Экосистемы, их оптимизация и охрана. 2014. Вып. 10. С. 21–29.

Биоценология и биология видов

UDC 663.131:663.252.41:663.262

BIODIVERSITY OF YEAST CULTURES ISOLATED FROM LITTORAL AREAS OF THE REGIONAL LANDSCAPE PARK «TILIGULSKIY»

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The identification of yeast cultures was done by polymerase chain reaction (PCR), using universal yeast primers. Following this, the morphology of each yeast culture was examined. The biochemical properties of each of these yeast cultures were determined during fermentation to carbohydrates (forming acid and gas), with the formation of hydrogen sulphide (H_2S) gas and sulphur dioxide (SO_2). Each yeast culture was identified according to its fermentation to the following carbohydrates: glucose, fructose, sucrose, maltose, lactose, galactose, sorbitol, mannitol, and xylose. The yeast cultures that were predominantly isolated from the Tiligul Estuary (benthic soils) littoral waters included: *Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis, Rhodotorula rubra*, *Saccharomyces cerevisiae.* The yeast cultures that were predominantly isolated and identified by PCR from the different grape varieties growing in the Tiligul estuary coastal area, following fermentation, included: *Metschnikowia* aff. *fructicola, Pichia barkeri, Pichia guilliermondii, Pichia kudriavzevii, Pichia* sp.*, Candida albicans, Candida tropicalis, Saccharomyces cerevisiae, Schizosaccharomyces pombe*. Yeast populations were collected from the surface of grape cultivars during harvesting season. Grapes were collected from Tiligul estuary coastal areas that included: the «Koblevo» and «Leninka» vineyards, and around the Chernvono-Ukrainka villages. Subsequently, a wide biodiversity of yeast species were shown to exist on the surfaces of ripe grapes obtained during harvesting season in coastal areas of the «Tiligulskiy» landscape park. Some of the isolated and identified yeast strains had the potential for biotechnological use in the wine industry. These included: *Saccharomyces cerevisiae,* and *Schizosaccharomyces pombe*. These yeast strains are currently in use in the wine industry, and are authorised for use in the food industry. Other yeast strains had potential for use as reference yeast strains, which could be used for testing sensitivity to anti-fungal agents, including: anti-fungal antibiotics and «azole» group agents.

Key words: *Saccharomyces cerevisiae, Schizosaccharomyces pombe*, biodiversity of yeast, biotechnology of yeast, landscape park.

INTRODUCTION

The biodiversity of yeast plays an important in nature role by supporting the biological balance between bacteria and fungi. Further, most yeasts ferment sugar in grape must, and have the potential for biotechnological use in the wine and alcohol industries [1]. The phylogenetic diversity of yeasts is shown by their assignment to two taxonomic classes of fungi: the ascomycetes and the basidiomycetes. Subdivision of taxa within their respective taxonomic classes is usually made comparing morphological and physiological features, whose genetic basis is often unknown [5, 8].

In protected areas, the issue of the biodiversity of yeast species remains as relevant as ever. The selection of yeast cultures and their detailed study make a significant contribution to the protection and conservation of wildlife. The biodiversity of yeast is of particular interest in yeast taxonomy, ecology, and biotechnology [2]. The role yeast plays in the ecology of estuaries and coastal areas is an important one in the ecosystems of waters and soils in these areas [4, 6].

A significant number of micro-organisms present on the surface of grapes and their crests are also present in pressed grape juice following processing. These micro-organisms represent a variety of genera and species of yeast, bacteria and fungi [12].

Strict biological selection takes place under the action of free acids, osmotic pressure and sugar solution contained within the grape juice. Microorganisms present that cannot tolerate the high acidity and high sugar concentration discontinue their growth and development, with some of them dying completely (i.e. bacteria and fungi) [10]. Acid-resistant micro-organisms engage in a struggle for dominance of the medium. As a result, spirituous fermentation manifests associated with the accumulation of ethanol.

Oxygen concentration in the fermented media decreases. Initially, this halts the activity of aerobic micro-organisms. Then, species and strains of yeast join the fray. These have differing ethanol-forming abilities and differing resistance to particular ethanol concentrations.

Asporogenous yeast, belonging to five genera and including eight species, participate in the fermentation process. In addition, six genera and 20 species of sporogenous yeast also participate [7].

During fermentation, yeast changes take place sequentially. Grape must fermentation often results in the presence of low-fermenting, but strongly budding yeasts, including *Hanseniaspora apiculata* and *Torulopsis bacillaris*. *Hanseniaspora apiculata* is primarily present during red grape fermentation, and is able to form 4 % to 5 % volume fraction of ethanol. *Torulopsis bacillaris* is a more stable yeast, and is able to form up to 10,7 % volume fraction of ethanol. These yeast species are, however, increasingly unstable in the presence of sulphur dioxide action. *Hanseniaspora apiculata* and *Torulopsis bacillaris* activity, therefore, decreases in sulphited musts.

In sulphited musts, *Saccharomyces cerevisiae*, which is more sulphite resistant and inhibits *Hanseniaspora apiculata* and *Torulopsis*, is able to multiply rapidly and completely. In addition, *Saccharomyces cerevisiae*, depending on yeast species and strain being used, is quite resistant to ethanol. They survive ethanol concentrations of between 8 % and 16 % volume fraction.

Other yeasts, including such species as: *Brettanomyces, Kluyveromyces, Schizosaccharomyces, Torulaspora*, and *Zygosaccharomyces*, may also be present during fermentation and can persist in the resultant wine. It is apparent, therefore, that spontaneous grape must fermentation encourages the presence of numerous yeast flora.

In the majority of cases, *Saccharomyces cerevisiae* yeasts have high ethanol resistance and are capable of fermenting residual sugar. Furthermore, they may be stored in a viable state for between months and several yeast [9].

Features relating to yeast distribution have been of great interest since the processes involved in traditional winemaking started. A question remains, however, regarding the sources and acquisition of wild yeast in fermenting substrates [3].

Saccharomyces cerevisiae are found in grapes. Also prevalent, however, are different yeast types that do not participate in subsequent grape juice fermentation. More rarely, *Saccharomyces cerevisiae* is encountered in the surrounding substrates; particularly so, in the soil underlying vineyards. Yeasts are also found on the soil surface, and up to a soil depth of between 20 and 30 centimetres throughout all seasons. As grapes ripen, a particularly large amount of yeasts are present [13].

Soil is not a media where active growth of yeast cultures is possible. For yeast, soil acts as a «trap» where yeast can be stored for a certain amount of time in a viable state. Thereafter, soil acts as a source of spores that infect grapes arising from new crops. Therein exists the cycle of yeast cultures in nature.

This study aims to identify and illustrate yeast biodiversity in grapes and in soils in the regional landscape park, «Tiligulskiy», in the Nikolaev region of Ukraine. In order to achieve this goal, the author led the cultivation and isolation of yeast cultures on selective yeast media following fermentation completion, and the identification of the isolated yeast cultures using PCR (Polymerase Chain Reaction) analysis. These measures aimed to establish the composition of yeast species, encouraging further debate and discussion around the biodiversity of yeast cultures occurring in the wild.

MATERIALS AND METHODS

Samples of several grape varieties that grow around the Tiligul estuary coastal area vineyards during the vintage season were obtained from vineyards of the «Koblevo» Agricultural Company in the Nikolaev region of Ukraine. Soil samples from different vineyards and littoral waters were obtained from the Tiligul estuary in the «Tiligulskiy» regional landscape park. A variety of industrial grapes were also selected for investigation.

The different grape cultivars, derived from the coastal territories of the «Tiligulskiy» regional landscape park, were put into sterile glass flasks up to approximately half of the 450 ml flask volumes. The flasks were carefully sealed with a rubber stoppers that were pierced by hollow needles to allow for the release of carbon dioxide, which is formed as a result of active anaerobic fermentation processes in the grape must.

Following grape must fermentation, pure yeast cultures were isolated using traditional microbiological methods (i.e. sample inoculation on Petri dishes with minor modifications of nutrient

selective agar for yeast isolation and cultivation). Primary yeast isolation was done using Inhibitory Mold Agar (IMA) medium, produced by the Becton Dickinson Company, USA.

Following primary yeast culture isolation, yeast morphological properties were analysed. Identification of yeasts was done using polymerase chain reaction (PCR) with universal yeast primers. Following yeast culture identification, yeast cultures were cultivated on Wort Agar medium, produced by the Becton Dickinson Company, USA. Each isolated and identified yeast culture was deposited, thereafter, in the NRRL Microbial Culture Collection (National Regional Research Laboratory, ARS, Peoria, USA), in the British National Collection of Yeast Culture (NCYC) in the Food Research Institute, Norwich, UK, and in Genebank of Japan in the Agrobiological Research Institute (NIAS), the MAFF (Ministry of Agriculture, Forest, and Fisheries) microbial culture collection, Tsukuba, Ibaraki, Japan.

Yeast isolate identification was carried out by amplification of ITS1-5.8S – ITS1-2b and D1 – D2 26S genome locus fragments that code ribosomal RNA with the next direct sequencing of received DNA fragments. The amplification of yeast cultures was done with the assistance of the Laboratory of Pharmacogenomics, the Research Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation.

Yeast isolate morphological, physiological and biochemical properties were determined [11]. Each yeast isolate was tested for several biotechnological characteristics, including: growth resistance at high temperature (+42°C) and low temperature (+6 to +8°C); growth in the presence of 5, 10 and 15 % ethanol (ethanol resistance); and growth in the presence of a high potassium bisulphite concentration (bisulphite resistance). Yeast isolate hydrogen sulphide (H_2S) gas synthesis was also studied.

The process of anaerobic fermentation commences following thorough mixing of the grape musts and stabilisation over a 24-hour period. Three days following initiation of the fermentation process, it is necessary to add 1.0 g diammonium phosphate to every 10 kgs of grape must as a nitrogen supplement for the yeast. Four days following fermentation initiation it is necessary to add another amino acid nutrient supplement for the yeast at a concentration of 2.0g for every 10 kgs of grape must.

Fermentation bio-processing continues over a period of 10 days. Following completion of fermentation, yeast cultures are then isolated on selective Inhibitory Mold Agar (IMA). The isolated yeast cultures are then grown in Wort Agar.

RESULTS AND DISCUSSION

Following fermentation, PCR was used to identify yeast cultures isolated from a variety of grape cultivars growing in Tiligul estuary coastal territory areas (see example in fig. 1). 82 yeast cultures were identified in this study. All were deposited in the aforementioned international microbial collections.

Following fermentation, the yeast cultures most frequently isolated from the grape musts studied included the following species: *Saccharomyces cerevisiae* (Meyen ex E. C. Hansen, 1883); *Schizosaccharomyces* pombe (Linder P., 1893); *Dekkera bruxellensis* (Van der Walt, 1964); *Candida tropicalis* (Berkhout, 1923); *Pichia kudriavzevii* (Kudriavzev, 1954); *Metschnikowia* aff. *fructicola* (T. Kamienski); *Pichia guilliermondii* (Wick, 1966); and *Pichia* sp., *Pichia barkeri,* and *Candida albicans* (Robin C. P., Berkhout, 1923).

The morphologies of yeast species isolated from different grape cultivars are shown in fig. 2 to fig. 9.

All of the isolated yeast cultures were deposited in the following, well-recognised, international collections:

The National Collection of Yeast Cultures (NCYC) in the Institute of Food research is the premier British yeast culture collection. The NCYC holds over 40,000 yeast strains collected over a period of around 65 years. The collection also has large collections of brewing yeasts, genetically-defined yeasts (used in many applications, including cancer research), yeasts associated with food spoilage, and yeasts of medical and industrial importance. The NCYC collects and preserves a wide variety of yeast cultures with applications in industry and academia. Research at NCYC has shed new light on yeast evolution and genetic inter-relationships, and resulted in novel tools for identifying and characterising yeasts. The NCYC makes yeast cultures in a variety of forms and tools NCYC has developed as services available to researchers. NCYC aims to grow both their collection and the know-how associated with it in order to

provide the most comprehensive representation and understanding of yeast biodiversity available, and to supply such materials and knowledge to industry and academia in an equitable and efficient way.

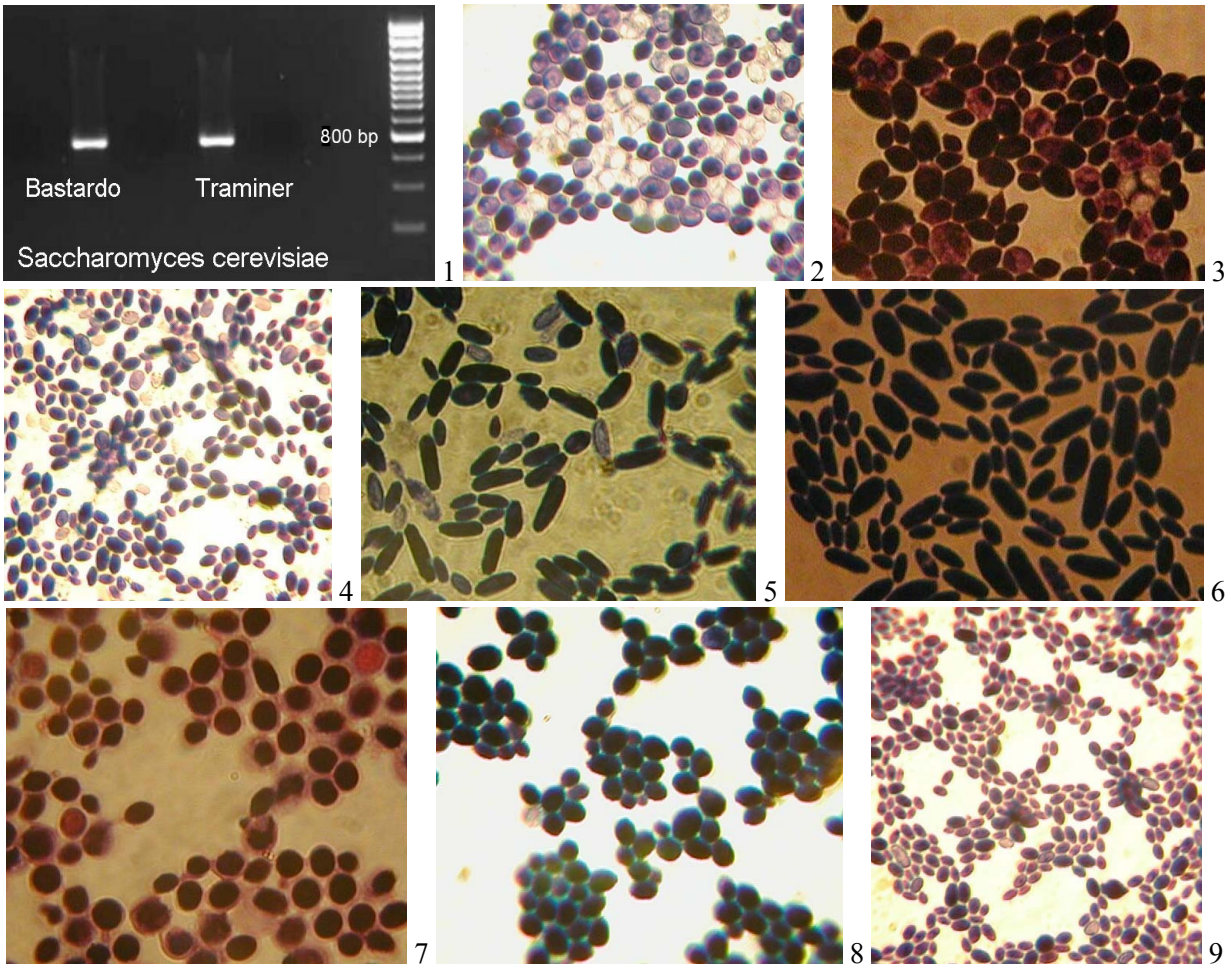

Fig. 1–9. Electrophoregram for identification of yeast cultures using PCR analysis with universal yeast primers (1) and the morphologies of yeast species isolated from different grape cultivars (2–9)

2 – morphology of *Saccharomyces cerevisiae* yeast culture isolated from red grape variety «Bastardo». MAFF-230125; NRRL Y-63641; USRCB Y-3486. Gram stained; Magnification ×720; 3 – morphology of *Dekkera bruxellensis* yeast culture isolated from grapes. MAFF-230063; USRCB Y-3341. Gram stained. Magnification ×720; 4 – morphology of *Pichia barkeri* yeast culture isolated from grapes. MAFF-230064; USRCB Y-3345. Gram stained. Magnification ×720; 5 – morphology of *Pichia sp.* yeast culture isolated from grapes. MAFF-230068; USRCB Y-3355. Gram stained. Magnification ×720; 6 – morphology of *Metschnikowia* aff. *fructicola* yeast culture isolated from grapes. MAFF-230070; USRCB Y-3357. Gram stained. Magnification ×720; 7 – morphology of *Pichia guilliermondii* yeast culture isolated from grapes. MAFF-230071; USRCB Y-3360. Gram stained. Magnification ×720; 8 – morphology of *Candida tropicalis* yeast culture isolated from grape variety «Aligote». MAFF-23073; USRCB Y-3362. Gram stained. Magnification ×720; 9 – morphology of *Pichia kudriavzevii* yeast culture isolated from grapes. MAFF-230067; USRCB Y-3347. Gram stained. Magnification ×720.

The ARS Culture Collection (also known as the NRRL Collection) is one of the largest public collections of micro-organisms in the wold. It currently contains approximately 96,000 strains of actinomycetes, bacteria, moulds and yeasts. The NRRL collection is housed in the Bacterial Food-borne Pathogens and Mycology Research Unit in the National Center for Agricultural Utilization Research in Peoria, Illinois, USA. The mission of the Bacterial Food-borne Pathogens and Mycology Research Unit is to enhance food safety and crop production in the USA and around the world. Researchers in this unit

integrate information from genetics, microbiology, chemistry and plant biology to develop novel approaches to limit mycotoxin contamination, control food-borne diseases and improve crop production.

Genebank of Japan is the central coordinating institute in Japan for conservation of plants, microorganisms, animals and DNA materials related to agriculture. The NIAS Genebank coordinates this activity in collaboration with a network of institutes throughout Japan. The NIAS Genebank actively participates in collaborative activities with other countries in relation to surveys and research related to agricultural related genetic resources. In all activities related to conservation of genetic resources the NIAS Genebank follows the international community norms and national regulations. Plant and microorganism germplasm within the NIAS Genebank system collected in Japan is available for research purposes. The micro-organism collection in Genebank of Japan is known as the MAFF collection.

Saccharomyces cerevisiae yeast cultures participate in the biodegradation of algal biomass, which contains enough carbohydrate for the fermentation process. This is especially the case over the summer period in the littoral waters of the Tiligul estuary. Yeast cultures, therefore, play an important role in the ecological support of littoral waters of the «Tiligulskiy» Regional Landscape Park.

Yeast cultures isolated from littoral soils of the Tiligul estuary degrade red and green macrophyte species, deposited by estuary waves on the shore, well. This aids the recovery of coastal waters from putrescent algal biomass and promotes self-purification of coastal waters in the Tiligul estuary.

The «Koblevo» winery is located on the coast of the Tiligul estuary on the shore of a village called «Leninka». The winery works in a closed cycle, processing grapes from vineyards growing on the coastal areas of the «Tiligulskiy» Regional Landscape Park. The close vicinity of the vineyards, the «Koblevo» winery and the «Tiligulskiy» Regional Landscape Park does not adversely disturb the environmental balance.

Indeed, the presence of the vineyards makes it possible to isolate wild yeast cultures with novel, regional properties. These may be of use to the wine industry; allowing for the production of regional, high-quality wines with specific tastes, aromas and bouquets.

Table 1

Cultures of yeast *Saccharomyces cervisiae* isolated from industrial grape cultivars of Agricultural Company «Koblevo», Nikolaev region of Ukraine, growing in the coastal area of the «Tiligulsky» Landscape Park

Grape Cultivar Name	Taxonomy (identified species)	Numbers deposited in the international microbial collections		
		NRRL	USRCB	MAFF
Aligote	*Saccharmomyces cerevisiae*	-	Y-3479	230118
Bastardo	*S. cerevisiae*	Y-63641	Y-3486	230125
Chardonnay	*S. cerevisiae*	Y-63636	Y-3480	230119
Cabernet Sauvignon	*S. cerevisiae*	Y-63642	Y-3487	230126
Isabella	*S. cerevisiae*	-	Y-3489	230128
Irshai Oliver	*S. cerevisiae*	Y-63643	Y-3488	230127
Merlot	*S. cerevisiae*	Y-63644	Y-3490	230129
Muscat Hamburg	*S. cerevisiae*	Y-63647	Y-3493	230132
Muscat Ottonel	*S. cerevisiae*	Y-63645	Y-3491	230130
Odessa Black	*S. cerevisiae*	Y-63646	Y-3492	230131
Rkatsiteli	*S. cerevisiae*	Y-63638	Y-3482	230121
Rhein Riesling	*S. cerevisiae*	Y-63637	Y-3481	230120
Sauvignon	*S. cerevisiae*	Y-63639	Y-3483	230122
Traminer	*S. cerevisiae*	Y-63640	Y-3484	230123

Note to the table: MAFF - Collection of microorganisms, Genebank of Japan. Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan; NRRL – National Regional Research Laboratory, ARS, Peoria, USA; USRCB – local yeast collection.

Some of these yeasts are subject to plant protection law. In such cases, it is necessary to get permission to isolate them and use them from the applicable plant pathogen governmental authorities. Yeasts that require this sort of permission include: *Candida albicans, Candida glabrata, Candida*

parapsilosis, Candida tropicalis, Metschnikowia fructicola, Pichia barkeri, Pichia guilliermondii, Pichia kudriavzevii, Pichia sp. This regulation applies as these yeasts are all phytopathogenic.

In addition to this list of phytopathogenic yeasts, other yeast species that are not phytopathogenic and have been isolated from these grapes include: *Dekkera bruxellensis*, *Hanseniaspora apiculata*, *Torulopsis bacillaris, Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe*.

The following yeast species were isolated and identified in the Tiligul estuary littoral benthic soils: Candida *albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Komagataea pratensis*, *Rhodotorula rubra*, *Saccharomyces cerevisiae*, and *Williopsis saturnus*.

The following yeast species were isolated and identified in the soils underlying the vineyards: *Hanseniaspora apiculata, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Torulopsis bacillaris,* and *Rhodotorula rubra*. The presence of these species is typical for black-coloured soils containing a high percentage of humus of between 7 and 15 %.

The yeast species most commonly isolated from grape musts, following fermentation, is *Saccharomyces cerevisiae* (wine yeast). The permitted use of *Saccharomyces cerevisiae* strains is of much importance to the food industry. The use of other less beneficial yeast strains, however, are forbidden in the food industry. These include: *Candida tropicalis*, *Candida albicans, Candida glabrata*, and *Candida parapsilosis*, among others.

Table 2

Biodiversity of yeast culture isolated from grape cultivars from around the coastal area of «Tiligulskiy» Landscape Park

Taxonomy (identified species)	MAFF	USRCB
Candida albicans	-	Y-3354
Candida tropicalis	-	Y-3350
Candida tropicalis	-	Y-3351
Metschnikowia aff. *fructicola*	230069	Y-3356
Metschnikowia aff. *fructicola*	230070	Y-3357
Pichia barkeri	230064	Y-3345
Pichia kudriavzevii	230067	Y-3347
Pichia sp.	230080	Y-3377
Pichia guilliermondii	230071	Y-3360
Saccharomyces cerevisiae	230072	Y-3361

The vines growing around the Tiligul estuary in the «Tiligulskiy» Regional Landscape Park are predominantly of French and Georgian varieties. Ukrainian, German and Hungarian varieties, however, also take part in maintaining the ecological balance and yeast biodiversity in «Tiligulskiy». It matters little from which country grape cultivars were introduced. Of most importance is that the grape cultivars thrive and grow over the years, give a good grape harvest, and actively participate in maintaining the ecological balance of the area on which they grow.

Of forty well-known and well-described species of yeast described in the scientific literature, twenty of these actively ferment carbohydrate in grape must.

Yeast cells of the *Saccharomyces* genus are of varying shape, including: round, oval, elongated or filamentary. They undergo vegetative reproduction, and exhibit multi-lateral budding. They can form pseudomycelium. Isogamous or heterogamic conjugation may or may not precede the formation of asci. *Saccharomyces* genus spores are generally rounded or oval, containing between one and four asci. A common feature of yeasts is that they rapidly ferment glucose and, often, other sugars. They are not assimilated nitrates. *Saccharomyces* yeasts are general agents of alcohol fermentation.

Yeast cells of the *Schizosaccharomyces* genus are of rod shape and vary in size from 3 to 4 microns in diameter and 7 to 14 micrometres in length. These cells grow only along their longitudinal axis and split in two. Their shape is, thereby, maintained. Classical wine yeasts are known to ferment malic acid, converting it into ethanol and carbon dioxide, without promoting volatile acidity. This process uses up between 16 and 37 % of the malic acid that had originally been present. In the same conditions, but in the

presence of various *Schizosaccharomyces* species, however, this process uses up between 65 and 80 of the malic acid in the grape must (i.e. the amount of malic acid can be reduced by up to 5 g/L). Of the *Schizosaccharomyces* species, *Schizosaccharomyces pombe* reduces the greatest amount of malic acid. Grape must with a total acidity of 9 g/L may decrease in wine acidity by up to 5 g/L.

The majority of studies cited on yeast interactions with their physico-chemical environment have highlighted interactions between yeast populations in the soils of the Tiligul estuary littoral waters, those in the soils of vineyards around the Tiligul estuary, and those in grape cultivars, as opposed to interactions between individual species or strains in this habitat. These studies have provided insight into the intrinsic abilities of particular species, and were essential in the rapid development of yeast biotechnology. Furthermore, these studies have shown the diversity of yeast species occurring in nature.

CONCLUSIONS

A variety of yeast species were isolated in cultures from benthic soils, vineyard soils, and grape musts following fermentation from the littoral waters of the Tiligul estuary in the «Tiligulskiy» Regional Landscape Park in the Nikolaev region of Ukraine.

The species of yeast isolated and identified in the Tiligul estuary benthic soils included: *Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis, Komagataea pratensis, Rhodotorula rubra, Saccharomyces cerevisiae*, and *Williopsis saturnus*.

The species of yeast isolated and identified in the Tiligul estuary vineyards included: *Hanseniaspora* apiculata*, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Torulopsis bacillaris, Rhodotorula rubra*.

The species of yeast isolated and identified in the Tiligul estuary grapes musts following fermentation included: *Candida albicans, Candida tropicalis, Dekkera bruxellensis, Hanseniaspora apiculata, Metschnikowia fructicola, Pichia barkeri, Pichia guilliermondii, Pichia kudriavzevii, Pichia sp., Saccharomyces cerevisiae, Schizosaccharomyces pombe*, and *Torulopsis bacillaris*.

The taxonomic composition of yeast microbiota was studied in the soils and grapes from the Tiligul estuary coastal lands and in the benthic soils in the Tiligul estuary coastal waters. These studies allowed for the identification of 19 species of yeast. The greatest abundance and taxonomic diversity of yeast was found in the Tiligul estuary vineyards and the soils and grapes around the «Koblevo» winery in harvesting season. This study showed that there existed a wide biodiversity of yeast species across the «Tiligulskiy» Regional Landscape Park territories.

All of the yeast strains isolated were deposited in the aforementioned international collections. These can be used for further wider research, and for research into possible yeast strains that could be utilised in wine biotechnology.

Clearly, the most important task is to preserve the abundant biodiversity of yeast species in nature. This role predominantly lies with the state and regional landscape/national parks.

The author wishes to thank: Vasiliy Cheban, Chief Agronomist of the Agricultural Company «Koblevo», Nikolaev region, for his help with the different varieties of grape samples; Ludmila Gerus, senior researcher the Department of Genetics and Selection, Tairov Research Institute of Viticulture and Oenology, Odessa region, for her information, help and material support during the preparation of this article; Ivan Azhder, Chief Agronomist of the Nursing Company cultivation of vine «Predunabian», Reni, Odessa region, for his information, help and materials; Alexander Baritsky, Chief Agronomist of the Experimental Company of the viticulture and winemaking «Tairovskoe», Odessa region, for his information and help; Maxim Filipenko, Laboratory of Pharmacogenomics, Research Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, R.F, for his help with PCR identification of the isolated yeast cultures; Mr.Chris Bond, Collection Manager of British National Collection of Yeast Cultures (NCYC), Institute of Food Research, for depositing the isolated yeast cultures in the NCYC open collection; Mr.James Swezey, Collection Manager of the American National Regional Research Collection of Microorganisms (NRRL) for depositing isolated cultures in the NRRL open collection; and Mr.Nagai Toshirou, Genetic Resources Center, National Institute of Agrobiological Sciences (NITE), Genebank of Japan, for depositing isolated yeast cultures in Genebank of Japan.

References

1. Bayraktar V. N. Magnetic field effect on yeast *Saccharomyces cerevisiae* activity at grape must fermentation / V. N. Bayraktar // Biotechnologia Acta. – 2013. – Vol. 6, N 1. – P. 125–137.
2. Barjeva I. Yeast Resources in Natural Habitats at Polar Circle Latitude / I. Barjeva, I. Reshetova // Food technol. biotechnol. – 1998. – Vol. 36, N 1. – P. 1–5.
3. Diaz C. Characterization and Dynamic Behavior of Wild Yeast during Spontaneous Wine Fermentation in Steel Tanks and Amphorae / C. Diaz, A. M. Molina, J. Nahring // BioMed Research International. – Vol. 2013. – Article ID 540465.
4. Kurtzman C. P. Molecular taxonomy of the yeast / C. P. Kurtzman // Yeast. – 1994. – Vol. 10, Iss. 13. – P. 1727–1740.
5. Kurtzman C. P. Phylogenetic relationships among species of Pichia, Issatchenkia and Williopsis determined from multigene sequence analysis, and the proposal of Barnettozyma gen. nov., 48 Lindnera gen. nov. and Wickerhamomyces gen. nov. / C. P. Kurtzman, C. J. Robnett, E. Basehoar-Powers // FEMS Yeast Research. – 2008. – N 8. – P. 939–954.
6. Milanovic V. Study of microbial diversity and yeast interactions using molecular methods / V. Milanovic // Dissertation thesises. Polytechnic University delle Marche. The Faculty of Life Science. – 2011. – 228 pp.
7. Motaung T. E. Identification and Potential Biotechnological Application of Yeast Isolates in the UNESCO-MIRCEN Biotechnological Yeast Culture Collection of the University of the Free State / T. E. Motaung // University of the Free-State Bloemfontein. South Africa. – 2011. – 135 pp.
8. Peterson S. W. Phylogenetic relationships among species of the genus Issatchenkia Kudriavzev. Antonie van Leeuwenhoek / S. W. Peterson, C. P. Kurtzman // 1990. –Vol. 58. – P. 235–240.
9. Sharma A. K. Comparison of Commercial and Locally Identified Yeast Strains in Relation to Young Wine Quality of Cabernet Sauvignon / A. K. Sharma, S. D. Savant, P. G. Adsule et al. // S. Afr. J. Enol. Vitic. – 2009 – Vol. 30, N 2. – P. 148–150.
10. Tikka C. Isolation and characterization of ethanol tolerant yeast strains / [C. Tikka, H. P. Osuru, N. Atluri et al.] // Bioinformation. – 2013. – Vol. 9, N 8. – P. 421–425.
11. Yamada, Y. The phylogenetic relationships of hatshaped ascospore-forming, nitrate assimilating Pichia species / Y. Yamada, K. Maeda, K. Mikata // Biotechnology and Agrochemistry. – 1994. –Vol. 58. – P. 1245–1257.
12. Zahavi T. Characterization of the yeast flora on the surface of grape berries in Israel / [T. Zahavi, S. Droby, L. Cohen et al.] // Vitis. – 2002. – Vol. 41, N 4. – P. 203–208.
13. Warnasurya D., Liyanage A. W., Weerawansa G. G. et al. Isolation and characterisation of yeasts of some fruits and fruit products of Sri Lanka / [D. Warnasurya, A. W. Liyanage, G. G. Weerawansa et al.] // J. Nat. Sci. Coun. Sri Lanka. – 1985. – Vol. 13, N 1. – P. 71–75.

Байрактар В. М. Біорізноманиття дріджових культур, виділеніх з прибережної території Тилігульського регіонального ландшафтного парку // Екосистеми, їх оптимізація та охорона. Сімферополь: ТНУ, 2014. Вип. 10. С. 21–29.

Були ідентифіковані дріжджові культури за допомогою полімеразної ланцюгової реакції (ПЛР) з використанням універсальних дріжджових праймерів. Була вивчена морфологія кожної культури дріжджів. Біохімічні властивості кожної культури дріжджів визначали по анаеробнму бродінню вуглеводів (утворення кислоти і газу), утворенню сірководню (H2S), формуванню діоксиду сірки (SO_2). Кожна дріжджова культура була досліджена по наступним вуглеводам: глюкоза, фруктоза, сахароза, мальтоза, лактоза, галактоза, сорбіт, маніт, ксиліт. Встановлено, що з різних сортів винограду виділені переважно віди дріжджів виділених з прибережних ділянок Тилігульського лиману: *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Dekkera bruxellensis, Candida tropicalis.* По завершенню ферментації культури дріжджів були виділені і ідентифіковані методом ПЛР такі види дріжджів як: *Pichia kudriavzevii, Metschnikowia* aff. *fructicola, Pichia guilliermondii, Saccharomyces cerevisiae, Pichia* sp.*, Pichia barkeri, Candida albicans, Candida tropicalis*. Популяція дріжджів була отримана з поверхні ягід винограду в сезон збору врожаю і включало прибережну територію Тилігульського лиману протягом виноградників у сіл: Коблево, Ленінка і до Червоно-Українки. Дослідження показало, що існує широке біорізноманіття видів дріжджів на поверхні зрілого винограду, отриманого в сезон збору врожаю на прибережній території де розташований ландшафтний регіональний парк «Тилігульський». Деякі виділені і ідентифіковані штами дріжджів можуть бути запропоновані для біотехнологічного використання за для виноробної промисловості, це переважно такі види, як *Saccharomyces cerevisiae, Schizosaccharomyces pombe*. які дозволено використовувати в харчовій промисловості. Інші штами можуть бути використані в якості типових культур дріжджів, як еталонні (референс) штами для вивчення чутливості до протигрибкових препаратів, включаючи протигрибкові антибіотики та препарати групи азолів.

Ключові слова: *Saccharomyces cerevisiae, Schizosaccharomyces pombe*, біорізноманіття дріжджів, біотехнологія дріжджів, ландшафтний парк.

Байрактар В. Н. Биоразнообразие дрожжевых культур, выделенных на прибрежной территории Тилигульского регионального ландшафтного парка // Экосистемы, их оптимизация и охрана. Симферополь: ТНУ, 2014. Вып. 10. С. 21–29.

Были идентифицированы дрожжевые культуры с помощью полимеразной цепной реакции (ПЦР) с использованием универсальных дрожжевых праймеров. Была изучена морфология каждой дрожжевой культуры. Биохимические свойства каждой культуры дрожжей определяли в процессе анаэробного брожения к различным углеводам (образование кислоты и газа), образованию сероводорода (H_2S), образование диоксида серы (SO_2). Каждая дрожжевая культура была исследована к следующим углеводам: глюкоза, фруктоза, сахароза, мальтоза, лактоза, галактоза, сорбит, манит, ксилит. Было установлено, что от различных сортов винограда выделены преимущественно

культуры следующих видов дрожжей выделенных из прибрежных участков Тилигульского лимана: *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Dekkera bruxellensis, Candida tropicalis.* По завершении ферментации культуры дрожжей были выделены и идентифицированы методом ПЦР такие виды дрожжей как: *Pichia kudriavzevii, Metschnikowia* aff. *fructicola, Pichia guilliermondii, Saccharomyces cerevisiae, Pichia* sp.*, Pichia barkeri, Candida albicans, Candida tropicalis*. Популяция дрожжей была получена с поверхности ягод винограда в сезон сбора урожая и включало прибрежную территорию Тилигульского лимана на протяжении виноградников у сел: Коблево, Ленинка и до Червоно-Украинки. Исследование показало, что существует широкое биоразнообразие видов дрожжей на поверхности спелого винограда, полученного в сезон сбора урожая на прибрежной территории где расположен ландшафтный региональный парк «Тилигульский». Некоторые выделенные и идентифицированные штаммы дрожжей могут быть предложены для биотехнологического использования в винодельческой промышленности, это преимущественно: *Saccharomyces cerevisiae, Schizosaccharomyces pombe,* которые разрешено использовать в пищевой промышленности. Другие штаммы могут быть использованы как типовые культуры дрожжей, как эталонные (референс) штаммы дрожжей для изучения чувствительности к противогрибковым препаратам, включая противогрибковые антибиотики и препараты группы азолов.

Ключевые слова: *Saccharomyces cerevisiae, Schizosaccharomyces pombe,* биоразнообразие дрожжей, биотехнология дрожжей, ландшафтный парк.

Поступила в редакцию 11.02.2014 г.