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Biodiversity of Yeasts Associated to Forest Ecosystem in Arasbaran-Iran

[1]Ahmadreza Hasani and [2]Sanam Alieva

[1]Ph.D. graduated of Microbiology Department, Biology Faculty, Baku State University and Natural Resources and Agriculture Research Center of East- Azerbaijan province, Tabriz-Iran.
[2]Microbiology Department, Biology Faculty, Baku State University, Baku-Azerbaijan Republic.

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ABSTRACT

The objective of the present study was to analyze the biodiversity of yeasts associated to soil and plants in Arasbaran Forest Research Station has been selected as a research platform with area of about 12,000 hectares in the basin of Sotan chay by East Azerbaijan Agriculture and Natural Resources Research Center. Sampling was carried out in two different regions of height. Site 1 and 2 were in above 1500-2000 meters and in low 1000 meters, respectively. The most of trees were including oak (*Quereus* L.), black hawthorn or cranberries (*Crataegus ambigus*) and blueberries (*ornus mas L.*). The soil type in this area was undeveloped podzol soil with depth from 2 to 20 cm. The horizon of F/FH as the forest soil cover were used for soil sampling. A total of 60 samples (30 samples from each area) were taken from different parts of forest trees (Including leaves and rotting fruits, blossoms and rotting branches and soil of around the trunk of the trees and the soil under the dry leaves and the like) during the months of April to December in 2009. Representatives of the different morphotypes were purified and identified by the conventional methodology. Species richness was calculated as the number of different species per sampling site. The analysis of biodiversity was done by means of the Shannon-Weaver index. The value of the Shannon-Weaver (H) index of biodiversity in sampling time was higher at research pilot of Arasbaran forest with frequency of (S=129), (H'=3.04 and $e^{H'}$=20.83) and at sampling site 1 (S=88) and 2 (S=41) was (H'=3.01 and $e^{H'}$=20.25) and (H'=2.72 and $e^{H'}$=15.22), respectively. The same occurred with the species richness (S), with the Site 1 presenting greater richness (S = 88) compared to the Site 2 (S = 41).

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INTRODUCTION

Yeasts are permanent residents of different substrates in natural ecosystems. Yeasts present a great biodiversity, playing crucial functions in the nature, as in ecosystem maintenance. Yeasts are generally found in fruits, cereals and other substrata that contain sugars. But they can also be isolated from air, ground, water, skin and intestine of animals, including associations with insects [10,20,21].

Yeasts produce metabolites including amino acids, enzymes and also provide carbohydrates, minerals and nutrients for growth of other microorganisms such as bacteria by utilizing of plant residues and play important and special role in nitrogen fixing in the soil and natural environment. Yeasts are eukaryotic classified microorganisms in fungi class with nearly 1500 species. In the most of yeasts asexual reproduction is by budding method, however, a few are enhanced by binary division. Of course, in some of yeasts can be seen sexually reproduction. Yeasts are unicellular but some isolates when budding are seen multicellular form, some of them have pseudo hyphs. The size of the cells are very wide depending on yeast strains and their usual size is 3-4 micron that in some yeasts could be over 40 microns [5,14,15,20,21].

Yeasts do not constitute a special phylogenetic or taxonomic groups. Currently, only one percent of all yeast species have been described. The term yeast often used as a synonym with Saccharomyces cerevisiae, but phylogenetic diversity of yeasts are expressed into two classified as basidiomycetes and ascomycetes. The budding yeasts or true yeasts are classified under the saccharomycetals class [15,20,21,22,26].

Biological diversity or biodiversity is a term we use to describe the variety of life on Earth. It refers to the wide variety of ecosystems and living organisms: animals, plants, their habitats and their genes. Biodiversity is

Corresponding Author: Ahmadreza Hasani, Ph.D. graduated of Microbiology Department, Biology Faculty, Baku State University and Natural Resources and Agriculture Research Center of East- Azerbaijan province, Tabriz-Iran.
E-mail: drhasani124@yahoo.com

the foundation of life on Earth. It is crucial for the functioning of ecosystems which provide us with products and services without which we couldn't live. Oxygen, food, fresh water, fertile soil, medicines, shelter, protection from storms and floods, stable climate and recreation - all have their source in nature and healthy ecosystems. But biodiversity gives us much more than this. We depend on it for our security and health; it strongly affects our social relations and gives us freedom and choice. Biodiversity is extremely complex, dynamic and varied like no other feature of the Earth. Its innumerable plants, animals and microbes physically and chemically unite the atmosphere (the mixture of gases around the Earth), geosphere (the solid part of the Earth), and hydrosphere (the Earth's water, ice and water vapour) into one environmental system which makes it possible for millions of species, including people, to exist. At the same time, no other feature of the Earth has been so dramatically influenced by man's activities. By changing biodiversity, we strongly affect human well-being and the well-being of every other living creature [13,16].

Iran has the privileged place in the world from the diversity of climatic and various ecosystems. Arasbaran, one of these ecosystems in the north west of country is appropriate to study and research. Arasbaran is one of the conserved regions as a particular region in terms of plant and animal species. Arasbaran forests after completing courses in conservation, some of which have the ability to be upgraded to a national park and will be exposed and accessible to nature enthusiasts. Elevation range of 256 to 2896 meters, average annual rainfall of 275 mm and mean of temperature 10°C, semi-arid and sub-humid climates of the region has taken temperate for Arasbaran forests. Arasbaran Forest Research Station has been selected as a research platform with area of about 12,000 hectares in the basin of *Sotan chay* by East Azerbaijan Agriculture and Natural Resources Research Center [1].

Yeasts distribution, location and change patterns for ecological conditions should be explored during the seasons of year to assess the role of them in various ecosystems.

For this reason, the preliminary research to evaluate the texture of yeast mycoflora for Arasbaran forest and rangeland carried out in the northern part of the pilot area, close to Aras River at Forest Research Station in enveloped of East-Azerbaijan Agricultural and Natural Resources Research Center.

MATERIALS AND METHODS

Sampling and isolation of yeasts:

Sampling was carried out in two different regions of height with considering the variety of ecological factors such as habitat diversity and richness of flora and fauna and different forming and types on plant cover in forests and rangelands structures at 400-2200 meters height above sea level. Site 1 and 2 were in above 1500-2000 meters and in low 1000 meters, respectively.

In this study, yeast mycoflora was identified in soil, forest plant cover, forest and rangeland plants surface.

In one experimental area, the plant cover was differed from other areas and mainly it was consists of tree herbs. There was almost bush plants and the forage cover was poor and non-dense.

The most of trees were including oak (*Quereus* L.), black hawthorn or cranberries (*Crataegus ambigus*) and blueberries (*ornus mas L.*). The soil type in this area was undeveloped podzol soil with depth from 2 to 20 cm. The horizon of F/FH as the forest soil cover were used for soil sampling [6,7,11,13].

In two experimental area (site 2), was similar to vegetation cover in one experimental area/site 1. Non-dense bushes of raspberries (Rubus L.), shrubs of black hawthorn (Crataegus L.) including Cranberry or black hawthorn (Crataegus ambigus) and red hawthorn (Crataegus meyer) were comprising for most of plants in the area of the pilot. The soil slices was prepared from podzol soil or humus, thickness of humus layer was 3 to 10 cm. In one experimental area carried out one sample from the sap of trees trunk and two samples from the nest of ants at both of experimental area.

A total of 60 samples (30 samples from each area) were taken from different parts of forest trees (Including leaves and rotting fruits, blossoms and rotting branches and soil of around the trunk of the trees and the soil under the dry leaves and the like) during the months of April to December in 2009.

Yeasts identification:

For isolation of yeasts in the samples, 5 g of each sample in 50 ml of malt extract broth (MEB) containing 0.1 g chloramphenicol were inoculated and incubated at 25°C for 24 h in the shaker incubator with 180 rpm. After incubation the yeasts isolates were studied by using light microscopy [2,3,5,15,20,21,26].

The isolates were phenotypically characterized by means of macro/micro-morphological and physiological features, as fermentation of glucose, maltose and sucrose; assimilation of the following carbon sources: D-Glucose, D-Galactose, D-Ribose, D-Xylose, L-Arabinose, D-Arabinose, L-Rhamnose, Sucrose, Maltose, Trealose, Cellobiose, Salicin, Melibiose, Lactose,Raffinose, Inulin, Starch, Glycerol, Erythritol, Ribitol, D-Glucitol, D-Mannitol, myo-Inositol, Lactate, Citrate,Tween 20, N-acetylglucosamine; assimilation of the following nitrogen sources: Nitrate, Nitrite, Ethylamine, Lysine, Creatine and Creatinine; starch formation; urea hydrolysis; Diazonium Blue B reaction; growth at 40 and 50°C and 50% D-Glucose, 10% NaCl/16% NaCl and in different temperatures. Identification was performed according to Barnett *et al*. [5] and Kurthzman *et al*. [14].

The used culture media for yeast identification:

1. MEPA Yeast Morphology Agar (YMA) Saboraud dextrose agar or SDA: contains all essential nutrients and required vitamins for yeast culture also a source of carbohydrate.
2. Yeast Carbon Base: contains all essential nutrients and required vitamins for yeast culture, except of the nitrogen source.
3. Yeast Nitrogen Base: contains all the essential vitamins and inorganic salts for yeast culture, except of the source of carbohydrate.
4. Yeast nitrogen base culture medium without amino acids (YNB w / o Amino Acids): contains all essential nutrients and vitamins needed for yeast culture, except of histidine, methionine, tryptophan and carbohydrate source.
5. Yeast nitrogen base culture medium without amino acids and ammonium sulfate (YNB w / o Amino Acids and Ammonium Sulfate): contains all essential nutrients and vitamins needed for the yeast culture. except of amino acids, nitrogen and carbohydrate.
6. Carbon Assimilation Tests: Liquid media for the yeast (sugar or carbohydrate) assimilation tests.
7. Nitogen Assimilation Tests: Liquid media for the yeast (amino acids) assimilation tests.

Analysis of biodiversity:

Species richness was calculated as the number of different species per sampling site. The analysis of yeast biodiversity was done by means of the Shannon-Weaver index [24].

H' = $-\sum pi$ **ln** *(pi)*

H' = The Shannon-Weaver Diversity Index

pi = The relative abundance of each group of organisms

But remember that the Shannon-Wiener index is usually expressed as $\boldsymbol{e^{H'}}$, the natural logarithm of e = 2.718.

The Shannon index, also known as the Shannon-Weaver Index and sometimes referred to as the Shannon-Wiener Index, is one of several diversity indices used to measure diversity in categorical data. It is simply the Information entropy of the distribution, treating species as symbols and their relative population sizes as the probability (Table 2).

This article treats its use in the measurement of biodiversity. The advantage of this index is that it takes into account the number of species and the evenness of the species. The index is increased either by having additional unique species, or by having a greater species evenness.

RESULTS AND DISCUSSION

The achieved results revealed that the number of yeasts vary quite obviously in the forest soils and plants depending on the season. Typically, the amount of yeasts in the pilot areas of forest soil (layer F) was higher than the A1 layer of soil and so the abundance of yeasts was higher.

In one third of investigated podzol soil samples for A1 and A2 horizons associated with blueberries, dependence of isolated yeasts did not follow from a specific rule on the seasons.

A total of 129 yeast isolates, about 33 genera and species of yeasts have been identified and their emissions were recorded on different substrates. 129 yeast strains were identified in about 16 species, at least 37 (27.67%) with ascomycetic affinity and 92 (71.32%) basidiomycetes associated to both sites (Table 1).

The genera more commonly found were *Candida* and *Aureobasidium pullulans* within the ascomycetous group and *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* within the basidiomycetes. The predominant species were *Candida curvata* (n=8) among the ascomycetes, and *Sporobolomyces roseus* (n=11), *Cryptococcus* sp. (n=13), *Cryptococcus albidus* (n=14), *Rhodotorula* sp. (n=17), and *Sporobolomyces roseus* (n=11) among the basidiomycetes. All these species were found in both sampling sites, although with minor differences in the frequencies. *S. roseus* and *C.curvata* were more common in Site 1, while *Cr. Albidus and Rhodotorula* sp. presented a slightly higher frequency in Site 2(Table 1-2).

Non pigment yeasts was dispersed in the soil and Lypomycets had the main position among them, pigment producing yeasts have a wide range spread in the live plants surface. Most of them, *Rhodotorula* (n=17) and *Sporobolomyces* (n=12) yeasts genera with red colony and *Aureobasidium pullulans* (n=6) as a like yeast with dark gray to black colonies were observed. Similar studies by other researchers have also confirmed the findings of the present study [7-9,11].

Yeast mycoflora are more diverse in forest ecosystems and isolates of *Cryptococcus* spp (n=13) especially *Cr. podzolicus* (n=8) are seen greater. *Debaryomyces* (n=6) and *Schwanniomyces* (n=1) genera in riverside grass soils were observed that, the results are almost consistent with the findings of other researchers [7-9,11].

Yeast-like with the dark colonies were observed in Rotting organic matter in forest soils and forage crops, such as: *Trichosporon pullulans* (n=3) and *Saccharomyces rosei* (n=2).

Table 1. Frequency of the yeast isolates associated with Arasbaran Forest Research Station

Yeasts	Total number of isolates	Sampling sites		Soil		Plants						The sap of trees trunk	the nest of ants
		1	2	Horizon A1 and A2	Horizon FH	Oak (*Quereus* L.)	Cranberries (*Crataeaus*	Raspberries (*Rubus* L.)	Blueberries (*ornus mas*	Forest plant cover	Rangelands		
	N	30	30	6	6	9	5	8	8	6	6	2	4
Ascomycetes													
Aureobasidium pullulans	6	4	2		1	1				1	1	1	1
Candida curvata	8	5	3	1	1	1	1	1	1		2		
Candida spp.	4	3	1	2						1	1		
Candida humicula	2	2	0	1								1	
Candida scottii	2	2	0	1								1	
Debaryomyces cantarellii	1	1	0										1
Debaryomyces castellii	1	0	1	1									
Debaryomyces formicarius	1	1	0		1								
Debaryomyces hansenii	3	2	1	2	1								
Lipomyces starkeyi	1	0	1	1									
Metschnikowia pulcherima	1	1	0			1							
Metschnikowia reukaufii	1	1	0								1		
Pichia bovis	1	1	0		1								
Pichia spp.	1	1	0		1								
Saccharomyces rosei	2	1	1		2								
Schwanniomyces alluvius	1	0	1	1									
Torulopsis ernobii	1	1	0		1								
Basidiomycetes													
Bullera alba	2	2	0			1	1						
Cryptococcus albidus	14	9	5	2	1	3	1	2	3		2		
Cryptococcus flavus	5	3	2		2				1	1	1		
Cryptococcus laurentii	3	3	0		1					1		1	
Cryptococcus podzolicus	8	5	3	2	3	1		1					1
Cryptococcus spp.	13	9	4		2	2		1	1	2	3	1	1
Cryptococcus terricolus	4	2	2	1						1	1		1
Rhodotorula graminis	1	0	1								1		
Rhodotorula minuta	3	3	0			1	1		1				
Rhodotorula spp.	17	11	6	1	2	3	1	3	2	1	2	1	1
Rhodotorula rubra	4	3	1		1					2	1		
Rhodosporidium diobovatum	3	2	1			1				1	1		
Sporobolomyces holsaticus	1	0	1								1		
Sporobolomyces roseus	11	7	4	1	3	2		1	1	1	2		
Trichosporon pullulans	3	3	0			1					2		
Total number	129	88	41	17	24	18	5	9	10	12	22	6	6

N= The total number of all individuals.

In Comparison of yeast mycoflora for different plants surface was observed a significant difference among mycoflora from falling leaves and fresh leaves of oak. Yeast strains were two times greater in forage crops and inside them, *Rhodotorula* and *Sporobolomyces* were predominant.

Aureobasidium pullulans had superiority in the soil cover with oak leaf (layer F) in all year round. *Candida* spp. (n=16) as other strain on trunk and leaves of oak trees and *Candida curvata* (n =1) on forage crops at flowering time were allocated to the high biodiversity. Amount of this yeast along with *Metschnikowia reukaufii* has increased tremendously in summer with flowering forage plants, presence of nectars and some pollinators [11,18,19].

Yeasts also exist in the air(14) and they belonged to surface yeasts exactly such as *Cryptococcus laurentii* (n=3) and *Sporobolomyces roseus* (n=11). Yeasts are distributed in the air by ballistospore that are seen in yeasts with dark gray colonies [16,17,18,23].

Certain genus of yeasts such as *Nadsonia* were isolated from the sap of trees trunk and the nests of ants that can be found less in other regions of forest ecosystems. Two species of *Debaryomyces* including *D. cantarelli* and *D. formicarius* are found permanently in the nests of ants, amounts of these yeasts reach to tens of thousands in soil [16,20,21,23].

The species frequency in each sampling site can also be seen in Table 1 and 2. The value of the Shannon-Weaver (H) index of biodiversity in sampling time was higher at research pilot of Arasbaran forest with frequency of (S=129), (H'=3.04 and $e^{H'}$=20.9) and at sampling site 1 (S=88) and 2 (S=41) was (H'=3.01 and $e^{H'}$=20.3) and (H'=2.72 and $e^{H'}$=15.2), respectively. The same occurred with the species richness (S), with the Site 1 presenting greater richness (S=88) compared to the Site 2 (S=41).

Table 2: The analysis of yeast biodiversity by means of the Shannon-Weaver index in Arasbaran Forest Research Station.

Yeasts	N	pi	LN pi	pi*Lnpi	S1	pi	LN pi	pi*Lnpi	S2	pi	LN pi	pi*Lnpi
Ascomycetes												
Aureobasidium pullulans	6	0.05	-3.07	-0.14	4	0.05	-3.09	-0.14	2	0.05	-3.02	-0.15
Candida curvata	8	0.06	-2.78	-0.17	5	0.06	-2.87	-0.16	3	0.07	-2.61	-0.19
Candida spp.	4	0.03	-3.47	-0.11	3	0.03	-3.38	-0.12	1	0.02	-3.71	-0.09
Candida humicula	2	0.02	-4.17	-0.06	2	0.02	-3.78	-0.09	0	0	0	0
Candida scottii	2	0.02	-4.17	-0.06	2	0.02	-3.78	-0.09	0	0	0	0
Debaryomyces cantarellii	1	0.01	-4.85	-0.04	1	0.01	-4.48	-0.05	0	0	0	0
Debaryomyces castellii	1	0.01	-4.85	-0.04	0	0	0	0	1	0.02	-3.71	-0.09
Debaryomyces formicarius	1	0.01	-4.85	-0.04	1	0.01	-4.48	-0.05	0	0	0	0
Debaryomyces hansenii	3	0.02	-3.76	-0.09	2	0.02	-3.78	-0.09	1	0.02	-3.71	-0.09
Lipomyces starkeyi	1	0.01	-4.85	-0.04	0	0	0	0	1	0.02	-3.71	-0.09
Metschnikowia pulcherima	1	0.01	-4.85	-0.04	1	0.01	-4.48	-0.05	0	0	0	0
Metschnikowia reukaufii	1	0.01	-4.85	-0.04	1	0.01	-4.48	-0.05	0	0	0	0
Pichia bovis	1	0.01	-4.85	-0.04	1	0.01	-4.48	-0.05	0	0	0	0
Pichia spp.	1	0.01	-4.85	-0.04	1	0.01	-4.48	-0.05	0	0	0	0
Saccharomyces rosei	2	0.02	-4.17	-0.06	1	0.01	-4.48	-0.05	1	0.02	-3.71	-0.09
Schwanniomyces alluvius	1	0.01	-4.85	-0.04	0	0	0	0	1	0.02	-3.71	-0.09
Torulopsis ernobii	1	0.01	-4.85	-0.04	1	0.01	-4.48	-0.05	0	0	0	0
Basidiomycetes												
Bullera alba	2	0.02	-4.17	-0.06	2	0.02	-3.78	-0.09	0	0	0	0
Cryptococcus albidus	14	0.11	-2.22	-0.24	9	0.1	-2.28	-0.23	5	0.12	-2.1	-0.26
Cryptococcus flavus	5	0.04	-3.25	-0.13	3	0.03	-3.38	-0.12	2	0.05	-3.02	-0.15
Cryptococcus laurentii	3	0.02	-3.76	-0.09	3	0.03	-3.38	-0.12	0	0	0	0
Cryptococcus podzolicus	8	0.06	-2.78	-0.17	5	0.06	-2.87	-0.16	3	0.07	-2.61	-0.19
Cryptococcus spp.	13	0.1	-2.29	-0.23	9	0.1	-2.28	-0.23	4	0.1	-2.33	-0.23
Cryptococcus terricolus	4	0.03	-3.47	-0.11	2	0.02	-3.78	-0.09	2	0.05	-3.02	-0.15
Rhodotorula graminis	1	0.01	-4.85	-0.04	0	0	0	0	1	0.02	-3.71	-0.09
Rhodotorula minuta	3	0.02	-3.76	-0.09	3	0.03	-3.38	-0.12	0	0	0	0
Rhodotorula spp.	17	0.13	-2.03	-0.27	11	0.13	-2.08	-0.26	6	0.15	-1.92	-0.28
Rhodotorula rubra	4	0.03	-3.47	-0.11	3	0.03	-3.38	-0.12	1	0.02	-3.71	-0.09
Rhodosporidium diobovatum	3	0.02	-3.76	-0.09	2	0.02	-3.78	-0.09	1	0.02	-3.71	-0.09
Sporobolomyces holsaticus	1	0.01	-4.85	-0.04	0	0	0	0	1	0.02	-3.71	-0.09
Sporobolomyces roseus	11	0.09	-2.46	-0.21	7	0.08	-2.53	-0.2	4	0.1	-2.33	-0.23
Trichosporon pullulans	3	0.02	-3.76	-0.09	3	0.03	-3.38	-0.12	0	0	0	0
	129			-3.04	88			-3.01	41			-2.72
			H' =	3.04			H' =	3.01			H' =	2.72
			$e^{H'}$ =	20.9			$e^{H'}$ =	20.3			$e^{H'}$ =	15.2

N: The abundance of total sites , S1: The abundance of area 1 , S2: The abundance of area 2 , The natural logarithm of $e = 2.718$.
LN: The short term for natural log. , Pi: The relative abundance of each species , LN pi: Logarithm for the relative abundance

It is observed that the value of biodiversity index for yeast mycoflora at site 1 or the altitude of 1500 meters above with considering high moisture and density of vegetation and substrate variety was higher than other area.

Conclusion:

According to the achieved results in this study and the reported results by other researchers can be concluded that in similar ecosystems from geographically, there are many similarities in the number of isolated yeast species. On the other hands, the differences should be judged and commented depending on special ecological and characteristics of the ecosystem and the type and density of substrates.

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