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Soil yeasts promoting plant growth: benefits for the development of common wheat and white mustard

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Abstract

A large number of soil microorganisms are characterized as plant growth promoting, but there seems to be a lack of comprehensive knowledge regarding plant growth promoting soil yeasts. The aim of the experiment was to analyse the properties of three yeast species: *Schwanniomyces occidentalis* BK0302D, *Cyberlindnera saturnus* CK2404I and *Candida tropicalis* 2TD2912B, important for plant growth (ammonium sulphate transformation, phosphorus, potassium and zinc dissolution), and to evaluate the effect of yeast on the growth of common wheat and white mustard seedlings after seeds' inoculation. Common wheat and white mustard seeds were inoculated with the selected yeasts. The final measurements showed that the highest amount of nitrate (10.40 μg mL^{-1} NO_3^-) was produced by *C. saturnus* CK2404I, while *S. occidentalis* BK0302D solubilized the largest amount of phosphorus (63.70 μg mL^{-1} P). All three strains are marked as potassium and zinc solubilizers with both acid and alkaline phosphatase activity. This is the first report on *S. occidentalis* and *C. tropicalis* ability to solubilize insoluble potassium and zinc, and *C. saturnus* ability to solubilize insoluble phosphorus, potassium and zinc. Also, *C. tropicalis* 2TD2912B exhibited high antagonistic activity (66% growth inhibition) toward *Botrytis cinerea*. *In vivo* trial was conducted in a low-nutrient substrate, and *S. occidentalis* BK0302D was found to have the most considerable influence on common wheat biomass production (34% increase). White mustard inoculation with *C. saturnus* CK2404I resulted in a 4-fold higher biomass production, while *S. occidentalis* BK0302D induced a 2-fold increase.

The presented results confirmed the multi-functional plant growth promoting characteristics of the tested yeasts and their potential for broad application from conventional agriculture on low-nutrient soils to revegetation of disturbed substrates.

Keywords: *Schwanniomyces occidentalis*, *Cyberlindnera saturnus*, *Candida tropicalis*, nitrogen, phosphorus, *Triticum vulgare*, *Sinapis alba*.

Introduction

Natural soils have large reserves of phosphorus, potassium and iron, unfortunately, most often in an insoluble form (Velázquez et al., 2016; Lu et al., 2018; Ali et al., 2019). A global problem of the soils under cereal production is zinc deficiency (Mumtaz et al., 2017). Commonly, nutrient deficiency is solved through chemical inputs of N, P and K, but such short term directed practices lead to further degradation. Agricultural reliance on mineral fertilizers faces the problem of low uptake efficiency. Globally, 50 million tons of phosphorus fertilizers are applied annually, while only 5% to 10% is available to crops. The majority stay trapped in an insoluble form representing potential environmental risk (Alori et al., 2017; Menezes-Blackburn et al., 2018; Ali et al., 2019). The same problem characterizes the application of potassium and zinc fertilizers (Etesami et al., 2017; Mumtaz et al., 2017), while nitrogen availability is strongly correlated with soil type, tillage practice, crop rotation and amount of precipitation (Di Benedetto et al., 2017).

Unavailable phosphorus, potassium and zinc compounds can be converted back to available forms through bioaugmentation of microbes having solubilization mechanisms such as the production of chelating ligands, secretion of organic acids, activation of oxido-reductive systems, etc. (Alori et al., 2017; Mumtaz et al., 2017). Their potential to raise the nutritional status of agricultural soils and improve the productivity of degraded, low-nutrient and marginal agricultural soils is exploited through a large number of commercial

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biofertilizers (Velázquez et al., 2016; Fernandez-San Millan et al., 2020).

Different groups of soil microorganisms have been referred to as plant growth promoting (PGP), mostly filamentous fungi and bacteria, but there seems to be a lack of comprehensive knowledge concerning the role of soil yeasts (Fernandez-San Millan et al., 2020). Yeasts represent common soil inhabitants involved in organic matter mineralization, nitrogen compound transformation and nutrient solubilization (Moreira, do Vale, 2018; Fernandez-San Millan et al., 2020). Even though yeast-plant interactions are neglected in the studies on PGP microorganisms, several species: *Saccharomyces* sp., *Candida tropicalis*, *Aureobasidium pullulans*, *Rhodotorula mucilaginosa*, *Williopsis californica* and *W. saturnus*, are characterized as plant growth promoting yeasts (PGPY) with the ability to produce indoles, siderophores, to mobilize essential nutrients, prevent disease occurrence and alleviate the level of stress effects (Al-Falih, 2005; Olanrewaju et al., 2017; Radić et al., 2018). All those characteristics have contributed to the perception of yeast as a cost-effective and eco-friendly, renewable alternative to mineral fertilizers applicable in sustainable farming practices (Fernandez-San Millan et al., 2020).

The objectives of the study were: (1) to detect plant growth promoting (PGP) characteristics of the selected yeasts (*in vitro*); (2) to determine ammonia transformation and phosphate solubilizing dynamics of the selected yeast strains; (3) to estimate PGP effects of yeast strains on common wheat and white mustard growth (*in vivo*) in a low-nutrient substrate.

Materials and methods

The experiments were conducted during 2018 at the Faculty of Agriculture, University of Belgrade, Serbia.

Yeast material and inoculum preparation. As starting material, soil yeasts *Schwanniomyces occidentalis* BK0302D, *Cyberlindnera saturnus* CK2404I and *Candida tropicalis* 2TD2912B were used with sequences deposited in the GenBank database: accession Nos. KP990664, KP990663 and KR013283, respectively. The strains are ammonia (NH_3) and indole-3-acetic acid (IAA) producers with confirmed phosphate solubilizing ability (qualitative assay) (Radić et al., 2018). Inoculums were prepared by separate cultures of yeast strains on a yeast extract-peptone-dextrose (YPD) medium at 28 ± 2°C/48 h/100 rpm in an incubator ES-20 (Biosan, Latvia). The suspensions were centrifuged at 6000× *g* for 10 min, 5804R (Eppendorf, Germany). The cells were washed and diluted in sterile physiological solution to 10^5 or 10^8 CFU mL^{-1} (0.35 and 0.5 OD, respectively) at 780 nm with a T70 UV/VIS spectrometer (PG Instruments LTD, UK).

Plant growth promoting (PGP) characteristics of the selected yeasts (*in vitro*). The ammonium sulphate ($(NH_4)_2SO_4$) transformation of the selected strains was detected by the method of Al-Falih (2006). Briefly, ammonium sulphate was added in 100 mL of the Czapek-Dox liquid medium to reach the final concentration of 0.1% w/v. The pH value of Czapek-Dox liquid medium was adjusted to 6. The treatments were as follows: Czapek-Dox medium inoculated with 1) *S. occidentalis* BK0302D, 2) *C. saturnus* CK2404I, 3) *C. tropicalis* 2TD2912B, and 4) uninoculated Czapek-Dox medium (control). The yeast inoculums contained 10^5 CFU mL^{-1}.

The experiment was set up in twelve repetitions per treatment. The flasks were incubated for 4 weeks at 28°C/100 rpm in an incubator ES-20 (Biosan). After each week, three replicates of each treatment were removed. The concentration of nitrates in the filtrate was determined by the ultraviolet spectrophotometric method 4500-NO_3^- B (https://www.standardmethods.org/doi/10.2105/SMWW.2882.089). The absorbance was measured at 220 and 275 nm with a spectrometer T70 UV/VIS (PG Instruments Ltd.), and nitrate absorbance (abs) was calculated by the formula: abs (NO_3^-) = abs 220 nm – 2 × abs 275 nm.

The concentration of nitrates in Czapek-Dox medium was determined using a calibration curve in the 0–100 mg L^{-1} NO_3^- range. The nitrate concentration produced by the yeasts was determined by subtracting the values of the treatments from the control. The pH value of the medium was measured with a pH meter Eutech pH 700 (Thermo, Canada). Finally, the yeast suspensions in Czapek-Dox medium were filtered through Whatman No. 1, and the yeast biomass retained on the paper was determined by drying the filter paper to a constant weight at 80°C temperature in a heating chamber ED 56 (Binder, Germany). This value was subtracted from the uninoculated medium (control).

Phosphate solubilization was detected by the method of Al-Falih (2005). Briefly, calcium phosphate ($Ca_3(PO_4)_2$) was added in 100 mL of the Czapek-Dox liquid medium to reach the final concentration of 1.0% w/v. The pH value of Czapek-Dox liquid medium was adjusted to 6. The treatments were as in the case of transformation of ammonium sulphate. The yeast inoculums contained 10^5 CFU mL^{-1}.

The experiment was set up in twelve repetitions per treatment. The flasks were incubated for 4 weeks at 28°C/100 rpm in an incubator ES-20 (Biosan). After each week, three replicates of each treatment were removed, and the yeast suspensions in Czapek-Dox medium were filtered through Whatman No. 1. After filtration, the yeast biomass retained on the paper was determined by drying the filter paper to a constant weight at 80°C temperature in a heating chamber ED 56 (Binder). The concentration of soluble phosphorus in the filtrate was determined by method 4500-P Phosphorus (https://www.standardmethods.org/doi/abs/10.2105/SMWW.2882.093). The absorbance was measured at 880 nm with a spectrometer T70 UV/VIS (PG Instruments Ltd.) using a calibration curve in the 0–250 μg mL^{-1} P range. The concentration of phosphorus solubilized by the yeasts was determined by subtracting the values of the treatments from the control. The pH of the medium was measured with a pH meter Eutech pH 700 (Thermo).

The phosphatase activity of the selected isolates was determined by API ZYM (BioMérieux, France) according to the manufacturer's instructions. The activity was semi-quantitatively determined by the API ZYM Colour Chart.

Potassium solubilization was detected on the modified Aleksandrov medium (Etesami et al., 2017). The colonies exhibiting clear zones after incubation at 28°C temperature for 72 h were considered positive. Potassium solubilization index (SI_K) was calculated by the formula:

$SI_K = Z / N$, where Z is halo zone diameter, N – diameter of colonies (Setiawati, Mutmainnah, 2016).

Zinc solubilization was detected on tris-minimal salt media (Mumtaz et al., 2017). The colonies exhibiting clear zones after incubation at 28°C temperature for 24 h were considered positive. Zinc solubilization index (SI_{Zn}) was calculated by the formula:

$SI_{Zn} = Z / N$, where Z is halo zone diameter, N – diameter of colonies (Rokhbakhsh-Zamin et al., 2011).

Siderophore production was detected on the CAS (chrome azurol S) (Fluka, USA) agar medium

(Alexander, Zuberer, 1991). The colonies exhibiting yellow-orange zones after incubation at 28°C temperature for 48–72 h were considered positive.

The lipase, N acetyl-*β*-glucosaminidase and *β*-glucosidase activities were determined by API ZYM kit (BioMérieux, France) according to the manufacturer's instructions. The activity was semi-quantitatively determined by the API ZYM Colour Chart. Protease production was determined using a skim milk agar (Chaiharn et al., 2008). The presence of a clear zone around the colonies after incubation at 30°C temperature for 5 days indicated protease activity. The cellulase activity was determined by the carboxymethyl cellulose (CMC) agar method (Romsaiyud et al., 2009). The activity unit (AU) of protease and cellulase production was defined by the diameter of the clear zone divided by the diameter of the zone with yeast colonies (Fu et al., 2016).

The antagonistic activity against *Pythium aphanidermatum* and *Botrytis cinerea* was evaluated using the confrontation assay on potato dextrose agar (PDA) plates. The plates were double inoculated with agar plugs (5 × 5 mm) of a 7-day-old culture of *P. aphanidermatum* or *B. cinerea* and yeast isolate (streak inoculation) at a 3 cm distance. Petri dishes were incubated at 25 ± 2°C temperature in the dark until the control fungus had reached the edge of the plate.

The pathogen mycelial growth inhibition percentage was calculated by the formula:

Mycelial growth inhibition (%) = ((R1 − R2) / R1)) × 100,

where R1 is the average diameters of pathogen colony of control, R2 – the average diameters of pathogen colony of dual-culture plates (Minova et al., 2015). Each treatment consisted of three replicates.

PGP effects of yeast strains on common wheat and white mustard growth (*in vivo*). Seeds of common wheat (*Triticum vulgare* L.) and white mustard (*Sinapis alba* L.) were surface-sterilized with 70% ethanol for 2 min, followed by 15 min exposure to 2% bleach. Afterwards, the seeds were 5-time washed with sterile distilled water and dried under aseptic conditions. The seeds were inoculated by immersion into 100 mL of the yeast suspension (10^8 CFU mL^{-1}) and shaken at room temperature for 1 h/100 rpm at the orbital shaker KS 260 basic (IKA, Germany). The treatments were as follows: 1) seeds inoculated with *S. occidentalis* BK0302D, 2) seeds inoculated with *C. saturnus* CK2404I, 3) seeds inoculated with *C. tropicalis* 2TD2912B, and 4) uninoculated seeds (control).

The prepared seeds were sown in 0.5 dm^3 plastic pots filled with overburden waste from the coal mine Kolubara (Lazarevac district, Serbia) characterized by low contents of nitrogen, organic carbon and humus (Radić et al., 2018). Prior to use, the overburden waste was air-dried, ground and sieved through a 2 mm diameter sieve. Plants were grown under controlled conditions at the maximum of 30°C and the minimum of 20°C temperature within a 12/12 hrs photoperiod at 14 000 lux 600 W (MH Philips) and relative air humidity of 60%. The experiment was set up in triplicate as a completely randomized design, 50 seeds per pot. After two weeks of the experimental period, shoot height, root length and dry biomass were recorded.

Statistical analysis. To detect a significant difference ($\alpha = 0.05$) between the mean values in all performed experiments, one-way analysis of variance (ANOVA) and Tukey's honestly significant differences test with the software package *Statistica*, version 8.0 (StatSoft Inc., USA) were used. To determine the correlation between the variables, the Pearson correlation coefficient calculator was used (www.socscistatistics.com/tests/pearson).

Results and discussion

Previous biochemical screening of the selected strains pointed to their PGP nature (Radić et al., 2018). Bearing in mind that such characteristics occur in association (Fu et al., 2016), this study aimed to reveal several other PGP attributes of the selected yeast strains. The characterization process started with the determination of ammonia transformation and phosphate solubilizing dynamics considering nitrogen and phosphorus as the ultimate and direct limiting nutrients in the plants (Elias et al., 2016).

Yeasts raise nitrogen availability through ammonium production and ammonium transformation to nitrate (Al-Falih, 2006; Fu et al., 2016). The results of the ammonium sulphate transformation test showed that *C. saturnus* CK2404I and *C. tropicalis* 2TD2912B are involved in nitrate (NO_3^-) production (Table 1). *S. occidentalis* BK0302D showed low biomass (0.025 g), which confirms its inability to use ammonium sulphate as a sole nitrogen source. Both yeasts produced the most nitrates during the 1st week: *C. saturnus* CK2404I 12.40 μg mL^{-1} NO_3^- and *C. tropicalis* 2TD2912B 11.20 μg mL^{-1} NO_3^-. During the whole experimental period, no biomass production was recorded in the control treatment, while the pH value and concentration of nitrate remained constant.

Table 1. The concentration of nitrate (NO_3^-), pH value and biomass production of soil yeasts grown in the Czapek-Dox liquid medium supplemented with ammonium sulphate

Yeast strain	Parameter	1st week	2nd week	3rd week	4th week	Biomass g
Schwanniomyces occidentalis BK0302D	NO_3^- mg L^{-1}	0.00 ± 0.00 a,A	0.00 ± 0.00 a,A	1.50 ± 0.30 a,A	1.85 ± 0.15 a,A	0.0250
	pH	3.03	3.08	6.04	6.01	
Cyberlindnera saturnus CK2404I	NO_3^- mg L^{-1}	12.40 ± 0.50 c,C	5.80 ± 0.90 b,B	9.80 ± 1.00 b,C	10.40 ± 1.30 b,C	0.2209
	pH	3.07	2.93	3.10	2.97	
Candida tropicalis 2TD2912B	NO_3^- mg L^{-1}	11.20 ± 1.60 b,B	10.60 ± 1.90 c,B	11.00 ± 1.70 b,B	10.00 ± 1.90 b,B	0.3473
	pH	3.81	3.04	2.96	2.96	

Note. Values in the same column with different lowercase letters differ significantly and values in the same row with different uppercase letters differ significantly (Tukey's test; $p < 0.05$); the concentration of NO_3^- produced by the yeasts was determined by subtracting the values of the treatments from the control; data are provided as mean ± standard deviation.

Al-Falih (2006) reported G*eotrichum candidum, G. capitatum* and *Rhodotorula minuta* role in ammonium sulphate transformation to nitrate. *R. minuta* produced 94 μg mL^{-1} NO_3^-, *G. capitatum* – 70 μg mL^{-1} NO_3^- and *G. candidum* – 55 μg mL^{-1} NO_3^-. The amounts recorded in our experiment at the end of the 4th week were much lower since *C. saturnus* CK2404I produced 10.40 μg mL^{-1} NO_3^- and *C. tropicalis* 2TD2912B produced 10 μg mL^{-1} NO_3^-. In parallel with nitrate accumulation, the pH value decrease occurs indicating organic acids accumulation (Al-Falih, 2006). On the other hand, the 3-week measurement revealed pH value increase in the medium with *S. occidentalis* BK0302D suggesting again no nitrate production. That can be related to its significantly lower biomass compared to the other two strains.

Interestingly, plants manifest a nitrogen-source preference, which is dependent on various ecological factors. Daryanto et al. (2018) pointed out the important question of why some agricultural and pioneer species prefer NO_3^- to NH_4^+, even if its acquisition and incorporation into the organic compounds requires an additional step. They reviewed NH_4^+ accumulation as one of the reasons causing toxic effects and affecting uptake of some cations, while NO_3^- toxicity is related to much higher soil concentrations. In that context, the role of the tested yeasts as heterotrophic nitrifiers has more importance.

Among the nutrients that most often restrict plant growth, phosphorus is right behind nitrogen with the least mobility among them (Ali et al., 2019). *S. occidentalis* BK0302D, *C. saturnus* CK2404I and *C. tropicalis* 2TD2912B were subjected to a quantitative phosphorus solubilization assay (Table 2).

The measurements made after the 1st week showed 21.40 μg mL^{-1} P solubilized by *S. occidentalis* BK0302D, while the amount of phosphorus was not increased by the other two strains. On the contrary, the first record of *C. saturnus* CK2404I medium revealed a significantly lower amount of solubilized phosphorus in comparison to the control. The pH value variations of the culture medium support the claim that acidification is the major mechanism of phosphate solubilization (Adhikari, Pandey, 2019). Namely, pH value decreased in the medium with *S. occidentalis* BK0302D and *C. tropicalis* 2TD2912B, while no change was recorded in the medium with *C. saturnus* CK2404I. The 2-week record confirmed that the solubilization process in the medium with *S. occidentalis* BK0302D and *C. tropicalis* 2TD2912B was continued. At this point, the dramatic decrease of solubilized phosphorus concentration occurred in the medium with *C. saturnus* CK2404I. Therewithal, an increase in biomass production was recorded (data not shown) and, according to Spagnoletti et al. (2016), the accumulation of phosphorus in the microbial biomass may cause the lower concentration of soluble phosphorus in the medium.

Naturally, microbial biomass represents a dynamic reservoir of phosphorus subjected to constant change (Richardson, Simpson, 2011). At the same time, the pH value in the medium with *C. saturnus* CK2404I increased to 7.5, which suggests that phosphorus integration into yeast biomass left behind a lot of available calcium causing solution neutralization. The 3rd week was the breaking point for *C. saturnus* CK2404I medium, when the concentration of phosphorus in the solution started to rise, while pH value dropped to 5.7.

Table 2. The concentration of soluble phosphorus (P), pH value and biomass production of soil yeasts grown in the Czapek-Dox liquid medium supplemented with calcium phosphate

Yeast strain	Parameter	1st week	2nd week	3rd week	4th week	Biomass g
Schwanniomyces occidentalis BK0302D	P μg mL^{-1}	21.40 ± 1.20 c,B	47.10 ± 1.80 c,C	44.80 ± 1.30 d,C	63.70 ± 1.50 c,D	0.115
	pH	5.60	5.55	5.50	5.40	
Cyberlindnera saturnus CK2404I	P μg mL^{-1}	−9.50 ± 0.00 a,B	−72.80 ± 1.70 a,A	32.90 ± 1.90 c,C	46 ± 1.50 b,D	0.090
	pH	6.10	7.50	5.70	5.60	
Candida tropicalis 2TD2912B	P μg mL^{-1}	−0.70 ± 0.00 b,A	25.20 ± 1.50 b,B	22.70 ± 1.30 b,B	48.30 ± 1.10 b,C	0.065
	pH	6.10	5.70	5.60	5.50	

Note. Values in the same column with different lowercase letters differ significantly and values in the same row with different uppercase letters differ significantly (Tukey's test; $p < 0.05$); the concentration of soluble P produced by the yeasts was determined by subtracting the values of the treatments from the control; data are provided as mean ± standard deviation.

The final results of the phosphorus solubilization measurement showed that the largest amount was solubilized by the *S. occidentalis* BK0302D (63.70 μg mL^{-1} P). At the same time, this strain produced the highest biomass suggesting that an increase in the phosphorus solubilization resulted in the biomass production increase and, *vice versa*, which is also confirmed by the correlation coefficient 0.5899 ($p \leq 0.01$). Also, the lowest pH value (5.4) was observed in the medium with *S. occidentalis* BK0302D after a 4-week incubation period. Similarly, *Drechslera* sp. solubilized 36.46 μg mL^{-1} P, while pH value dropped to 5.5, whereas other dark septate fungi had much lower pH value (<3.6) and much higher amount of solubilized phosphorus (Spagnoletti et al., 2016). *C. tropicalis* 2TD2912B solubilized 48.30 μg mL^{-1} P, while *C. saturnus* CK2404I solubilized 46 μg mL^{-1} P, despite the initial stagnation. Elias et al. (2016) reported a decrease of pH value to 5 by *Aspergillus* sp. JUFbF58 and *Penicillium* sp. JUHbF94, but their solubilization efficiency was much higher (170 μg mL^{-1} P) compared to the studied strains. During the phosphorus solubilization assay soil yeasts exhibited different modes of adaptation to given conditions resulting in different solubilization dynamics. But, at the bottom line, all of them confirmed the phosphorus solubilizing feature, which has already been documented for *S. occidentalis* and *C. tropicalis* (Al-Falih, 2005; Gizaw et al., 2017). This study represents the first report of *C. saturnus* ability to solubilize insoluble phosphorus.

Another way to increase the availability of phosphorus in the soil is through extracellular phosphatase activity, since a large portion of phosphorus

is bound in organic matter (Spagnoletti et al., 2016; Alori et al., 2017). Enzymatic profiles of all three isolates confirmed the production of acid and alkaline phosphatase (Table 3), which is important in variable and heterogenic soil conditions. The activity of those enzymes was characterized as very strong, except for *C. saturnus* CK2404I, whose activity of alkaline phosphatase was shown to be low. Also, *C. tropicalis* 2TD2912B showed strong activity of naphtol-AS-BI-phosphohydrolase.

Table 3. Plant growth promoting attributes of yeasts

Yeast strain		*Schwanniomyces occidentalis* BK0302D	*Cyberlindnera saturnus* CK2404I	*Candida tropicalis* 2TD2912B
Solubilization	Potassium solubilization index (SI_K)	4.89	6.98	3.42
	Zinc solubilization index (SI_{Zn})	2	1.1	1.14
Production	Alkaline phosphatase	+++	+	+++
	Acid phosphatase	+++	+++	+++
	Naphtol-AS-BI-phosphohydrolase	–	–	+++
	Siderophores	–	–	–
	Lipase	–	–	–
	N-acetyl-*β*-glucosaminidase	–	–	+
	β-glucosidase	–	–	–
	Protease (AU)	–	–	–
	Cellulase (AU)	2	3	–

AU – activity unit; enzymatic activity: – – no, + – low (<5 nmol), ++ – moderate (5–20 nmol), +++ – strong (>20 nmol)

Potassium is the third essential element, right behind nitrogen and phosphorus. Regardless, the ability of PGP microorganisms to raise its supply is not common in *in vitro* PGP tests (Velázquez et al., 2016). Up to now, only a few yeast species (*Torulaspora globosa*, *Pichia anomala* and *Rhodotorula glutinis*) have been recognized as potassium solubilizers (Sattar et al., 2018). A qualitative assay of potassium solubilization marked all three strains as solubilizers with indexes ranging from 3.42 to 6.98 noted for *C. saturnus* CK2404I. This is the first report on *S. occidentalis*, *C. saturnus* and *C. tropicalis* ability to solubilize insoluble potassium. All three isolates showed zinc solubilizing capacity with solubilization indexes ranging from 1 to 2. Similar values were reported by Fu et al. (2016), who tested yeasts isolated from *Drosera spatulata*, and zinc solubilization index (SI_{Zn}) had the largest number of positive isolates ranging from 1 to 2, except *Dothideomycetes* sp. JYC385 (2.18). Fernandez-San Millan et al. (2020) reported 69 zinc solubilizing yeast strains with *Candida pimensis* Cpi-27 (SI_{Zn} = 6.25) and *C. apicola* Ca-40 (SI_{Zn} = 5.83) having the most outstanding efficiency. This is the first report on *S. occidentalis*, *C. saturnus* and *C. tropicalis* ability to solubilize zinc.

Siderophores are among the principal mechanisms of iron acquisition involved in phosphate solubilization too (Alori et al., 2017). Regardless of this fact, no production of siderophores was detected on the CAS agar medium.

The enzymatic profiling of soil yeasts was mainly directed toward the detection of compounds involved in phytopathogen inhibition. The ability of the studied yeasts to inhibit the growth of plant pathogens may significantly, even though indirectly, contribute to better adaptation and survival of plants. The biocontrol potential of *S. occidentalis* BK0302D, *C. saturnus* CK2404I and *C. tropicalis* 2TD2912B was estimated through the production of fungal cell wall-degrading enzymes and inhibition of pathogen growth. Semi-quantitative analysis of chitinolytic enzyme N-acetyl-*β*-d-glucosaminidase marked *C. tropicalis* 2TD2912B as the only producer. The cellulase activity was observed in medium of *S. occidentalis* BK0302D and *C. saturnus* CK2404I with 2 and 3 AU, respectively. Similar values were reported by Fu et al. (2016), where AU had the largest number of positive isolates ranging from 2 to 3, except for two isolates of *Aureobasidium pullulans* with 3.17 and 3.2. Such a feature may be of great help against *Oomycetes*, whose cell walls consist of cellulose and glucan (Fernandez-San Millan et al., 2020).

One of the representatives of *Oomycetes*, a large group of plant pathogens causing seedling blights, damping-off, seed rot, root rots and downy mildew, is *P. aphanidermatum* (Rahman, Sarowar, 2016) used in a confrontation test with the selected yeast strains. *C. saturnus* CK2404I was the only one that exhibited some level of antagonistic activity with 32% inhibition (Table 4) at the same time showing the highest cellulase activity (Table 3).

Confrontation test with *B. cinerea*, an important pathogen attacking a wide range of hosts and almost all plant parts (Radić, 2017), revealed new levels of antagonism. *C. tropicalis* 2TD2912B inhibited *B. cinerea* growth by 66% showing high antagonistic activity according to Sookchaoy et al. (2009) classification. The enzymatic profile of this strain confirmed chitinolytic activity, which has been shown to inhibit spore germination and germ tube elongation of *B. cinerea* (Muniappan, Heinrichs, 2016). *S. occidentalis* BK0302D antagonistic activity towards *B. cinerea* was qualified

Table 4. Mycelial growth inhibition (MGI) of *Pythium aphanidermatum* and *Botrytis cinerea* by soil yeasts

Yeast strain	MGI %	
	P. aphanidermatum	*B. cinerea*
Schwanniomyces occidentalis BK0302D	NI	56 ± 2.96 b
Cyberlindnera saturnus CK2404I	32 ± 1.29	46 ± 2.41 a
Candida tropicalis 2TD2912B	NI	66 ± 1.76 c

Note. NI – no inhibition; values followed by the different letters differ significantly (Tukey test, $p < 0.05$); data are provided as mean ± standard deviation.

as moderate, while *C. saturnus* CK2404I expressed low antagonistic activity according to Sookchaoy et al. (2009) classification.

A series of *in vitro* tests confirmed the PGP nature of soil yeast strains, which were capable of expressing both, indirect and direct, mechanisms. *In vivo* tests were conducted to verify the stimulative effects of yeasts on plant growth. For this part of the study, coal-mine overburden waste was used as a model for low-nutrient, no-structure and highly disturbed substrates. *In vivo* trials were conducted on common wheat and white mustard inoculated with *S. occidentalis* BK0302D, *C. saturnus* CK2404I and *C. tropicalis* 2TD2912B. The effects were monitored through biomass production, shoot height and root length (Table 5).

Table 5. Biomass production, shoot height and root length of common wheat and white mustard inoculated with soil yeasts

Treatment	Common wheat			White mustard		
	biomass g	shoot height cm	root length cm	biomass g	shoot height cm	root length cm
Control	2.28 ± 0.14 a	14.05 ± 3.68 a	9.52 ± 3.24 a	0.46 ± 0.08 a	7.60 ± 1.50 a	1.94 ± 1.00 a
Schwanniomyces occidentalis BK0302D	3.05 ± 0.13 c	16.13 ± 3.92 b	10.15 ± 3.70 b	0.93 ± 0.08 b	9.32 ± 1.30 b	2.11 ± 0.74 a
Cyberlindnera saturnus CK2404I	2.69 ± 0.19 b	17.97 ± 2.94 b	11.21 ± 2.54 b	2.02 ± 0.12 c	9.70 ± 1.30 b	3.01 ± 2.26 b
Candida tropicalis 2TD2912B	2.31 ±0.20 a	13.75 ± 3.7 a	9.28 ± 2.71 a	0.82 ± 0.10 b	9.30 ± 1.90 b	2.50 ± 1.59 a

Note. Values in the same column followed by the different letters differ significantly (Tukey test, $p < 0.05$); data are provided as mean ± standard deviation.

After 2-weeks, common wheat inoculated with *S. occidentalis* BK0302D produced the highest biomass (3.05 g), which represented an increase of 34%. This effect is supported by 15% higher shoots (16.13 cm) and 7% longer roots (10.15 cm) compared to the control (14.05 and 9.52 cm, respectively). Inoculation with *C. saturnus* CK2404I resulted in 18% higher biomass (2.69 g), 28% (17.97 cm) higher shoots and 18% (11.21 cm) longer roots. *C. tropicalis* 2TD2912B showed no plant growth promoting effects on wheat.

White mustard inoculation caused an enormous increase in biomass production by all strains. The most pronounced effect was recorded in treatment with *C. saturnus* CK2404I, where 4-fold higher biomass production (2.02 g) was recorded accompanied by 28% (9.7 cm) higher shoots and 55% (3.01 cm) longer roots. Among the studied yeast strains, this one exhibited the highest potassium solubilizing efficiency and nitrate production (Table 3). Inoculation with *S. occidentalis* BK0302D, the strain with the highest phosphorus and zinc solubilizing ability, caused 2-fold higher biomass production (0.93 g) and 23% (9.32 cm) higher shoots of white mustard. *C. tropicalis* 2TD2912B induced 78% (0.82 g) higher biomass production and 22% (9.3 cm) higher shoots of white mustard compared to the control (0.42 g and 7.6 cm, respectively).

In vivo trial confirmed the ability of the selected strains to promote common wheat and white mustard growth. According to Radić et al. (2018), *C. saturnus* CK2404I showed growth promoting effects on biomass production (19%), shoots (29%) and roots (67%) of red clover. Our results agree with those of Amprayn et al. (2012), who reported a 16–35% biomass increase of rice with *Candida tropicalis* HY (CtHY). Kuo et al. (2018) reported the stimulating activity of *Cryptococcus laurentii* (JYC370), which raised phosphorus content in plant tissues proving that phosphorus dissolved by particular yeast can be absorbed and converted into plant biomass. Fernandez-San Millan et al. (2020) reported a 10% higher leaf dry weight of maize 15 days after inoculation with *Saccharomyces cerevisiae* and *Lachancea thermotolerans* characterized as phosphorus and zinc solubilizers as well as cellulase and IAA producers.

The differences in yeast performance among plant species are a well-known problem of PGP microorganisms (Fernandez-San Millan et al., 2020), which was encountered in our experiment too. While *S. occidentalis* BK0302D significantly increased biomass production of wheat, *C. saturnus* CK2404I showed full potential in stimulating white mustard biomass production. On the other hand, *C. tropicalis* 2TD2912B significantly stimulated white mustard growth with no effects on wheat. The fact that the substrate chosen for the *in vivo* test is unfavourable for plant growth due to mechanical structure, low nitrogen and humus content increases the significance of results obtained by yeast inoculation. According to Egamberdiyeva (2007), such substrates help in reaching the full potential of PGP microorganisms.

Despite the broad biotechnological applications, the full potential of soil yeasts in sustainable agriculture and soil restoration is not completely exploited. On the other hand, yeast biotechnology has been comprehensively mastered by humans enabling the facilitated production of biofertilizers. Along with that, interest in eco-friendly agricultural practices highlights the importance of soil microflora and its contribution to ecosystem functioning and restoration. In that context, the obtained results may help the formulation of novel soil yeast-based bioinoculants that can be included in nutrient management regimes for wheat and white mustard and potentially other crops on low-nutrient soils.

Conclusions

1. The biochemical characterization confirmed the multifarious plant growth promoting attributes of soil yeasts. They are capable of conducting solubilization of phosphorus, potassium and zinc and increasing nitrogen supply. Those features make them effective nutrient suppliers to plants. They express indirect plant growth promoting mechanisms reflected through the production of cell wall-degrading enzymes and antagonistic activity toward *Botrytis cinerea*, one of the most important pathogens.

2. *Schwanniomyces occidentalis* BK0302D, *Cyberlindnera saturnus* CK2404I and *Candida tropicalis* 2TD2912B can solubilize insoluble potassium and zinc. This fact indicates their potential to solve one of the major agricultural problems potassium and zinc deficiency.

3. *C. saturnus* CK2404I can solubilize insoluble phosphorus and potentially represent an eco-friendly alternative to P-fertilizers.

4. The applied yeast inoculums stimulated common wheat and white mustard growth in an unfavourable substrate indicating their potential usage in wheat and mustard production in organic, chemical-free production.

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Augalų augimą skatinančių dirvožemio mielių rūšių poveikis baltųjų garstyčių ir paprastųjų kviečių daigams

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Santrauka

Žinoma, kad dirvožemio mikroorganizmai gali skatinti augalų augimą, tačiau pasigendama išsamesnių žinių apie atskirų grupių, taip pat ir dirvožemio mielių, poveikį augalams. Tyrimo tikslas – išanalizuoti mielių rūšių trijų padermių: *Schwanniomyces occidentalis* BK0302D, *Cyberlindnera saturnus* CK2404I ir *Candida tropicalis* 2TD2912B, augalų augimui svarbias savybes (pagal amonio sulfato transformaciją, fosforo, kalio ir cinko tirpinimą, fermentų sudėtį, antagonistinį aktyvumą prieš *Pythium aphanidermatum* ir *Botrytis cinerea*) ir įvertinti jų poveikį paprastųjų kviečių bei baltųjų garstyčių daigų augimui, sėklas inokuliavus mielėmis. Palyginus trijų rūšių mieles nustatyta, kad *C. saturnus* CK2404I pagamino didžiausią kiekį nitratų (10,4 μg mL^{-1} $NO3^{-}$), o *S. occidentalis* BK0302D ištirpdė daugiausia fosforo (63,7 μg mL^{-1} P). Visos tirtos mielės pasižymėjo rūgštiniu ir šarminiu fosfataziniu aktyvumu. Tai pirmasis tyrimas, kuriuo nustatyta *S. occidentalis* bei *C. tropicalis* geba tirpinti netirpų kalį ir cinką, o *C. saturnus* – netirpų fosforą, kalį ir cinką. Be to, *C. tropicalis* 2TD2912B pasižymėjo dideliu antagonistiniu aktyvumu prieš *Botrytis cinerea* (66 % augimo slopinimas). Mielėmis inokuliuotas sėklas daiginant mažai maisto medžiagų turinčioje terpėje nustatyta, kad po inokuliavimo *S. occidentalis* BK0302D praėjus dviem savaitėms labiausiai (34 %) padidėjo paprastųjų kviečių daigų masė; baltųjų garstyčių daigų masė padidėjo keturis kartus inokuliavus *C. saturnus* CK2404I ir du kartus – *S. occidentalis* BK0302D.
Eksperimento rezultatai patvirtino įvairias augalų augimą skatinančias tirtų mielių savybes ir jų plataus pritaikymo galimybes ir nederlinguose, ir atkuriamuose pažeistuose dirvožemiuose.

Reikšminiai žodžiai: *Schwanniomyces occidentalis*, *Cyberlindnera saturnus*, *Candida tropicalis*, azotas, fosforas, *Triticum vulgare*, *Sinapis alba*.