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Plant growth-promoting and antifungal activity of yeasts from dark chestnut soil

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ABSTRACT

538 yeast strains were isolated from dark chestnut soil collected from under the plants of the legume family (*Fabaceae*). The greatest number of microorganisms is found at soil depth 10–20 cm. Among the 538 strains of yeast 77 (14.3%) strains demonstrated the ability to synthesize IAA. 15 strains were attributed to high IAA-producing yeasts (above 10 μg/ml). The most active strains were YA05 with 51.7 ± 2.1 μg/ml of IAA and YR07 with 45.3 ± 1.5 μg/ml. In the study of effect of incubation time on IAA production the maximum accumulation of IAA coincided with maximum rates of biomass: at 120 h for YR07 and at 144 h for strain YA05. IAA production increased when medium was supplemented with the L-tryptophan. 400 μg/ml of L-tryptophan showed maximum IAA production. 10 strains demonstrated the ability to inhibit the growth and development of phytopathogenic fungi. YA05 and YR07 strains formed the largest zones of inhibition compared to the other strains – from 21.6 ± 0.3 to 30.6 ± 0.5 mm. Maximum zone of inhibition was observed for YA05 against *Phytophtora infestans* and YR07 strains against *Fusarium graminearum*. YA05 and YR07 strains were identified as *Aureobasidium pullulans* YA05 (GenBank accession No JF160955) and *Rhodotorula mucilaginosa* YR07 (GenBank accession No JF160956).

1. Introduction

The PGPM (plant growth-promoting microorganisms) have a number (direct and indirect) beneficial effects on plants, such as: nitrogen fixation, production of plant hormones like auxins, gibberellins, cytokins, synthesis of vitamins, antifungal and antibiotic compounds, ability to solubilize minerals like phosphorus and other nutrients, capability to degrade numerous toxic chemicals, etc. (Pérez-Montaño et al. 2014; Yang et al. 2009; Amprayn et al. 2012; Martínez-Viveros et al. 2010; Kim et al. 2011; Vessey 2003; Nutaratat et al. 2014).

The most attention is paid to the role of auxin in the stimulation of plant growth and nutrition, because the ability to produce indole-3-acetic acid (IAA) is widespread among soil organisms (Rao et al. 2010; Limtong and Koowadjanakul 2012; Limtong et al. 2014; Nassar et al. 2005; El-Tarabily 2004; Bilkay et al. 2010). The positive effect of microbial auxins on the initiation and elongation of roots and stems (Nassar et al. 2005; El-Tarabily and Sivasithamparam 2006), on the development of lateral roots and root hairs is shown, that may be important for plant growth promotion, the uptake of nutrients and the formation of plant resistance to stress (Glick et al. 1999; Halliday et al. 2009; Moller and Weijers 2009). The synthesis of auxins by soil and rhizosphere microorganisms is largely determined by the composition of root exudates containing the major metabolic precursor L-tryptophan (Lynch 1985; Bharucha et al. 2013; Maslov et al. 2011).

It is noteworthy that effects of PGPM are preserved not only in natural communities, but also after plants treatment with microorganisms in laboratory and field conditions (Agamy et al. 2013; Bennett and Whipps 2008; Ji et al. 2014). Many studies have noted the increase in germination, length and biomass of seedlings after seed inoculation with yeast strains, enhance plant growth, increase of photosynthesis productivity (Hu and Qi 2013; Amprayn et al. 2012; Agamy et al. 2013; Nassar et al. 2005). The positive effect of biologically active compounds produced by microorganisms on agricultural plants begins at the earliest stages of plant development and further is expressed in the suppression phytopathogens and increase productivity.

Biocontrol of plant pathogenic organisms by soil yeast is carried out, on the one hand, by improving the uptake of water and mineral elements nitrogen, phosphorus, potassium by the plant, on the

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Fig. 1. Soil profiles.

other hand – through the production of antifungal agents and the displacement of pathogenic bacteria and fungi in the rhizosphere by inhibiting their growth (Santos et al. 2004; Compant et al. 2005; Nutaratat et al. 2014; Vero et al. 2002; Chanchaichaovivat et al. 2007; Hatoum et al. 2012; Golubev 2006).

2. Materials and methods

2.1. Sampling

Samples of dark chestnut soil were collected in the foothill areas of Almaty region of Kazakhstan in the period from August to September 2013. The soil samples were collected from under the plants of the legume family (*Fabaceae*): alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*), clover (*Trifolium pratense*), sweet clover (*Melilotus officinalis*), and soybean (*Glycine max*). Material was taken from 0 to 10 cm, 10 to 20 cm and from 20 to 40 cm depth (Fig. 1).

The soil samples were analyzed for a variety of physical and chemical characteristics (Table 1).

2.2. Isolation of yeasts

Yeasts were isolated from soil samples using serial dilution and pour plate method. Sabouraud dextrose agar (SDA), Chapek-Dox agar, Ashby-sucrose agar were used for the isolation.

2.3. Screening of indole-3-acetic acid (IAA) producing yeasts

Yeast isolates were grown in Sabouraud dextrose broth supplemented with 0.1% (1000 μg/ml) L-tryptophan. Strains were incubated on a shaker (180 rpm) at 26 °C. At the end of the incubation cultures were centrifuged at 8000 g for 10 min and the supernatants were collected. One ml of supernatant was mixed with 2 ml of the Salkowski reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$). The mixture was allowed to stand for 30 min for color development. The intensity of the color developed was measured at a wavelength of 530 nm using a spectrophotometer (Gordon and Weber 1951). Calibration curve using authentic IAA was established for calculation of IAA concentration. The high IAA-producing strains were selected for further studies.

2.4. Study of effect of incubation time and L-tryptophan concentration on IAA production

2.4.1. Influence of incubation time on IAA production

The effect of incubation time on IAA production by yeast strains was studied in Sabouraud dextrose broth amended with 1% (1000 μg/ml) L-tryptophan. Samples were drawn every 12 h up to 192 h. Yeast biomass was determined by dry weight. Yeast culture samples (10 ml) were filtered over preweighed filters. The filters were dried at 85 °C for 2 h and weighed. IAA production was determined as described previously in Section 2.3.

2.4.2. Influence of L-tryptophan concentration on IAA production

Tryptophan is a main metabolic precursor for indole-3-acetic acid biosynthesis pathways in microorganisms (Martens and Frankenberger 1993; Mano and Nemoto 2012; Spaepen et al. 2007). L-Tryptophan in an amount of 50, 200, 400, 600, 800 and 1000 μg/ml was added into the medium. IAA production was also detected in the absence of tryptophan. IAA production was determined as described previously in Section 2.3.

2.5. Study of antifungal activity

Phytopathogenic fungi from the collection of the Institute of Microbiology and Virology of the MES RK *Fusarium graminearum*, *Cladosporium* sp., *Phytophtora infestans* and *Botrytis cinerea* were used as test objects. Yeast strains were grown on SDA medium in tubes for 5 days. 0.1 ml of aqueous suspensions of yeasts with concentration of 10^6 cells/ml was plated on the surface of SDA plates. Yeasts were grown as a lawn on a Sabouraud agar surface for 5–7 days and then disks of 8 mm in diameter were cut. Phytopathogenic fungi were cultured for 4–5 days. Suspensions containing 10^4 conidia/ml were prepared from grown cultures and then lawns of phytopathogenic fungi were made on plates. On the surface of the Petri dishes with phytopathogenic fungi disks with yeasts cultures were placed. Antifungal activity was determined by measuring zone of inhibition produce by yeasts against phytopathogenic fungi.

2.6. Molecular identification of strains

Molecular identification of yeast strains was performed in National Scientific Laboratory of Biotechnology in National Biotechnology Center of the MES RK (Astana, Kazakhstan). Yeast cells were grown under aerobic conditions at 24 °C for 1 day. After extraction and purification of genomic DNA the DNA concentration was measured using a NanoDrop spectrophotometer at a wavelength of 260 nm. The DNA concentration was normalized

Table 1
General physical and chemical properties of soil.

Sampling depth (cm)	pH	Organic C (%)	Total *N* (%)	Moisture content (%)	Horizon	Texture
0–10	8.40	2.84	0.20	3.9	A	Light loam
10–20	8.36	2.65	0.18	6.1	A	Light loam
20–40	8.24	2.47	0.17	7.6	B	Light loam