ASSESSMENT OF YEAST DIVERSITY IN DIFFERENT SOIL TYPES UNDER VARIOUS MANAGEMENT REGIMES IN MOLDAVIA REGION, ROMANIA

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Abstract

This study examined the diversity and abundance of yeasts population isolated from soils sample under different agricultural regimes (permanent grassland, arable land under agricultural rotation, forest land etc) from Moldavia region located in northeastern Romania, which covers an area of 35,806 km^2 and has a population of approximately 4.3 million inhabitants. Twenty sites were common to the two samplings in May and September, 2015 and 2016. Soil samples were represented by typical chernozem, greic chernozem, psamic chernozem, cambic chernozem, faeoziom chernozem, preluvosoil, albic luvosoil, psamic luvosoil, solonchak, solonetz, planosoil, molic saline aluviosoil, vertosoil and gleiosoil. At each site, five replicate bulk samples were taken, consisting of 10 randomly collected sub-samples from the surface soil (10-15 cm horizon). The samples were transported to the laboratory, stored overnight at 4°C, air-dried at room temperature and sieved (2-mm mesh) prior to further use in the experiment. The soils pH values covered a range between 5.6 (planosoil) and 8.7 (solonchak). Yeast isolation and obtaining of the pure cultures was done mainly through inoculums dissemination and loop exhaustion techniques on solid nutrient media. We isolated and identified molecularly a total of 33 yeast species, including several possible new species. Occurrence and distribution of yeasts isolated from these soils provide new insights into ecology and niche specialization of several soil-borne species. Species composition of different localities was very heterogeneous and many of the species were found in a single site only.

Key words: yeast diversity, soil types, different agricultural regimes

Soils are heterogeneous and discontinuous environments, where physical, chemical and biological parameters are keys to the creation of habitats available for microbial communities to survive and proliferate (Campos *et al*, 2012; Dumbrell *et al*, 2010).

Yeast are unicellular fungi, usually present among diverse microorganisms inhabiting soils, which participate in important ecological processes, such as decomposition of organic matter (including pesticides and fertilizers), mineralization, cycle of nutrients in the soil (carbon, nitrogen, phosphorus and sulphur), and stabilization of soil aggregates (El-Tarabily, 2004; Cloete *et al*, 2009; Botha, 2011; Mestre *et al*, 2011, Deng *et al*, 2015).

The occurrence, composition and quantity of of yeasts flora in soils depends on many factors: type of soil, soil organic matter content, water availability, management regimes, plant diversity, and seasonal fluctuations (Vishniac, 2006; Slaviková and Vadkertiová, 2003; Yurkov *et al*, 2012). In agricultural soils, the quantity of the yeast population ranges from a few to several thousands of cells/g soil (Slavikova and Vadkertiova, 2003; Yurkov *et al*, 2012; Lopez-Pineiro *et al*, 2013). The abundance of yeasts in vineyard soil depends on the management regime, the season and soil water content. The quantity of yeasts ranged from 5.8 x 10^3 to 3.5 x 10^4 CFU/g soil in sandy low-nutrient vineyards which contained about 15% water (Vreulink *et al*, 2007). Yeast abundance in soils decreases with the depth. A significant reduction in the yeast quantities and diversity was noted at a depth up to 20–30 cm (Wuczkowski and Prillinger, 2004).

The overall aim of the present research was to investigate the occurrence and diversity of yeasts population in various types of soils under different management regime in the northeast of Romania, because only little information about yeasts associated with soil on the territory of Romania is available. The diversity and the role of communities of yeast present in soils are still little explored because of difficulties of cultivation. This work is a part of a broader survey of the presence and diversity of yeasts flora in all types of soils in Romania.

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MATERIAL AND METHOD

The soils in this study were sampled from the Moldavia region located in north-eastern Romania, which covers an area of 35,806 km^2 and has a population of approximately 4.3 million inhabitants (Morariu, 2015). Co-ordinates for the coverage area extended from 45.844 latitude N (UTM 5076871 m) to 48.096 N (UTM 5327016 m) and from 26.304 longitude E (UTM 447538 m) to 28.123 E (UTM 585843 m). Four samplings were taken, two in May (2015, 2016) and two in September (2015, 2016) from twenty different study sites. Five replicate bulk samples were taken from each sampling site (*Table 1*), each consisting of 10 randomly collected sub-samples from the surface soil (10-15 cm horizon). Approximately 1,000 g of soil were thus collected in sterile plastic bags, from each site, by means of removing any organic residue from the surface before sampling

Soil samples were collected from 15 different soil types from different land regimes: arable land under crop rotation, permanent grassland (P), hardwood forest (F), vegetable crop (VC), hay crop (HC) and tilled vineyard (TV). The 15 soil types (Florea and Munteanu, 2012) are classified in 6 classes according to World Reference Base for Soil Resources (WRB, 2014). In the organically managed arable farms, compost and animal green manures are permitted with a view to build up the soil fertility, whereas in the conventionally managed soils synthetic pesticides and fertilizers have been used. The altitude in the sampling region ranges from 61 to 443 metres above sea level (Table 1).

The samples were transported to the laboratory, stored overnight at 4°C, air-dried at room temperature and sieved (2-mm mesh) prior to further use in the experiment. Microbiological analyses were done within 24 hours after soils sampling. Soils pH was determined with a glass electrode in a 1:2.5 soil to water ratio.

Soil serial dilutions were prepared. One gram of soil was mixed with 9 mL sterile water (dilution 10^{-1}) and then 1 mL of the dilution 10^{-1} was poured into 9 mL sterile water (dilution 10^{-2}). Following a successive tenfold dilution series, 10^{-2} to 10^{-6} dilutions were prepared. Aliquots (1 mL) of 10^{-2} to 10^{-6} dilution were pleated into nutritive media (yeast extract malt extract medium supplemented with 200 mg/l chloramphenicol).

For yeast identification DNA was extracted from individual colonies obtained after incubation interval at 25°C in complete darkness. DNA fragments were amplified by PCR and sequence analysis of the 26S rRNA gene was necessary.

Table 1

Characteristic of the tested soils

Soil sample	*Soil type*	*Soil class*	*Land use[a] and management[b]*		*Alt. m*	*GPS location Lat. N/Long. E*
1	Typical chernozem 1	Chernisols	A	C	138	46.722 / 28.123
2	Typical chernozem 2		A	O	66	46.438 / 28.193
3	Greyic chernozem		F	-	296	46.401 / 27.866
4	Psamic chernozem		VC	O	96	45.844 / 27.478
5	Cambic chernozem		A	C	121	47.123 / 27.501
6	Greyic Phaeozem		P	O	423	47.419 / 26.314
7	Cambic Phaeozem		A	C	390	46.958 / 26.565
8	Preluvosol 1	Luvisols	TV	C	343	47.356 / 26.918
9	Preluvosol 2		VC	C	144	47.623 / 26.820
10	Preluvosol 3		F	-	365	47.063 / 27.688
11	Albic Luvosol		A	C	370	47.371 / 26.327
12	Psamic Luvosol		P	O	439	47.003 / 26.538
13	Planosol		F	-	443	47.883 / 26.329
14	Solonchak 1	Salsodisols	P	-	70	47.289 / 27.501
15	Solonchak 2		P	-	72	47.260 / 27.536
16	Solonetz 1		P	-	61	47.100 / 27.826
17	Solonetz 2		P	-	68	47.100 / 27.826
18	Molic saline aluviosol	Protisols	HC	O	119	46.366 / 27.789
19	Vertosol	Pelisols	A	C	243	48.096 / 26.787
20	Gleiosol	Hydrisols	P	O	418	47.419 / 26.314

[a]arable land under crop rotation (A), permanent grassland (P), hardwood forest (F), vegetable crop (VC), hay crop (HC) and tilled vineyard (TV); [b]under conventional (C), organic (O) and unmanaged (-) agricultural condition

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RESULTS AND DISCUSSION

A total of 27 yeast species including several possible new species, were isolated from 20 soil samples collected from different managed biotopes. During this study 18 yeast species were identified. According to the genetic distances and the physiological profiles, nine yeast taxa represented potential new species. They belong to four lineages of *Fungi*, *Agaricomycotina* (ten species), *Saccharomycotina* (three species), *Pucciniomycotyna* (three species) and *Pezizomycotina* (two species). *Table 2* provides a list of isolated species. *Cryptococcus terricola* and *Cryptococcus terreus* were the most frequently isolated species from the samples taken in all types of soils. Yeasts from *Cryptococcus* genera are capsulated and survive better in habitats poor in

nutrients and with periods of desiccation. Capsules may serve to protect cells from physical and biological stresses encountered in their natural habitat and may influence the ability of the cells to survive low moisture conditions. These insoluble extracellular polysaccharides can also act as soil binding agents (Slaviková and Vadkertiová, 2003).

The maximum number of yeast species isolated from one location was four. Only in one case arable land under crop rotation have the same number of species (4) as permanent grasslands. In rest of the locations, with regard to land use intensity, the arable land under agricultural rotation showed no presence of yeasts or only one species was isolated. In general, extensively managed areas (permanent grasslands) showed increased species richness in comparacy to intensively managed areas. Natural forests have a lower or equal number of yeasts species with permanent grasslands (*Table 2*).

Table 2

Yeast species isolated from different types of managed soils

Soil class	Soil type	Places for soil sampling	Agricultural regime	pH value	Isolated yeasts
Chernisols	Typical chernozem 1	Râşeşti (VS)	arable land under agricultural rotation	8.4	*Debaryomyces subglobosus*
	Typical chernozem 2	Vetrişoaia (VS)	arable land under agricultural rotation	8.2	*Guehomyces pullulans*
	Greyic chernozem	Roşieşti (VS)	forest land	7.4	*Cryptococcus terricola* *Trichosporon spp.*
	Psamic chernozem	Matca (GL)	arable land under agricultural rotation	7.0	*Rhodotorula graminis* *Sporobolomyces roseus* *Cryptococcus terreus* *Cryptococcus terricola*
	Cambic chernozem	Ezăreni (IŞ)	arable land under agricultural rotation	7.3	-
	Greyic Phaeozem	Fălticeni - Spătăreşti (SV)	permanent grassland	6.97	*Schwanniomyces occidentalis* *Cryptococcus terreus*
					T1413-1 T1413-2 *Rhodosporidium azoricum*
	Cambic Phaeozem	Girov (NŢ)	Rapeseed field (2013)	7.2	*Williopsis saturnus*
			Wheat field (2014)		P1407 *Holtermanniella takashimae*
Luvisols	Preluvosol 1	Cotnari (IS)	vineyard	6.6	*Holtermanniella watticus* *Cryptococcus terricola*
					Cryptococcus aerius
	Preluvosol 2	Copălău (BT)	arable land under agricultural rotation	8.1	-
	Preluvosol 3	Bucium (IŞ)	hardwood forest	6.6	*Guehomyces pullulans*
	Albic Luvosol	Boroaia (SV)	arable land under agricultural rotation	6.6	T1401
	Psamic Luvosol	Ştefan cel Mare (NŢ)	permanent grassland	8.2	*Schwanniomyces occidentalis* *Rhodotorula graminis* *Hannaella zeae* *Cryptococcus adeliensis*
	Planosol	Vârful Câmpului (BT)	hardwood forest	8.7	*Cryptococcus terricola*
Salsodisols	Solonchak	Vânători (IS)	permanent grassland	8.6	P1410 P1413 P1415 *Aureobasidium pullulans*
	Solonetz	Osoi (IŞ)	permanent grassland	5.6	*Tetracladium spp* *Debaryomyces subglobosus*
Protisols	Molic saline aluviosol	Sârbi (VS)	alfalfa field	8.4	T1405 T1406 *Cystofilobasidium macerans*
Pelisols	Vertosol	Coţuşca - Mileanca (BT)	arable land under agricultural rotation	7.4	-
Hydrisols	Gleiosol	Fălticeni - Spătăreşti (SV)	permanent grassland	7.6	*Williopsis saturnus* *Cryptococcus terricola* *Guehomyces pullulans*

CONCLUSIONS

During this study 21 yeast species were identified. They belong to the following subphyla: *Agaricomycotina* (*Cryptococcus terricola, Cryptococcus terreus, Cryptococcus aerius, Cryptococcus adeliensis, Hannaella zeae, Trichosporon* sp, *Holtermanniella takashimae, Holtermanniella watticus, Cystofilobasidium macerans, Guehomyces pullulans*), *Saccharomycotina* (*Debaryomyces subglobosus, Williopsis saturnus, Schwanniomyces occidentalis*), *Pucciniomycotyna* (*Sporobolomyces roseus, Rhodosporidium azoricum, Rhodotorula graminis*) and *Pezizomycotina* (*Aureobasidium pullulans, Tetracladium sp*)

According to the genetic distances and the physiological profiles, nine yeast taxa represented potential new species. Further studies are necessary to improve the knowledge about the

influence of soil types, management regimes and seasonal fluctuations on the yeasts occurrence and diversity in soils.

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