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BIOSYNTHETIC AND BIOCONTROL POTENTIAL OF ENDOPHYTIC YEAST STRAINS YP6 AND YBS14 FOR IMPROVEMENT THE GROWTH AND DEVELOPMENT OF SOLANACEAE PLANTS

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Abstract

YP6 was isolated from Triticum aestivum L. seeds and YBS14 was isolated from the roots of Helichrysum italicum L. Partial sequence analysis of ITS5-5.8-ITS4 region of the nuclear ribosomal DNA with universal primers identified YP6 as Pichia fermentans and YBS14 as Saccharomyces cerevisiae. Both yeast strains produced indole-3-acetic acid when cultivated in a medium supplemented with 0.2 % L-tryptophan. The antimicrobial activity of yeast strains against plant pathogenic fungi was determined. YP6 and YBS14 were tested for endophytic colonization of Solanaceae plants by soil drenching and leaf spraying. To establish colonization in the various tissues of tested plants, samples were taken and explants were inoculated on yeast malts agar. The effect of the microbial endophytes on photosynthesis, stomatal conductivity, and transpiration intensity was analyzed by using the portable photosynthetic system for CO_2 analysis in plants. In all treated plants photosynthesis was intense and growth stimulation was observed. The final aim of the present study is to evaluate endophytic yeast and demonstrate their PGP activity.

Key words: *endophytic yeast, Solanaceae, biosynthetic potential, physiological parameters, antimicrobial activity.*

INTRODUCTION

Local varieties of Solanaceae plants and populations obtained as a result of long evolutionary development are of significant potential interest in this direction. They are well adapted to different agro-ecological conditions and are carriers of valuable economic, taste and technological qualities. This is confirmed by the enormous interest shown by foreign breeders, scientific institutes and private companies in genetic material from local Bulgarian varieties. Unprecedented advances in science and new biotechnologies, including microbiology, microbiomes, etc., enable the development of flexible management models involving local genetic resources, research and the development of innovative biopreparations for pathogen control and yield enhancement.

Yeasts inhabit all aerobic habitats, but most species are known for their biotechnological applications and/or medical relevance, rather than their potential application in agricultural production systems or their biological function in the environment (Buzzini et al., 2012; Cantrell et al., 2011; Sundh et al., 2011). Yeast endophytes are found in intercellular space or inside cells of host plants causing no apparent damage (Saikkonen et al., 1998). They benefit plants by promoting plant growth (Dai et al., 2008), improving resistance to multiple stress (Lewis, 2004; Malinowski et al., 2006, protecting from diseases and insects (Wilkinson et al., 2000; Tanaka et al., 2005; Vega et al., 2008). Relatively few yeast-based products for plant protection have reached advanced developmental stages and were suggested as commercial products for postharvest

applications. *Saccharomyces cerevisiae* strain LAS02, is also being evaluated for its biological activities (Droby et al., 2016; EFSA, 2015; Liu et al., 2013). These examples clearly demonstrate the potential use of yeasts for plant production and protection, but also indicate their limited exploitation for commercial applications in agriculture. In addition to biocontrol, yeasts have been implicated in benefiting soil structure and plant nutrition, and biostimulation of growth, development, and stress resistance (Yakhin et al., 2016). The elucidation of the modes of action underlying all bio-activities is a prerequisite for the development of science-based, reliable products. Until recently, however, the mode of action underlying the beneficial activities of yeast and yeast-based products has been little understood.

Endophytes may contribute to their host plant defences against phytopathogenic organisms through plant physiology control (Giménez et al., 2007). An increase in plant growth will prevent a variety of abiotic and biotic stresses, reflecting plant vigour or persistence and being considered as potential protection against pathogen challenges (Kuldau and Bacon, 2008; Kim et al., 2006). Many studies demonstrate that plants infected with endophytes obtain growth promotion (Barka et al., 2002; Petkova et al., 2022), resistance to drought stress (Swarthout et al., 2009) and tolerance to unsuitable soil conditions (Belesky and Fedders, 1995; Malinowski et al., 2006). Previous research has examined the effect of single and double treatment with yeast *Z. bailii* YE1 and *S. cerevisiae* YD5 inoculum on the development and biochemical parameters of tobacco (Petkova et al., 2022). According to this publication the effect of leaf spraying versus root drench and the effect of single treatment versus duplicate treatment, *Z. bailii* YE1 strain was the most effective by foliar inoculation, while for *S. cerevisiae* YD5 application was better with single soil treatment.

In the current study, the focus of research is the biosynthetic and biocontrol potential of two endophytic yeast strains YP6 and YBS14 on four different Solanaceae plants. Endophytic inoculation with yeast strains was done by soil drenching and leaf spraying and the observation of the physiological and biometric parameters of treated plants was accomplished. Finally, current experiments must also assess whether it is worthy or useful to promote crop growth, development, photosynthesis, and disease protection.

MATERIALS AND METHODS

1. Isolation and molecular identification of yeast

YP6 was isolated from surface-sterilized *Triticum aestivum* L. seeds and YBS14 was isolated from surface-sterilized roots of *Helichrysum italicum* L., grown on the training-experimental field Institute of Plant Genetic Resources "K. Malkov"- Sadovo. The surface sterilization to remove the adhering microorganisms was done according to Petkova et al. (2022). Yeast colonies, isolated from each explant were subculture on separate yeast malts agar (YMA) (Himedia, Mumbai, India) plates at room temperature, morphologically analyzed by microscope observation and then identified. To demonstrate the success of surface sterilization, the wash water from the last wash was inoculated with YMA without antibiotics and the plates were incubated at 27°C for 5 days.

For molecular identification, DNA was isolated with a HiPurA fungal DNA purification kit (Himedia, Mumbai, India) (Petkova et al., 2022). Control of the purity and concentrations of genomic DNA is performed by agarose gel electrophoresis. The protocol for DNA extraction of filamentous fungi was conducted according to Rosa et al. (2009). The internal transcribed spacer (ITS) domains of the rRNA gene were amplified with the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), as described by White et al. (1990) and 0.5 µL Taq polymerase mix (5 U/µL, Canvax, Spain). Amplification of the ITS region was performed as follows: 95°C for 5 min; followed by 35 cycles consisting of 94°C for 1 min, 58.5°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 5 min. Amplified PCR products were sent to Macrogen (Seoul, South Korea) for DNA sequencing. The nucleotide sequences of endophytic yeast isolates were aligned using the BLAST

software package (http://www. https://blast.ncbi.nlm.nih.gov) and submitted to the GenBank database.

2. The biosynthetic potential of the studied strains

2.2.1. *Production of indole-3-acetic acid, phosphate solubilization, ZnO solubilization, and proteolytic activity*

Yeast inoculum was prepared by cultivation in a 5 ml sterilized broth which contains in one litre of water 10 g/l yeast extract, 20 g/l peptone, and 20 g/l dextrose and the culture was incubated at 30°C for 48 h. Quantification of indole-3-acetic acid was accomplished by the addition of an equal volume of Salkowski reagent. Measurement was done spectrophotometrically according to Limtong and Koowadjanakul (2012). Statistical analysis data are calculated as mean standard deviation. Student t-tests were used for the expression of the differences between groups and variance was considered statistically significant at $p<0.05$ (Silva-Hughes et al., 2015).

The inoculum density was adjusted to an optical density at 600 nm (OD600) and 100 µl was spotted onto Pikovskaya's agar (Zaidi et al., 2006) and incubated at 30°C for 5 days. The formation of a clear zone around the colonies was considered a positive result for phosphate solubilization. PSE (Phosphate Solubilization Efficiency) = Z/C x 100, where, Z is the clearance zone including microbial growth and C is the colony diameter (Nagaraju et al., 2017).

To determine the zinc solubilization ability, research strains YP6 and YBS14 were plated into the 9 mm agar wells of ZnO agar according to Rokhbakhsh-Zamin et al., 2011. The plates were incubated at 30°C for 3 days and observed for showing a zone of enlightenment around the colonies. The halo area was measured to determine the zinc solubilization efficiency (ZnE) of the strains using the method of Dinesh et al., 2018: ZnE = (HZ/C) × 100, where HZ is the diameter of the solubilizing halo area, and C is the diameter of the colony.

The phosphate and zinc oxide solubilization efficiency was calculated as a ratio between the diameter of the halo zone and the diameter of the colonies.

Proteolytic activity was determined from a clear zone in skimmed milk agar (Himedia, India) (Petkova et al., 2022). The agar plates were prepared and then YP6 and YBS14 were plated into the 9 mm agar wells and inoculated at 30°C for 3 days. The halo zone around the colony was measured and accepted as positive for cell wall degrading enzyme production.

2.2.2. *Screening for siderophore production*

The production of siderophore from yeast was performed in 2% yeast malts agar with the addition of chrome azurol S (CAS) (Himedia, India) according to Schwyn and Neilands (1987). After inoculation of the medium, the yeast was incubated at 27°C for 5 days in the dark. The development of a yellow-orange halo around the colony was considered a positive result for siderophore production producing ability.

2.2.3. *Antifungal activity of YP6 and YBS14 against Fusarium, Rhizoctonia, and Alternaria*

The studied yeast strains were tested to determine their antifungal action against *Fusarium solani*, *Rhizoctonia solani*, and *Altenaria solani* as was performed by the agar diffusion method (Cardenas et al., 1999). Phytopathogenic fungi have been isolated from damaged tobacco plants. Pathogenic species were identified by morphology-based identification of spore and mycelium characteristics (Matute et al., 2019). The fungal plant pathogens were tested for their ability to cause host damage under the application of Koch's postulates.

3. Plant colonization by endophytic *P. fermentans* YP6 and *S. cerevisiae* YBS14

For the purposes of the experiment, seedlings of well-developed tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.) and tobacco (*Nicotoana tabacum* L.) plants were used. On the 7^{th}, 14^{th}, and 21^{st} day after treatment (DAT), some samples of the studied plants were taken to determine the presence of yeast in them by inoculating explants from leaves, stems and roots on yeast extract agar (Merck, Germany) as was described by Petkova et al., 2021. The isolation frequency (IF) of the

colonization of tobacco by yeast strains is calculated by the following formula published by Petrini and Fisher (1986).

The soil application was done by the root application and the leaf spraying was made on all leaves with a suspension at a concentration of 1×10^4 yeast cells, separately for each strain as was well-defined by Petkova, 2021. Control plants were sprayed only with water without treatment with yeast suspension. The effect of yeast isolates on the roots of tobacco was studied after treatment, using a magnification of 4 x on light microscope Leika M320.

4. Biometrical and physiological analysis of tobacco plants after the first and the second treatment with *P. fermentans* YP6 and *S. cerevisiae* YBS14

Biometrical analysis by the important traits such as stem height, root and leaf number, and leaves biomass of the aboveground part of plants were taken weekly. Measurements were conducted in three biological replicates and the statistical data were calculated with MS Excel 2010.

Physiological measurements were performed on three fully developed leaves by the utilization of a portable photosynthetic system Q-box CO650 - Plant CO_2 Analysis Package (Qubit Systems Inc., Canada). The intensity of photosynthesis (A, μmol m-2 s-1), stomatal conductance (Gs, μmol m-2 s-1), and intensity of transpiration (E, μmol m-2 s-1) were monitored and the results are presented as arithmetic mean ± SD. Parameters of photosynthesis and transpiration measurements were done by using a portable photosynthetic system Q -box CO650 - Package for CO_2 analysis in plants (Qubit Systems Inc., Canada). Statistica 7.0 software was used for the statistical evaluation (Stat Soft Inc. 2004).

RESULTS AND DISCUSSIONS

1. Identification of yeast strains

To isolate endophytes from selected plants, healthy and well-developed plants were selected. YP6 was isolated from *Triticum aestivum L.* seeds. YBS14 was isolated from the roots of *Helichrysum italicum L.*, which is mainly used for its limited habitat and importance as a medicinal plant. DNA-based methods are used to classify and identify yeast and are extremely valuable in the study of microbial species isolated from natural habitats. Using molecular identification methods, the nucleotide sequences of the resulting 950 bp PCR fragments were sequenced. After processing the results of sequencing and performing BLAST analysis with the available data in GenBank, the identity of the strains is determined. Partial sequence analysis of ITS5-5.8-ITS4 region of the nuclear ribosomal DNA with universal primers identified YP6 as *Pichia fermentans* and YBS14 as *Saccharomyces cerevisiae* with accession number in the Gene bank MZ798453 and MZ798454, respectively.

2. Biosynthetic potential of the studied strains

The result in table 1 revealed that the isolated strains possess high (solubilization zone greater than 2 cm) phosphate-solubilizing activity, measured as the phosphate-solubilization index (PSI) on PVK medium. The highest dissolution index was observed in YBS14 (135.37%), followed by YP6 (134.50%). Acid phosphatases and phytases synthesized by rhizosphere microorganisms are involved in the organic solubilization of soil phosphorus (Thaller et al. 1995). The established ability of three of the investigated strains to improve the solubility of inorganic phosphates is an important characteristic of PGP-microorganisms. Several fungi and bacteria have been shown to be phosphate- and Zn - solubilizers (Young et al. 2013; Sharma 2011; Kumar et al. 2009) but few of them exhibited also antimicrobial activities. The ZnO dissolution index for *P. fermentans* YP6 was calculated to be 132.85%. When inoculation was performed with *S. cerevisiae* YBS14, no solubilization of zinc oxide was observed.

Phytohormone IAA is the most common naturally occurring and methodically examined plant growth regulator. The amino acid tryptophan is a prominent precursor of IAA. The current experiment showed that the concentration of IAA production was strain specific. In the current study, yeasts were able to produce IAA. The tested yeasts have a medium level of IAA synthesis ranging from 28.3 ± 0.0134 mg/L by *P. fermentans* YP6 strain to 15.0 ± 0.026 mg/L by *S. cerevisiae*

YBS14 strain when there is 0.1% L-tryptophan in the medium (Table 1). Plant growth hormones are well-known to increase crop growth and yield (Saharan and Nehra, 2011).

Yeast can produce plant growth hormones such as auxin, gibberellic acid, and ethylene, which promote plant growth and yield (Amprayn et al. 2012; Nassar et al. 2005).

Table 1. Phosphate-solubilization index and IAA production, and qualitative determination of proteolytic activity and synthesis of siderophores of tested yeast strain

Yeast strains	Clear zone of phosphate-solubilization activities in mm ± SD, N=3	Phosphate-solubilization index (PSI) in %	ZnO solubilization as a zone in mm ± SD, N=3	ZnO solubilization index in %	IAA production ron tryptophan	Proteolytic activity, measured as zone in mm ± SD, N=3
P. fermentans YP6	27.6 ± 0.095	134.50	23.5 ± 0.086	132.85	28.3 ± 0.0134	23.53 ± 0.238
S. cerevisiae YBS14	28.3 ± 0.134	135.37	ND	ND	15.0 ± 0.026	18.63 ± 0.087

*SD - standard deviation; N-number of replicates. ND - not detected. The results are presented as arithmetic mean of three replicates (N = 3).

Synthesis of siderophores by yeasts in the rhizosphere also leads to the difficulty of iron access to harmful microflora and is reported as a significant PGP feature (Deshwal and Kumar, 2013). In the present study, the ability of strains to produce siderophores was determined on a solid medium after the addition of Chrome Azurol S (CAS) according to Schwyn and Neilands (1987). The synthesis of siderophores by the yeast strains was detected by a change in the colour of the culture medium (from blue to red-purple or yellow). Only *P. fermentans* YP6 showed activity when cultivated on CAS solid medium (Figure 1). Synthesis of siderophores leads to the deterrence of iron access to harmful microflora and is reported as a significant PGP feature (Deshwal and Kumar, 2013).

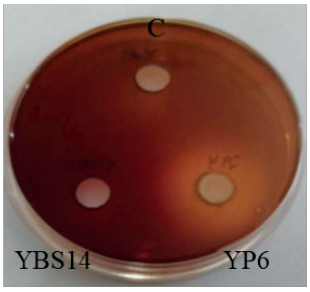

Figure 1. Synthesis of siderophores by yeasts YP6 and YBS14 strains

The main focus was to assess PGP effects on the nightshade family of plants. During seed bioassay, yeast inoculations improved root development as compared to the control. A study of the effect of YP6 and YBS14 culture filtrates on the tomato root showed a significant positive impact. S. cerevisiae YBS14 exhibited lower IAA production and as a result a smaller amount number of root hairs (Figure 2). In contrast, *P. fermentans* YP6 produced a 53% higher amount of IAA and in a co-cultivation experiment with *S. cerevisiae* YBS14, tobacco exhibited the development of a dense complex of root hairs. Hsu et al. published in 2010 that IAA stimulate plant cell enlargement, cambium cell, division, differentiation of phloem and xylem, root initiation and lateral root formation. This statement was confirmed by the results of the present study.

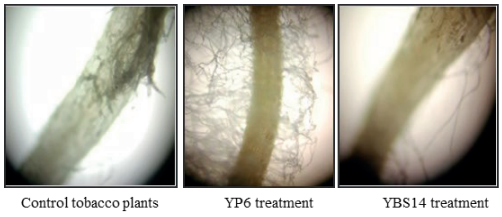

Figure 2. The interaction of yeast isolates with the roots of tobacco (*N. tabacum* L.*)* was studied after treatment, using a magnification of 4 $_x$ of a light microscope Leika M320

3. Antifungal activities of yeast strains against *Alternaria solani, Rhizoctonia solani,* and *Fusarium solani*

A large group of active yeast strains have been described as biocontrol agents as a result of their common ability to produce various antifungal metabolites (Silva-Hughes et al., 2015; Petkova et al., 2022). The antifungal activity of the tested yeast strains against

Fusarium solani, *Rizoctonia solani* and *Alteraria solani* and the demonstration of their inhibition effects are presented in Figure 3.

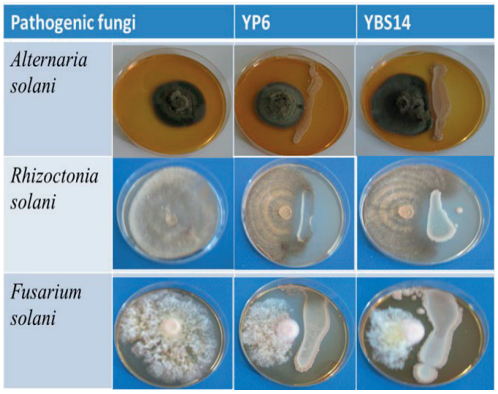

Figure 3. Antimicrobial activities of yeast strains against pathogenic fungi (*Alternaria solani, Rhizoctonia solani*, and *Fusarium solani*)

From the obtained results, it can be noted that two strains YP6 and YBS14 inhibited the growth of three of the test moulds *Fusarium solani*, *Rhizoctonia solani,* and *Alternaria solani.* The strongest effect of yeast is shown against *Fusarium* fungus. The weakest effect of YP6 and YBS14 is shown towards *Alternaria solani*. Their effect is probably due to the production of lytic enzymes and in the case of YP6 synthesis of siderophore. This result is similar to the previously published data by Petkova et al., 2022 with dought yeast strains YD5, YE1, and YSW1 which have been shown to inhibit the same phytopathogens and stimulate tobacco growth and development. Earlier findings reported the use of siderophores to control several pathogenic fungi such as *Pythium ultimum*, *Sclerotinia sclerotiorum*, and *Phytophthora parasitica* causing plant diseases (Mcloughlin et al., 1992). A large group of active yeast strains have been described as biocontrol agents due to their combined ability to produce various antifungal metabolites (Tian et al., 2009). Among these compounds, yeast produces low molecular weight antimicrobial molecules. Endophytes may contribute to their host plant defences against phytopathogenic organisms through plant physiology control.

4. Conducting pot and trials test cultures to track the effect of the tested strains separately

The highest colonization rates of leaves and stems of 85% to 100% were reported for YP6 and YBS14 of colonization root of tested plants (Figure 4). After data processing, the higher incidence rate in leaves and stems was impressive, even in the soil-inoculated experimental plants. Both downstream and upstream yeast migration was demonstrated up to the 21st DAT, where the percentage of colonization in different tissues of soil-treated and foliar-treated tobacco plants was quite similar to our previous results published by Petkova et al. (2022) (Figure 4). No colonization of plants was detected in control plants from the *Solanaceae* family.

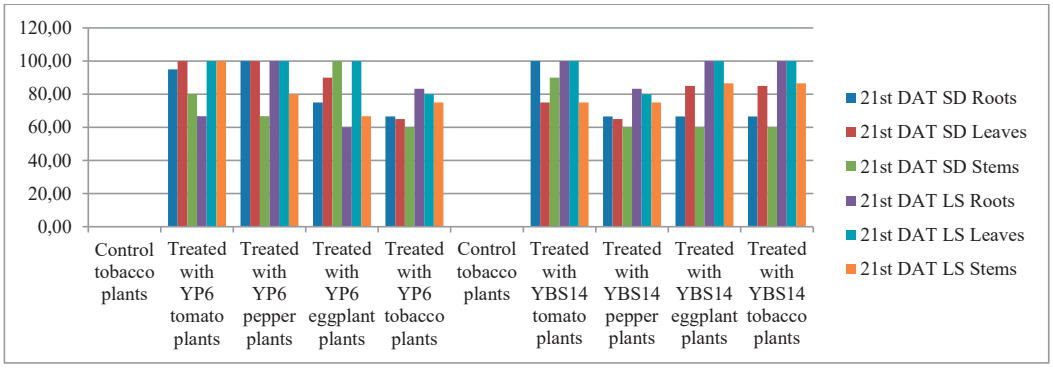

Figure 4. Percent colonization of tobacco plants at 21st days after treatment (DAT) with YP6 and YBS14 introduced by direct soil inoculation (SD) and leaf spray (LS)

From the results in Table 2, it can be seen that by the 21st day, the greatest increase in above-ground parts was recorded in tomato plants leaf-spraying treated with YP6, which reached a height of 29.45 cm, followed by YP6 treatment by soil application of the yeast 27.42 cm.

Soil-treated plants with YBS14 showed stem height close to that of control untreated plants. Both strains when applied by soil drench cause stress on root and lateral root growth.

Above-ground biomass (stem and leaves) was higher compared to control plants.

In the case of the experiments with pepper plants, there was variation in the height of the aboveground mass, but a threefold increase in root growth compared to the control (2.7 cm), after treatment with both yeast strains and in both treatments (12.3 to 16.6 cm) (Table 2). This leads to an increase in the biomass of the aerial part of the plants and the number of leaves.

Table 2. Biometry of tomato, pepper, eggplant, and tobacco to monitor the effect of the studied strains (YBS14 and YP6) separately on their growth and development

Plant	Days after treatment	Yeast Strain	Method of treatment	Stem height, cm ± SD*	Root length, cm ± SD*	Above-ground biomass (stem and leaves) in gram ± SD*	Number of leaves ± SD*
Tomato	21 DAT	Control	No treatment	21.83±1.14	5.96±0.15	6.55±1.79	8.00±1.29
		YBS14	LS	20.16±1.27	12.52±0.64	5.84±1.35	7.50±1.42
		YBS14	SD	27.83±2.34	24.86±1.56	6.66±1.12	7.33±1.25
		YP6	LS	29.45±2.56	17.71±1.42	2.96±0.07	6.50±1.67
		YP6	SD	27.42±2.16	11.6±1.06	7.5±0.43	8.00±1.36
Pepper	21 DAT	Control	No treatment	15.00±1.44	2.7±0.25	0.6±0.12	5.33±2.33
		YBS14	LS	14.03±1.92	12.13±0.84	1.86±0.35	7.00±2.16
		YBS14	SD	16.86±1.85	11.80±0.75	2.9±0.26	8.50±1.27
		YP6	LS	14.53±2.03	13.25±0.63	2.19±0.39	7.33±0.97
		YP6	SD	14.45±1.38	16.65±0.46	2.46±0.17	7.66±1.02
Eggplant	21 DAT	Control	No treatment	14.37±1.89	5.70±0.23	2.26±0.34	4.33±0.69
		YBS14	LS	15.55±1.72	8.25±0.42	2.05±0.29	5.30±1.49
		YBS14	SD	14.17±2.03	7.03±0.59	2.6±0.46	5.50±1.22
		YP6	LS	14.66±1.47	8.35±0.71	3.45±0.22	3.60±0.84
		YP6	SD	16.33±1.68	16.63±1.36	4.43±0.39	6.30±0.79
Tobacco	21 DAT	Control	No treatment	22.60±3.16	9.20±0.62	10.65±0.45	7.00±0.84
		YBS14	LS	18.00±2.67	17.63±0.53	13.2±0.51	8.33±1.27
		YBS14	SD	23.80±2.80	10.02±0.72	12.54±0.62	7.66±0.93
		YP6	LS	22.70±3.06	11.66±0.79	12.48±0.53	5.35±0.78
		YP6	SD	19.26±1.85	17.89±0.83	11.86±0.76	4.39±0.72

Legend: LS-leave spraying; SD-soil drenching. *SD - standard deviation; N-number of replicates was 10 plans for each treatment and 10 control plants.

Soil treatment with YP6 of eggplants showed a stimulating effect on plant biometric parameters at 21st DAT.

In contrast to the results with YP6, the soil treatment with the other tested strain YBS14 showed the weakest stimulation of stem, root, and leaf biomass growth in eggplant (Table 2).

The longest root length was recorded in soil treatment of tomato with YBS14, in pepper and eggplant in the soil treatment with strain YP6, in tobacco with strain foliar treatment with YP6 and soil treatment with YBS14.

Biometry data show that different yeast species have different effects on plants.

In the case of tobacco, the growth of the above-ground part of the plants in the initial stages had similar values in the soil and foliar treatments, but on the 21st day after treatment, it was seen that the plants with the foliar application of YP6 had almost twice higher growth (Table 2).

The greatest effect on stimulating stem length was shown by YP6 in leaf-spraying treatment of tomatoes and tobacco. Strain YBS14 showed better results in soil application of the yeast inoculum on tobacco growth. Biometric and quality indicators of plants also depend on applied agricultural techniques and growing conditions, which also contribute to changing

the composition of microorganisms and the nature of interactions in general. This shows that it would be good to carry out additional studies in the future under more and different conditions in order to use the metabolic properties of the yeasts under investigation as effectively as possible.

Because the quality of Solanaceae plants depends on the specific qualities formed in the leaves and fruits depending on a number of factors. This requires additional research and in different conditions in order to use the metabolic qualities of the studied yeasts as effectively as possible. It is more specific to tested plants and should be investigated in the future because the biometric and quality indicators vary depending on the applied agricultural techniques and growing conditions.

The use of the biochemical properties of microorganisms in agriculture is a promising and rapidly developing field in modern agriculture, which has the potential to help solve important problems related to plant protection, fertilization and, respectively, environmental protection.

Periodical measurements on 7th, 14th, 21st, and 21st day after yeast inoculation on the intensity of photosynthesis, transpiration and stomatal conductivity of experimental plants clearly indicated that mutual plant-yeast coexistence causes no stress to the plant organism. More expressed significant positive impact on studied physiological traits exerted by the YP6 strain than YBS14 strain.

When regarding the effect of leaf spraying versus root drench the statistical evaluation of all studied biometric and physiological parameters highlighted that for YP6 strain the most effective is foliar, while for YBS14 is single soil and single leaf treatment ($p<0.05$) (Table 3). Photosynthesis in almost all experimental variants was recorded at a higher rate 21 days after treatment with the respective yeast inoculum. When measuring stomatal conductance and transpiration intensity, higher values were found in almost all investigated variants compared to the control plants.

Based on these findings it could be concluded that there is no inhibitory impact on plant health status and physiology caused by the endophytic yeast strains colonization. Furthermore, treatment with YP6 and YBS14 enhance the photosynthetic potential as well as the plant productivity which is well demonstrated also by data for photosynthesis rate monitoring (Table 2 and 3).

Table 3. The intensity of photosynthesis (A. µmol m-2 s-1) stomatal conductance (Gs. µmol m-2 s-1) and intensity of transpiration (E. µmol m-2 s-1) in the different experimental conditions variants on the 7th and 21st days after treatment of tomato, pepper and eggplant with the strains YBS14 and YP6

Tested *Solanacea e* plant	Days after treatment (DAT)	Yeast strain	Method of treating plants	Photosynthesi. mol m^{-2} s^{-1}	Stomatal conductance. mol m^{-2} s^{-1}	Transpiration intensity. mol m^{-2} s^{-1}
Tomato	7TH DAT	Control	No treatment	8.444±2.76	0.678±0.06	73.71±5.06
		YBS14	LS	12.039±3.39	0.149±0.05	16.94±2.48
		YBS14	SD	6.813±2.78	0.145±0.03	14.73±1.78
		YP6	LS	9.717±2.01	0.511±0.05	71.03±4.53
		YP6	SD	6.787±1.97	0.358±0.08	44.51±3.12
	21ST DAT	Control	No treatment	3.384±0.68	0.296±0.09	38.23±6.68
		YBS14	LS	4.901±0.98	0.133±0.08	16.74±3.07
		YBS14	SD	3.189±1.03	0.321±0.06	48.28±3.64
		YP6	LS	6.489±1.66	0.191±0.05	18.69±3.43
		YP6	SD	1.368±0.28	0.132±0.05	12.79±2.17
Pepper	7TH DAT	Control	No treatment	15.153±1.45	1.11±0.07	14.16±2.87
		YBS14	LS	8.922±1.32	0.183±0.04	19.06±3.54
		YBS14	SD	11.509±2.03	0.163±0.05	18.39±2.90
		YP6	LS	8.635±1.32	0.146±0.03	15.27±3.18
		YP6	SD	11.723±1.63	0.221±0.02	23.5±4.64
	21ST DAT	Control	No treatment	2.937±0.16	0.098±0.01	11.68±2.06
		YBS14	LS	8.671±1.56	0.26±0.02	36.88±3.78
		YBS14	SD	4.209±0.48	0.107±0.01	12.64±2.38
		YP6	LS	8.924±1.36	0.156±0.02	17.67±3.03
		YP6	SD	7.301±0.14	0.188±0.02	23.74±2.77

Eggplant	7TH DAT	Control	No treatment	9.096±0.98	0.417±0.03	43.01±3.54
		YBS14	LS	8.937±1.06	0.371±0.04	49.07±3.96
		YBS14	SD	11.681±2.03	0.596±0.04	96.01±4.25
		YP6	LS	16.31±2.56	0.848±0.06	146.97±6.78
		YP6	SD	18.831±2.67	0.622±0.08	83.61±5.74
	21ST DAT	Control	No treatment	3.03±0.676	0.11±0.02	12.31±2.58
		YBS14	LS	7.09±1.23	0.438±0.03	74.41±4.48
		YBS14	SD	4.502±0.690	0.293±0.05	40.97±3.28
		YP6	LS	11.578±1.93	0.227±0.03	27.65±2.49
		YP6	SD	13.474±2.36	0.158±0.02	17.82±3.63
Tobacco	7TH DAT	Control	No treatment	4.665±2.28	0.648±0.04	18.98±3.78
		YBS14	LS	6.265±1.36	0.581±0.09	17.35±2.99
		YBS14	SD	7.887±1.96	0.644±0.06	18.02±3.01
		YP6	LS	4.683±1.93	0.192±0.03	6.04±1.34
		YP6	SD	6.685±1.32	0.534±0.05	15.50±2.87
	21ST DAT	Control	No treatment	12.086±4.33	0.136±0.01	4.14±1.69
		YBS14	LS	13.402±2.76	0.139±0.01	3.32±1.10
		YBS14	SD	15.528±2.58	0.182±0.01	4.65±1.25
		YP6	LS	12.689±3.54	0.257±0.03	6.51±2.30
		YP6	SD	15.931±3.91	0.391±0.06	10.25±3.55

Legend: LS-leave spraying; SD-soil drenching

CONCLUSIONS

In conclusion, the newly isolated strains were characterized by physiological-biochemical and molecular-genetic studies. In both inoculation methods, colonization was observed in all parts of the tested plant. This testifies to the ability of the tested yeasts to move up and down in plants. *P. fermentans* YP6 and *S. cerevisiae* YBS14 are capable of endophytic colonization of Solanaceae plants without damaging their tissues. YP6 and YBS14 can produce and synthesize physiologically active substances (indole-3-acetic acid. siderophores) and stimulate plant growth and reduce pathogen infection as well as biotic and abiotic stress. They have a stimulating effect on photosynthesis, stomatal conductivity, and transpiration intensity. YP6 and YBS14 have a high potential to be used as a biocontrol agents in agriculture. The results are of interest for the development of multifunctional biological preparations with different biological activities. YP6 and YBS14 strains are promising for inclusion in commercial PGP products for sustainable agriculture.

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