Soil yeast abundance and diversity assessment in a hot climatic region, semi-arid ecosystem

Mohammed Khalid Al-Atrash*, Zwida K. Khadur, Anwar A. Khadim

Department of Nursing, Baquba Technical Institute, Middle Technical University, Baghdad, Iraq

Received: November 2020, Accepted: March 2021

ABSTRACT

Background and Objectives: Yeasts are an important portion of microbial communities of soil due to their bioactivity for ecosystem safety. Soil yeast abundance and diversity are likely to be affected under harsh environmental and climatic conditions. In Iraq, human activity and climatic changes especially high temperature which may alter microbial communities in soil. Very little is known about yeast abundance and diversity in a hot climatic region.

Materials and Methods: By PCR technique, soil yeast abundance and diversity were investigated under extreme environmental and climatic conditions, as well as the effects of soil properties and vegetation cover in semi-arid lands.

Results: In all, 126 yeast strains were isolated and identified as belonging to 13 genera and 26 known species. The maximum quantity of yeast was $8 \times 10^5$ CFU g$^{-1}$ of soil, with significantly varied in abundance and diversity depending on soil properties and presence of vegetation.

Conclusion: The results show that soil yeast abundance in these regions was significantly decreased. However, semi-arid lands are still rich in yeast diversity, and many species have adapted to survive in such conditions.

Keywords: Soil yeasts; Yeasts diversity; Semi-arid lands; Microbial adaptation

INTRODUCTION

Although several studies have been conducted on the occurrence of microorganisms in temperate and cold habitats (1-3), our knowledge of the abundance and microbial diversity in high-temperature soil is still very limited. Furthermore, most research in the cold and temperate regions has focused on new species (3-4). Iraq is one of the warmest countries on the planet, mainly because of the range of extreme environmental and climatic conditions (5). Iraq's ecosystems faced a number of unique challenges during the recent decades such as extremely high temperatures, strong sunlight, decrease annual rainfall, desertification, soil salinity, and extended years of wars. Moreover, many agricultural regions have extensively transformed either into arid or urban lands, generating impacts on many natural habitats and biodiversity (6). Culturable land area in Iraq is estimated at only about 22% of the total area of the country. However, no more than 12% has been actually cultivated (7).

The climate of Baghdad is semi-tropical continental, with mean annual temperature and rainfall of 22.9°C and 120 mm, respectively. Maximum and minimum temperature during the summer (June, July and August) are 28°C and 50°C, respectively (5). *Suaeda aegyptica* was the perennial main plant in the study region, which is a low sprawling shrub and
grows naturally in saline lands (halophytes), with some other types of plants such as *Alhagi maurorum*, *Salsola longifolia* and *Haloxylon articulatum*, all of which are classified as perennial halophytes (8). There is a continuous decline for agricultural lands in Iraq due to many reasons, including the urban expansion, land mismanagement and the ongoing wars and conflicts in the country for decades, as well as the impact of global climate change.

Temperature is one of the most important factors affecting the growth of microorganisms (3). Karhu et al. (9) and Chen et al. (10) found that high temperature has led to an increase in microbial abundance in a cold area, whereas Delgado-Baquerizo et al. (11) described that semi-arid lands are particularly sensitive to temperature and precipitation. With increasing climate change, there are many ecosystems that have been affected worldwide.

Yeasts are widespread in nature, including extreme environments, and often soils were perceived largest as a reservoir for yeasts (3, 12). Fungi play a vital role in maintaining the balance of ecosystems such as decomposition, nutrient recycling and they interact with ecosystem ingredients and providing nutrients through their biological activity to improve soil health (13, 14). Studies showed microbial abundance in soil has a significant impact on organic matter decomposition in nature (10, 15). Therefore, changing the abundance of microbial communities as a result of climate change may have a significant impact on the future of life on earth (16), and this depends on the rate at which the microbes are consumed for nutrients (14). As showed by Chen et al. (10) that abundance and diversity of microorganisms in the soil vary depending on climatic zones and ecosystem types. Many factors can greatly affect microbial activity such as temperature, pH, moisture, nutrients, soil type, ecosystem type, human activities and vegetation type (17).

Yeast abundance and diversity in high-temperature soils are still unknown. Thus, the study of these zones is needed. The main aim of the present study was to assess the abundance and diversity of soil yeast in a hot climatic zone and the effect of soil properties on its biological activity.

**MATERIALS AND METHODS**

**Study site and sampling.** Soil samples were collected from different sites in Baghdad, Iraq, which is located in the Middle East (33°25′-33°44′N, 44°16′-44°29′E) with a total area of 2042.2 km² (about 1.5% of Iraq area).

Sampling was carried out between September and October 2018. The sites were selected to represent homologous samples of the semi-arid ecosystem. All the lands from which samples were collected have not been managed for decades, they are also not grazing lands. In total, 150 samples were collected from 10 different sites. Soil samples were collected from each plot at the corners and center of 20 m² using a soil column cylinder, this means 5 samples for each plot. Half of the samples were taken from the soil with vegetation cover (SWV) (perennial plant cover was no less than 50%), while the other half was from the soil without vegetation cover (SWOV). Herbs root, stones and other impurities were removed from the samples. Soil samples were put quickly into a sterilized glass container and transported to the laboratory on ice for analyses.

**Isolation of yeasts.** Yeasts were isolated using glucose yeast extract peptone agar (GYP). One g from each soil sample was placed in tube and 5 – fold diluted with sterile water, the suspension was shaken in an orbital shaker at 200 rpm for 1 hr. 100 µl of suspension was cultured. 3 replicates (subsamples) were analyzed from each diluted sample. Based on environmental conditions at the sampling sites, the selected incubation temperature was 27 ± 1°C. Colonies were examined and counted after 3 days, the values were analyzed statistically using ANOVA.

**DNA extraction, amplification and sequencing.** Yeast total genomic DNA was extracted using a FastDNA®kit (MP Biomedicals LLC, Irvine, CA) according to the manufacturer instruction. The D1 / D2 domain of the 26S rRNA gene was amplified using the primers NL1 (5'-GCA TAT CAATAA GCG GAG GAA AAG-3') and NL4 (5'-GGT CCG TGT TTC AAG ACG G-3') (MWG Biotech). The internal transcribed spacer (ITS) sequences were obtained using the forward primer ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and the reverse primer ITS 4 (5'-TCC TGC TAT TGA TAT GC-3') (MWG Biotech).

Sequences were carried out using an Applied Biosystems DNA sequencer using standard protocols. Sequence alignment was made using Meg Aliga DNA STAR Lasergene. All sequences obtained were
compared with sequences from the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST) using the BLASTN search algorithm.

**Soil analysis.** All soil samples were analyzed for moisture, pH, temperature, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and salinity using standard methods (18). All experiments were carried out in triplicate, and the statistical analysis was done using ANOVA strategy.

**RESULTS**

At the time of sampling, the soil temperature was within the range 25.8-27.4°C and the soil pH was (7.0-7.7). Soil samples exhibited significant (P < 0.01) differences in some of their characteristics, the moisture content and nutrients in soil with vegetation cover were higher than in soil without vegetation cover. The electrical conductivity (EC_e) of soil samples was within range (2.7-8.7 dSm⁻¹) with significant (p < 0.01) differences between the samples, but no significant effect on the yeast community (Table 1).

In all analyzed soils, yeast numbers ranged from 0.2 × 10 to 0.8 × 10² CFU g⁻¹ of soil, with significant (p < 0.01) differences between SWV and SWOV (Fig. 1). The relative abundance of yeast communities was significantly higher in SWV than in SWOV. These quantities did not differ significantly between the study sites. While no significant effect was observed on yeast abundance by vegetation type. Without vegetation, yeast abundance decreased significantly at all the study sites and represented a small proportion (about 34%) of the yeast community in soil (Figs. 1 and 2 and Table 2).

In all, 126 yeast strains were isolated and identified during this study. 83 yeasts from SWV and 43 from SWOV. Results of the sequence analyses of 126 yeasts showed that the obtained yeast strains belonged to 13 genera and 26 known species (Table 2). Yeast community composition in this study significantly varied depending on the presence or absence of plants, thirteen species were present exclusively in SWV, while only one species (*C. variovaarae*) was present exclusively in SWOV (Table 2). Thus, yeast diversity was higher in SWV than in SWOV (Fig. 2).

![Relative distribution of soil yeasts community between SWV and SWOV (above), and relative dominance between Ascomycetous and Basidiomycetous fungi (below).](image)

![Relative abundance of yeast genera, based on molecular sequence data, dominating in soil with vegetation cover and soil without vegetation cover. The above data represents the yeast community in semi-arid land, Baghdad, Iraq.](image)
Table 2. Species list and number of soil yeast strains isolated from semi-arid lands. Total number and species richness are provided.

| Species                        | SWV strains | SWOV strains | Total (%) |
|--------------------------------|-------------|--------------|-----------|
| Ascomycetous yeasts            |             |              |           |
| Candida maritima               | 1           | -            | 1 (<1%)   |
| C. parapsilosis                | 3           | 2            | 5 (4%)    |
| C. tropicalis                  | 7           | 4            | 11 (9%)   |
| C. variotaurae                 | -           | 1            | 1 (<1%)   |
| Hansenula holstii              | 2           | -            | 2 (2%)    |
| H. polymorpha                 | 8           | 3            | 11 (9%)   |
| Hanseniaspora valbyensis       | 3           | 1            | 4 (3%)    |
| Pichia delftensis              | 6           | 2            | 8 (6%)    |
| P. kluyveri                    | 3           | 1            | 4 (3%)    |
| P. philogaea                   | 1           | -            | 1 (<1%)   |
| Saccharomyces boulardii        | 4           | 2            | 6 (4%)    |
| Schizoblastosporon gracil      | 4           | 4            | 8 (6%)    |
| Basidiomycetous yeasts         |             |              |           |
| Asterotremella humicola        | 2           | -            | 2 (2%)    |
| Cryptococcus. aerius           | 1           | -            | 1 (<1%)   |
| Cr. albidas                    | 7           | 10           | 17 (13%)  |
| Cr. diffluens                  | 2           | 1            | 3 (2%)    |
| Cr. skinneri                   | 13          | 11           | 24 (19%)  |
| Cr. terreus                    | 1           | -            | 1 (<1%)   |
| Cr. terricola                  | 1           | -            | 1 (<1%)   |
| Guehomyces pullulans           | 3           | -            | 3 (2%)    |
| Piskurozyma filicatus          | 1           | 1            | 2 (2%)    |
| Trichosporon porosum           | 4           | -            | 4 (3%)    |
| T. dulcicum                    | 1           | -            | 1 (<1%)   |
| Rhodospiridium babjevae        | 2           | -            | 2 (2%)    |
| Rhodotorula colostrri          | 2           | -            | 2 (2%)    |
| Rh. laryngis                   | 1           | -            | 1 (<1%)   |
| Total quantity                 | 83          | 43           | 126       |
| Species richness               | 25          | 13           | 26        |

Generally, the most frequent genera were Cryptococcus (37% of total isolates), Candida (15%), Pichia (10%), Hansenula (9%) and Schizoblastosporon (6%). While at the species level, Cryptococcus skinneri (19%) and Cr. albidas (13%) were most frequent of all strains. About 50% of the total isolates in SWOV were Cryptococcus, followed by Candida (19%). Of 26 species isolated, eight species (about 1/3 of total isolates) occurred as single isolates (Table 2). Moreover, that average observed yeast species values ranged from 1 to 3 species per sample. Rhodotorula laryngis, Rh. colostrii and Rhodospiridium babjevae were only obtained as pigmented species in this study, where they represented fewer than 5% of the total isolates.

Basidiomycetous yeasts were slightly higher than Ascomycetous (approximately equal in dominance). For example, Candida, Hansenula, Hanseniaspora, Pichia, Saccharomyces and Schizoblastosporon were observed in about 49% of total isolates, belonging to the Ascomycetous group. Furthermore, the diversity of Ascomycetous yeasts was higher than Basidiomycetous in SWOV (nine species of Ascomycetous yeast were obtained, while only four species of Basidiomycetous were found) (Table 2).

**DISCUSSION**

Several studies reported that ecosystems in Iraq...
are retreating progressively, and more environmental degradation will occur in the future, due to the impact of global climate change and land mismanagement (7). Accordingly, the semi-arid ecosystems in Iraq can be considered a suitable site for studying climate change impact and extreme environmental conditions on the microbial activity in soil.

Physical and chemical analysis of soils collected in semi-arid lands showed that concentrations of TOC, TN and TP less than those formerly observed in temperate and cold soils (1, 2, 19). The semi-arid soil is characterized by low nutrients and moisture (3). Carbon, nitrogen and phosphorous concentrations in soil affect the diversity and the relative abundance of the microbial communities (13, 15). At the same time, the study showed a significant decrease in soil moisture content. Low moisture can significantly impact microbial activity and restrict growth (13, 20). Whereas de Nijis et al. (21) mention that microorganisms can adapt to lower moisture levels. Soil moisture content varied among the samples collected in this study, and this may be related to the amount and depth of groundwater in the area. According to Abbas et al. (22), soil analysis data indicate that soil salinity was slightly to moderate in terms of the electrical conductivity (EC) index, which is restricted of many plants. Isolated yeasts from salt environments were reported by Butinar et al. (23). The optimum temperature for microbial growth in soil range from 25-30°C (24), thus, the temperature in the study area was within the optimum range.

Most studies conducted on yeast communities were from temperate or cold sites, especially in temperate forests and glacial sites (10, 17, 19, 25). This is the first study to assess abundance and diversity of soil yeasts in a hot climatic regions.

Although yeasts represent less than a quarter of fungal communities in soil (19), but they are an important part of microbial soil communities around the world (4). On the whole, the average number of yeasts obtained in semi-arid lands under study were lower than previously reported about temperate forests soil and grassland (17, 25) and glacial sites (1, 2). These results are in agreement with data reported by Allison et al. (26) who found that climate change reduced microbial abundance. As shown by Yurkov et al. (17), global climate change and human activities have a great impact on microbial abundance. Some authors mention that increasing temperature had a positive effect on the microbial abundance in colder regions (10), while the effect was negative in the current study (in a hot region). The relative abundance of yeasts in SWOV was significantly decreased compared with SWV. Plants prevent soil erosion and increase water storage capacity (27). Life often depends on the presence of water and nutrients (28).

These results are also mentioned by Vaidkertiová et al. (29) that the yeast abundance in soil depends on soil water content and vegetation cover. Also this is in agreement with Li et al. (15) that plants greatly affect the microbial community and provide nutrients. The diversity of species observed in this study may help us to understand more substantially about yeast adaptations, and also better understand their capacity for surviving in different types of soils. Several studies indicate that yeast has the ability to adapt in various soils worldwide (17, 30). Based on the analysis of the diversity results reported here and including results from previous studies (2, 17, 19, 26), it is possible to conclude that the semi-arid soils in hot climatic regions still retain yeast diversity regardless of the environmental and climatic conditions. Soil microbial communities have the ability to make structural and functional changes when responding to different environmental factors (31). This result is in agreement with data reported by Rasporn and Zupan, (12) that yeasts are able to adapt to a wide range of extreme environmental conditions. The results also showed that the diversity of Ascomycetes yeasts are being dominant in SWOV. The results also showed that the diversity of Ascomycetous yeasts is substantially greater than Basidiomycetous yeasts in SWOV. These results are in agreement with Gorbushina and Broughton (32), who found that Ascomycetous yeasts were more resistant to abnormal conditions than Basidiomycetes. The occurrence of many yeast species as single isolates in this study could indicate that such species may be more sensitive to abnormal conditions (Table 2).

This work support previous researches (2, 17, 25), that the most frequently isolated yeast genus was Cryptococcus. This is consistent with previous reports that Cryptococcus species can be considered the predominant yeast in most habitats. For example, Cryptococcus was isolated from cold environments as predominant species in a wide range of locals: Argentina (26), Italy (2), Norway (33), European glaciers and Antarctica (2). These are in addition to results from temperate habitats (17, 30). This is the first study reporting that Cryptococcus as predomi-
nant species in a hot habitats. The large presence of Cryptococcus in most environments is interesting, because this may indicate an important role for this yeast that has not yet been determined.

Carotenoid, melanin and mycosporine are pigments produced by pigmented yeasts, and they are antioxidant compounds that play an important role in UV radiation and strong sunlight protection (34, 35). Some authors have reported the presence of a relationship between pigment production and extreme environments (36, 37). However, few pigmented isolates were recorded in this study, this might be due to low vegetation cover in semi-arid lands, where pigmented yeasts are usually associated with the phylloplane (17, 37).

Generally, the current study showed that semi-arid soils in a hot climatic regions are rich in many species of yeasts but with smaller quantities than in other soils. Thus, reduced yeast abundance can be related to semi-arid soil properties, which are characterized by low nutrient concentrations, low water content, high temperature and scarcity of vegetation cover. These factors mainly resulted from the global warming and harsh environmental conditions. Diversity also indicates that yeasts have the potential to reduce metabolic activity as a physiological response to such environmental factors to survive.

Despite the fact that abnormal factors in the semi-arid ecosystems are a major challenge for many organisms, the microorganisms are still remain essential populations in soil even in abnormal environments. In the present paper, Since as the plants play a major role in conserving soil water and provide essential nutrients, it is concluded that the plants are closely associated with increasing resident soil-dwelling microbial abundance. The return of vegetation cover to Iraqi lands will restore the balance of the ecosystem by providing a suitable environment for microbial activity.

REFERENCES

1. Branda E, Turchetti B, Diolaiuti G, Pecci M, Smiraglia C, Buzzini P. Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy). FEMS Microbiol Ecol 2010;72:354-369.
2. Turchetti B, Goretti M, Branda E, Diolaiuti G, D’Agata C, Smiraglia C, et al. Influence of abiotic variables on culturable yeast diversity in two distinct Alpine glaciers. FEMS Microbiol Ecol 2013;86:327-340.
3. Yurkov AM. Yeasts of the soil—obscure but precious. Yeast 2018;35:369-378.
4. Yurkov AM, Chernov Iu, Tiunov AV. Influence of Lumbriicus terrestris earthworms on the structure of the yeast community of forest litter. Mikrobiologiya 2008;77:121-125.
5. Salman SA, Shahid S, Ismail T, Chung ES, Al-Abadi AM. Long-term trends in daily temperature extremes in Iraq. Atmos Res 2017;198:97-107.
6. Jabbar MT, Zhou JX. Environmental degradation assessment in arid areas: a case study from Basra province, southern Iraq. Environ Earth Sci 2013;70:2203-2214.
7. Food and Agriculture Organization (FAO). Iraq Agriculture sector note.2012; Available at: http://www.fao.org/3/i2877e/i2877e.pdf
8. Mehrabian A, Naqinezhad A, Mahiny AS, Mostafavi H, Liaghati H, Kouchekzadeh M. Vegetation mapping of the Mond protected area of Bushehr province (southwest Iran). J Integr Plant Biol 2009;51:251-260.
9. Karhu K, Auffret MD, Dungait JA, Hopkins DW, Prosser J, Singh BK, et al. Temperature sensitivity of soil respiration rates enhanced by microbial community response. Nature 2014;513:81-84.
10. Chen J, Luo Y, Xia J, Jiang L, Zhou X, Lu M, et al. Stronger warming effects on microbial abundances in colder regions. Sci Rep 2015;5:18032.
11. Delgado-Baquerizo M, Maestre FT, Escolar C, Galardo A, Ochoa V, Gozalo B, et al. Direct and indirect impacts of climate change on microbial and biocrust communities alter the resistance of the N cycle in a semiarid grassland. J Ecol 2014;102:1592-1605.
12. Raspor P, Zupan J (2006). Yeasts in extreme environments. In: Biodiversity and ecophysiology of Yeasts. 1st ed. Springer, Berlin, Heidelberg, pp.371-417.
13. de Vries FT, Shade A. Controls on soil microbial community stability under climate change. Front Microbiol 2013;4:265.
14. Rousk J, Bengtson P. Microbial regulation of global biogeochemical cycles. Front Microbiol 2014;5:103.
15. Li Y, Bezemer TM, Yang J, Lü X, Li X, Liang W, et al. Changes in litter quality induced by N deposition alter soil microbial communities. Soil Biol Biochem 2019;130:33-42.
16. Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken LR, Baylis M, et al. Scientists’ warning to humanity: microorganisms and climate change. Nat Rev Microbiol 2019;17:569-586.
17. Yurkov AM, Klemel M, Begerow D. Assessment of yeast diversity in soils under different management regimes. Fungal Ecol 2012;5:24-35.
18. Carter MR, Gregorich EG (2007). Soil sampling and
methods of analysis. 2nd ed. CRC press. Boca Raton FL.
19. Mašínová T, Bahnmann BD, Větrovský T, Tomšovský M, Merunková K, Baldrian P. Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS Microbiol Ecol* 2017;93:fiw223.
20. Kravchenko A, Chun HC, Mazor M, Wang W, Rose JB, Smucker A, et al. Relationships between intra-aggregate pore structures and distributions of *Escherichia coli* within soil macro-aggregates. *Appl Soil Ecol* 2013;63:134-142.
21. de Nijs EA, Hicks LC, Leizeaga A, Tietema A, Rouk J. Soil microbial moisture dependences and responses to drying–rewetting: The legacy of 18 years drought. *Glob Chang Biol* 2019;25:1005-1015.
22. Abbas A, Khan S, Hussain N, Hanjra MA, Akbar S. Characterizing soil salinity in irrigated agriculture using a remote sensing approach. *Phys Chem Earth* 2013;55:43-52.
23. Butinar L, Santos S, Spencer-Martins I, Oren A, Gund-Cimerman N. Yeast diversity in hypersaline habitats. *FEMS Microbiol Lett* 2005;244:229-234.
24. Pietikäinen J, Pettersson M, Bååth E. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiol Ecol* 2005;52:49-58.
25. Mestre MC, Rosa CA, Safar SV, Libkind D, Fontenla SB. Yeast communities associated with the bulk-soil, rhizosphere and ectomycorrhizosphere of a Nothofagus pumilio forest in southwestern Patagonia, Argentina. *FEMS Microbiol Ecol* 2011;78:531-541.
26. Allison SD, Lu Y, Weihe C, Goulden ML, Martiny AC, Treseder KK, et al. Microbial abundance and composition influence litter decomposition response to environmental change. *Ecology* 2013;94:714-725.
27. Brady NC, Weil RR (2008). The nature and properties of soils. 14th ed. NJ: Prentice Hall. New York.
28. Aerts JW, van Spanning RJM, Flahaut J, Molenaar D, Bland PA, Genge MJ, et al. Microbial communities in sediments from four mildly acidic ephemeral salt lakes in the Yilgarn Craton (Australia)–terrestrial analogs to ancient Mars. *Front Microbiol* 2019;10:779.
29. Vadkertiö R, Dudašová H, Baláščaková M (2017). Yeasts in agricultural and managed soils. In: *Yeasts in Natural Ecosystems: Diversity*. 1st ed. Springer Cham, Switzerland AG, pp.117-144.
30. Botha A (2006). Yeasts in soil. In: *Biodiversity and ecophysiology of yeasts*. 1st ed. Springer, Berlin, Heidelberg. pp.221-240.
31. Nie M, Pendall E, Bell C, Gasch CK, Raut S, Tamang S, et al. Positive climate feedbacks of soil microbial communities in a semi-arid grassland. *Ecol Lett* 2013;16:234-241.
32. Gorbushina AA, Broughton WJ. Microbiology of the atmosphere–rock interface: how biological interactions and physical stresses modulate a sophisticated microbial ecosystem. *Annu Rev Microbiol* 2009;63:431-450.
33. de Garcia V, Zalar P, Brizzi S, Gunde-Cimerman N, van Brooek M. Cryptococcus species (Tremellales) from glacial biomes in the southern (Patagonia) and northern (Svalbard) hemispheres. *FEMS Microbiol Ecol* 2012;82:523-539.
34. Roy S (2000). Strategies for the minimisation of UV-induced damage. In: *The effects of UV radiation in the marine environment*. 1st ed. Cambridge University press, UK, pp.177-205.
35. Moliné M, Libkind D, Diéguez Mdel C, van Brooek M. Photoprotective role of carotenoids in yeasts: response to UV-B of pigmented and naturally-occurring albino strains. *J Photochem Photobiol B* 2009;95:156-161.
36. Libkind D, Moliné M, Sampiao JP, Van Brooek M. Yeasts from high-altitude lakes: influence of UV radiation. *FEMS Microbiol Ecol* 2009;69:353-362.
37. Fonseca A, Inácio J (2006). Phylloplane yeasts. In: *Biodiversity and ecophysiology of yeasts*. 1st ed. Springer, Berlin, Heidelberg. pp.263-301.