728168	Ectomycorhizosphere	*Nothofagus nervosa*	LNP
Trichosporonales					
Trichosporon sp.	L12.20	KU728171	Rhizosphere	*Vaccinium ashei* var. *Becky Blue*	BF
Ascomycota					
Dothideales					
Aureobasidium pullulans[§]	YF8.3	HQ629551	Dry fruit	*Nothofagus nervosa*	LNP
Candida cf. ralunensis[†]	CRUB1774	KU693289	Rhizosphere	*Nothofagus pumilio*	NHNP
Candida maritima[†]	CRUB1775	KF826532	Bulk-soil	*Nothofagus pumilio*	NHNP
Saccharomycetales					
Candida saitoana	CRUB1770	KF826529	Bulk-soil	*Nothofagus antarctica*	LNP
Candida sp.	T18.4b	KU728169	Ectomycorhizosphere	*Nothofagus nervosa*	LNP
Wickerhamomyces sp.[‡]	CRUB1773	KU693288	Ectomycorhizosphere	*Nothofagus pumilio*	NHNP

References: [†]Mestre et al. [13], [‡]Fernández et al. [12], [§]Mestre et al. [8]. NHNP, Nahuel Huapi National Park; LNP, Lanin National Park; BF, Blueberry farm; El Hoyo (Chubut).

Colorimetric determination was performed at 530 nm in a BioTEK Synergy MX, Monochromator-Based Multi-Mode Microplate Reader. Commercial Indole-3-Acetic Acid (Sigma) was used as the reference curve and included in the same microplate.

Siderophores compound production was performed on MMS solid medium ($(NH_4)SO_4$ 2 g/L, KH_2PO_4 2 g/L, $CaCl_2$ 0.075 g/L, $MgSO_4$ 0.5 g/L, Glucose 10 g/L, Yeast extract 1 g/L; Agar 15 g/L) modified by the addition of Chrome Azurol Sulphonate (CAS) and Pipes Buffer [24]. MMS medium pH was adjusted with NaOH until a pH of 6.8 (Pipes buffer pKa) and then 30.24 g/L of Pipes buffer (Sigma) was added into the medium. CAS 10× solution was made according to Tortora et al. [25]. MMS modified medium and CAS solution were autoclave separately at 121 °C for 15 min and then mixed after cooling and poured into Petri dishes. Yeast strains were inoculated onto the plates using sterile tooth-picks, in triplicate, incubated at 20 °C and examined at 24, 48 h, and weekly for 1 month. Siderophore production was observed as orange-red halos around yeast colony and semi-quantitative determination was performed comparing colony and halo diameter. The yield of siderophore production (% Y) was determined as [(halo diameter-colony diameter)/colony diameter] ×100.

Phosphate solubilization was tested on solid YPD medium (Yeast extract 10 g/L, Bacto Peptone 20 g/L, Glucose 20 g/L, and Agar 15 g/L) amended with $Ca_3(PO_4)_2$ 0.5%, in triplicate. Plates were incubated at 20 °C, in triplicate, and examined after 24, 48 h, and weekly for 1 month. Positive results were observed as lighter-color halos around the colony.

Biocontrol characteristics

Pathogenic strains were acquired from the National Collection of Fungi, Pretoria, South Africa. Tested strains used were *Verticillium dalhiae* PPRI5569, *Pythium aphanidermatum* PPRI 9009, and *Fusarium oxysporum* PPRI5457, all isolated from tomato.

A co-inoculation assay was performed on PDA solid medium (Potato extract 4 g/L, dextrose 20 g/L, and agar 15 g/L; Merck) on 90 mm Petri dishes, at 20 °C in triplicate and cultures were examined at 24, 48 h, and weekly for 1 month. A 6 mm diameter fungal plug (from a 1 week old culture) was placed in the center of the plate, simultaneously with yeast inoculation. One yeast strain was used per plate and the inoculum consisted of 20 μL of a 72 h MYP culture (OD_{600}nm = 0.3). Each yeast strain was inoculated in four positions equidistant from the center of the plate. The control plate with an individual pathogenic culture was cultivated at the same

conditions. Pathogen growth was evaluated by measuring the diameter of pathogens' colony and the percentage of growth reduction was determined as [(diameter control-diameter treated)/ diameter control] × 100.

Yeast culture supernatant effect on fungal growth was evaluated on solid medium using either 72 h or 1 week MYP culture supernatant filtered through a sterile 0.22 μm membrane, in replacement of yeast cultures. Inoculation method was as described above. This assay was performed to evaluate the production of yeast extracellular compounds and their ability to inhibit fungal pathogen growth.

Statistical analysis

One-way ANOVA was used to evaluate any differences in PGP traits between yeast strains. One-way ANOVA was also used to evaluate effect on pathogen colony diameter affected by each yeast treatment, both for co-culturing and supernatant assay. Effects were considered to be statistically significant at the level $p < 0.05$. Tukey's post-hoc test was used to form homogenous groups.

Results

The identification to species level of the yeast strains used in the present study is shown in Table 1. The species identified belong to both Ascomycota (6) and Basidiomycota (6) Phyla and included different taxonomical orders and genera. These species were isolated from plant related substrates: dry fruits, bulk soil, rhizosphere, and ectomycorrhizosphere samples; and were associated with three *Nothofagus* species from native forest, and two *Vaccinium* species from a blueberry plantation (Table 1).

Plant growth promotion characteristics

All strains were *in vitro* tested for three characteristics related to plant growth promotion: production of auxin-like compounds, production of siderophore compounds, and solubilization of inorganic phosphate (Table 2).

The evaluation of auxin-like compounds showed different levels of production for each yeast strain. The main producer strain was YF8.3 identified as *Aureobasidium pullulans* (9.15 ± 0.87 μg/ml IAA units). Strain CRUB1775, identified as *Candida maritima*, and strain CRUB1772, identified as *Guehomyces pullulans*, showed intermediate levels of production. (4.01 ± 0.46 and 3.67 ± 0.35 μg/ml IAA units, respectively).Strains L5.6 and L8.12 were identified as *Holtermanniella* sp. (2.21 ± 0.10 μg/ml IAA units) and *Holtermanniella takashimae* (1.95 ± 0.43 μg/ml AIA units), and showed low production levels; while strains identified as *Cryptococcus cylindricus* CRUB1771, *Asterotremella albida* T9.1, *Trichosporon* sp. L12.20, *Candida cf ralunensis* CRUB1774, *Candida saitoana* CRUB1770, *Candida* sp. T18.4b, and *Wickerhamomyces sp*.CRUB1773 showed minimal production (Table 2).

Siderophore production was detected on MMS + CAS plates by the presence of an orange halo. Three strains developed strong halo formation after 2 weeks: *A. pullulans* YF8.3 production yield was about sevenfold more than the other two positive strains, *A. albida* T9.1 and *C. saitoana* CRUB1770. The remaining six strains showed weak halos after 1 month of incubation, which could not be measured (Table 2).

The ability to solubilize inorganic phosphate was observed on *H. takashimae* L8.12, *C.saitoana* CRUB1770, and *Wickerhamomyces* sp. CRUB1773; *A. albida* T9.1, *Trichosporon* sp. L12.20, and *C.* cf. *ralunensis* CRUB1774 showed no halo formation, while other strains showed variable results among replicates. Solubilization halos

Table 2. Plant growth promoting characteristics evaluated *in vitro* for 12 yeast strains.

Species	Auxin (μg/ml)	Siderophores (%yield)	Phosphate solubilization[†]
Guehomyces pullulans CRUB1772	3.68 ± 0.35^{d}	w	v
Cryptococcus cylindricus CRUB1771	0.19 ± 0.19^{a}	w	v
Holtermanniella sp. L5.6	2.21 ± 0.10^{c}	w	v
Holtermanniella takashimae L8.12	1.95 ± 0.44^{bc}	w	+
Asterotremella albida T9.1	0.23 ± 0.15^{a}	43.5 ± 10.7	−
Trichosporon sp. L12.20	0.53 ± 0.03^{a}	−	−
Aureobasidium pullulans YF8.3	**9.15 ± 0.87[e]**	**292.63 ± 27.2**	v
Candida cf ralunensis CRUB1774	0.69 ± 0.03^{ab}	w	−
Candida maritima CRUB1775	4.01 ± 0.46^{d}	w	−
Candida saitoana CRUB1770	0.51 ± 0.27^{a}	35.19 ± 3.21	+
Candida sp. T18.4b	0.12 ± 0.06^{a}	−	nd
Wickerhamomyces sp. CRUB1773	0.05 ± 0.35^{a}	w	+

Letters indicate different homogeneous groups calculated by Tukey test. Bold characters indicates the highest level of production.
[†]Only qualitative data are available from two separate assays; −, negative; +, positive; v, variable, w, weak; nd, not determined (no test were performed for that strain).

were usually too small for measuring, so only qualitative data are shown (Table 2).

Biocontrol characteristics

All strains were evaluated for pathogen growth inhibition against *V. dalhiae* PPRI5569, *P. aphanidermatum* PPRI 9009, and *F. oxysporum* PPRI5457, by co-culturing and supernatant-culturing assays. All strains were able to inhibit strain PPRI5569 of *V. dahliae* grown in co-culturing assays, with statistically significant reduction of fungal diameter up to 68%, *Candida* sp. T18.4b being the main inhibitor species (Table 3). Six strains were able to inhibit strain PPRI 9009 *P. aphanidermatum* growth in co-culturing assays, with statistically significant reduction of fungal diameter from 17 to 25%, the main inhibitor being *C. saitoana* CRUB1770 (Table 3). The co-culturing assay with strain PPRI5457 of *F. oxysporum* showed no reduction in pathogen growth but changes in fungal mycelium color and colony morphology were observed in some plates. The supernatants from species *Trichosporon* sp. L12.20, *G. pullulans* CRUB1772 and *Holtermanniella* sp. L5.6 were able to inhibit growth of strain *V. dalhiae* PPRI5569 by 11.9%, 13.7, and 17.5, respectively (Table 4). None of the culture supernatants were able to inhibit the growth of strains PPRI 9009, *P. aphanidermatum* or strain PPRI5457 of *F. oxysporum*.

Discussion

The yeast strains evaluated correspond to diverse taxonomical and environmental origins. Production of auxin-like compounds was observed from yeast strains representing both Ascomycota and Basidiomycota Phyla isolated from dry fruits and rhizospheric soil. Evaluation of auxin-like compounds production was performed on DEV-Tryptophan broth and all yeasts strains were able to grow in this medium; but, the growth was slow which might led to an under-estimation of the potential production. The main producer of auxin-like compounds corresponded to strain YF8.3 isolated from dry fruits of *Nothofagus* and identified as *A. pullulans*. Its level of production was two-fold higher than the highest level of production reach by the strains isolated from soil. The production levels for soil strains ranged from 1.95 to 4.01 μg/ml IAA units. These values are similar to *Candida tropicalis* production values (2.55 μg/ml IAA units) reported by Amprayn et al. [19], but much lower than *Lindnera saturnus (Williopsis saturnus)* (22.51 μg/ml IAA units) reported by Nassar et al. [18]. These yeast strains were able to increase the dry weight of inoculated rice seedling roots or the length of maize roots, respectively, in in vivo assays [18, 19]. The levels of auxin-like compounds produced by the Patagonian isolates used in this study are promising and further *in vivo* assays are required.

Detection of siderophore compounds was performed on MMS-CAS plates, and siderophores production was evident in three strains recovered from different *Nothofagus* related environment. *A. pullulans* YF8.3 was isolated from the surface of *Nothofagus nervosa* dry fruits, while strain CRUB1770 identified as *C. saitoana*, and strain T9.1 identified as *A. albida* were isolated from the bulk soil in *N. pumilio* forest and from the

Table 3. Pathogen growth, measure as colony diameter, after yeast-pathogens co-culture assay, and inhibition of pathogen growth, expressed as percentage diameter reduction.

	Verticillium dahliae PPRI5569		*Pythium aphanidermatum* PPRI 9009	
Treatment	**Diameter (mm)**[†]	**% Reduction**	**Diameter (mm)**[†]	**% Reduction**
Control without yeast	70.4 ± 2.4^{i}		38.9 ± 2.6^{d}	
Guehomyces pullulans CRUB1772	49.3 ± 0.6^{acd}	30	32.3 ± 1.5^{abc}	17
Cryptococcus cylindricus CRUB1771	47.2 ± 1.6^{ac}	33	37.2 ± 1.6^{d}	4
Holtermanniella sp. L5.6	52.3 ± 1.7^{bd}	26	Nd	Nd
Holtermanniella takashimae L8.12	56.3 ± 2.5^{b}	20	Nd	Nd
Asterotremella albida T9.1	28.7 ± 2.6^{g}	59	30.7 ± 2.9^{abc}	21
Trichosporon sp. L12.20	43.0 ± 1.0^{e}	39	Nd	Nd
Aureobasidium pullulans YF8.3	36.8 ± 0.3^{h}	48	30.7 ± 4.2^{abc}	21
Candida cf *ralunensis* CRUB1774	47.0 ± 2^{ae}	33	30.2 ± 0.8^{ab}	22
Candida maritima CRUB1775	48.0 ± 1.7^{ae}	32	31.8 ± 1.8^{abc}	18
Candida saitoana CRUB1770	52.5 ± 0.7^{bcd}	25	29.2 ± 4.1^{a}	25
Candida sp. T18.4b	22.3 ± 1.7^{f}	68	37.8 ± 2.4^{d}	3
Wickerhamomyces sp. CRUB1773	54.3 ± 2.5^{b}	23	36.4 ± 1.0^{d}	6

Letters indicate different homogeneous groups calculated by Tukey, for each pathogen. Nd, not determined (no test were performed for that strain).
[†]Average value from three replicates.

Table 4. Pathogen growth, measure as colony diameter, after culture supernatant assay and inhibition of pathogen growth, expressed as percentage diameter reduction.

	Verticillium dahliae PPRI5569		*Pythium aphanidermatum* PPRI 9009	
Treatment	**Diameter (mm)**[†]	**% Reduction**	**Diameter (mm)**[†]	**% Reduction**
Control without yeast	30.5 ± 0.3^{a}		18.4 ± 3.3	
Guehomyces pullulans CRUB1772	26.3 ± 0.6^{c}	14	21.0 ± 2.8	−14
Cryptococcus cylindricus CRUB1771	29.0 ± 0.0^{a}	5	16.0 ± 1.5	13
Holtermanniella sp. L5.6	25.2 ± 0.8^{d}	17	16.5 ± 2.0	10
Holtermanniella takashimae L8.12	29.5 ± 0.5^{a}	3	15.0 ± 0.5	19
Asterotremella albida T9.1	30.4 ± 1.2^{a}	0	18.1 ± 2.4	2
Trichosporon sp. L12.20	26.9 ± 0.2^{c}	12	14.8 ± 2.0	19
Aureobasidium pullulans YF8.3	31.4 ± 0.2^{a}	−3	18.0 ± 1.3	2
Candida cf *ralunensis* CRUB1774	29.8 ± 0.3^{a}	2	16.7 ± 0.8	9
Candida maritima CRUB1775	29.0 ± 0.0^{a}	5	16.8 ± 0.8	9
Candida saitoana CRUB1770	28.3 ± 0.1^{a}	7	15.9 ± 2.7	14
Candida sp. T18.4b	31.1 ± 0.5^{a}	−2	19.2 ± 5.9	−4
Wickerhamomyces sp. CRUB1773	30.1 ± 0.1^{a}	1	17.0 ± 1.0	8

Letters indicate different homogeneous groups calculated by Tukey, for each pathogen.
[†]Average value from three replicates.

ectomycorrhizosphere in *N. nervosa* forest, respectively. *A. pullullans* is a known siderophore producer and has been associated with antimicrobial activity, which may be linked to the antimicrobial activity of siderophores [26, 27]. Low siderophore production was observed for other strains isolated from soil related substrates. Micronutrients, such as Fe are present at low concentrations on aerial parts of the plants and the production of siderophores may be a strategy to survive this environment [12] by enhancing Fe uptake, and/or by increasing competitive ability against other inhabitants on the fruit surface [5, 6, 27].

Solubilization of inorganic P is usually attributed to CO_2 and weak organic acids derived from biological activity in soils [5]. In a previous study, we observed that Patagonian soil yeast communities were able to produce weak acids *in vitro* which suggested that inorganic P solubilization was to be expected in these communities [13]. The yeasts strains isolated from rhizosphere and bulk soil, *Holtermaniella takashimae* L8.12, and *C. maritima* CRUB 1775, were able to solubilize phosphate when included in YPD medium as $Ca_3(PO_4)_2$. Four strains showed variable results among replicates and solubilization halos were too small for measuring.

The present study, showed that several yeasts strains were able to reduce the growth of the common soil-borne pathogens, *V. dalhiae* PPRI5569 and *P. aphanidermatum* PPRI 9009. Members of genus *Pythium* are the most important responsible for post- and pre-emergence damping-off disease; these pathogens affects seed and seedlings before emergence [28]. *Verticillium* spp. members are responsible for verticillium-wilt disease; the pathogen enters the root tissue through surface wounds and the infection is more severe when the pathogen reaches the internal xylem vessels [29]. *F. oxysporum* species complex cause vascular wilt, root rot, and damping-off in more than 120 host plants including ornamentals and vegetables of economic importance [30]; the pathogen penetrates the root and colonizes the cortex were it could lead to occlusion of host vessels [31]. Members of these three pathogenic genera are present in Argentina affecting blueberry [32, 33] and *Pinus* spp. [34, 35] which are of economic importance in Patagonia and other cold-temperate environments. The ability of the yeast strains to reduce pathogen growth in *co-culturing assay* varied with pathogen strains. *V. dahliae* PPRI5569 seems to be more susceptible to any yeast treatment than *P. aphanidermatum* PPRI 9009. While no yeasts strains were able to inhibit *F. oxysporum* PPRI5457 growth (colony diameter), some of them affect colony appearance. Changes in *F. oxysporum* colony appearance have been associated with variation in the C/N ratio in culture media [36]. While no obvious growth inhibition was observed, the change in colony appearance might indicate change in nutrient distribution in the plate due to the growth of yeast strains. Jabnoun-Khiareddine et al. [37] working with *Trichoderma* and several *V. dahliae* isolates, shown that *in vitro* reduction of mycelia growth of 30–70% was associated with an effective suppression of *Verticillium* wilt in tomato plant. Similar growth reduction percentages were found on the present study, suggest that these yeast strains could be effective on suppression of *Verticillium* wilt but further *in vivo* assays are required. The *yeast culture supernatant assay* was performed to evaluate the production of extra-cellular

compounds that may be associated with fungal pathogen growth inhibition. Only supernatant from *Trichosporon* sp. L12.20, *G. pullulans* CRUB1772, and *Holtermanniella* sp. L5.6 were able to reduce pathogen growth at a low percentage. Siderophore production (extracellular) by microorganisms has been associated with pathogen biocontrol effect as this compound may sequester Fe ion making this nutrient unavailable for the pathogen [6, 27]. Nevertheless, *Trichosporon* sp L12.20, *G. pullulans* CRUB1772, and *Holtermanniella* sp. L5.6 showed weak production of extracellular siderophores, which indicates that supernatant inhibition of pathogen growth may be related to other inhibitory compounds produced by yeasts and these extracellular compounds should be further studied. For most of the yeasts strains studied, the pathogen growth inhibition could be attributed to competition rather than the production of inhibitory compounds. The co-occurrence of pathogen and other microorganisms cause competition for space (i.e., host tissue entrance wound) or nutrients, which result in pathogen growth reduction and/or reduced virulence [6, 38]. The yeast-pathogen competence mechanism has been a strategy used successfully on post-harvest disease control on different fruit and it has been recommended as it avoids the production of resistant strains [27, 38]. A similar strategy could be used to develop an efficient protection against soil-borne pathogens with some of the yeast species evaluated in the present study: seed coating prior to sowing or promoting the colonization of the root surface by biocontrol yeast strains could reduce the severity of pathogen infection under field or greenhouse conditions. The use of biocontrol agents with plant growth promoting features could be more convenient as these agents not only reduce the impact of the pathogen on crops but also enhance plant growth [38]. *A. pullulans* YF8.3 strain isolated from *N. nervosa* dry fruits, showed the highest production of auxin-like compounds and siderophores, and was able to out compete *V. dahaliae* PPRI5569 and *P. aphanidermatum* PPRI 9009 in *in vitro* test. The species *A. pullulans* is well known by the ability of producing *pullulan*, a linear polysaccharide that protects from desiccation or help to adhere to environmental substrates [39]. Therefore, strain *A. pullulans* YF8.3 is a suitable candidate to be used in seed coating as it may attach to seed more easily than soil species and endure seed storage conditions (i.e., low humidity); while it may also prevent the entrance of pathogens to seeds and during seed germination in soil. *G. pullulans* CRUB1772 and *C. maritima* CRUB1775 strains showed good production of auxin-like compounds and effectively inhibited growth of pathogens in co-culture, making these strains good candidates for direct inoculation on seedlings prior to sowing in soil.

Our results show that yeast species present in different environments in Northwestern-Patagonia have physiological features *in vitro*, compatible with plant growth promotion, and they may be actively influencing plant growth in these environments. The ability of these species to effectively compete with soil-borne pathogens shows how yeast communities could regulate or influence other soil microorganism communities. *In vivo* assays for detection of plant growth enhancement are required and will be performed to validate the *in vitro* results. In the Northwestern-Patagonian part of Argentina there is a nucleus of plant producers dedicated to forestry and organic-fruit production for local and foreign markets. The development of biological products from native yeast (which are able to produce auxin-like compounds and siderophores, solubilize inorganic phosphate, and reduce plant pathogen growth), is an attractive biotechnological tool to improve organic production in cold-temperate regions.

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Conflict of interest

All authors declare not to have any financial/commercial conflicts of interest.

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