Phylogenetic biodiversity of Yeasts

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ABSTRACT

The authors identified yeast strains from the following grape cultivars: Rhein Riesling -*DEN1- (7-2018)*; Cabernet -*ZH-3 (9-2018)* and *L-3 (6-2018)*; Flora -*DEN-5 (10-2018)*. According to the analysis results of nucleotide sequence that codes part of rRNA genes, performed in BRC VKPM NRC "Kurchatov Institute" – State Research Institute of Genetics, it was found that DEN-1 (7-2018) strain belongs to the species *Metschnikowia pulcherrima*; ZH-1 (9-2018) strain is closest to the species *Pichia kluyveri;* L-3 (6-2018) strain is the representative of the species *Metschnikowia pulcherrima* and DEN-5 (10-2018) belongs to the species *Pichia kudriavzevii.* The studies confirm that the grape cultivars – Rhein Riesling, Cabernet, Flora, introduced in the Republic of North Ossetia-Alania, are promising natural resource of systematic yeast diversity.

Introduction

Yeast cells are microbial eukaryotes that belong to ascomycetes, which are a good source of vitamin B and protein (Madigan, 2003). Yeasts are unicellular fungi. As a living organism, fungi require heat, water, protein, nitrogenous substances, and sugar to stay alive (the Artisan, The Yeast Treatise, 2002).

The yeast cell averages about 8 µm in diameter (Ando, 2006). Each cell has a two-layer wall that is porous and semi-permeable to certain substances and thus through the wall nutrients enter the cell and metabolites leave it (Madigan, 2003).

The use of yeasts (*Saccharomyces cerevisiae*) in the production of wine and beer, as well as bread has been known since the Bible (Cocolin, 2001).

Currently, biotechnology plays an important role in the fermentation of various substrates and in the production of ethanol, as well as in the production of biodiesel and many other biofuels (Madigan and Martinko, 2006). Advances in biotechnology have shed the new light on ancient and well-proven technologies, having provided humanity for both a new, clean, sustainable biofuel and bright future (Orelli, 2006).

The yeasts strain – "Baker's Yeasts" is scientifically known as *Saccharomyces cerevisiae*, 5-10 µm-sized in diameter (Halasz, 1988). Saccharomyces cerevisiae is the most famous and popular yeast species, as it is used to make alcohol and bread. It is known for being the most commonly used as an alcoholic beverages fermenter and the most com-

monly used for making bread (Halasz, 1991). The yeast species *Saccharomyces cerevisiae* is also safe for humans as it is not pathogenic and does not negatively affect the environment.

Under certain conditions some yeast fungi can show their negative for human health properties (Nasseri, 2011).

Experimental

The research material was yeast strains: DEN-1 (7-2018), ZH-3 (9-2018), L-3 (6-2018), DEN-5 (10-2018) isolated from berries of Rhein Riesling, Cabernet, and Flora grape cultivars.

The primary identification of the selected strains was performed in the Research Institute of Biotechnology of Gorsky State Agrarian University, using traditional research methods. (Babyeva, 1979).

For the final identification based on the analysis of ribosomal genes sequence, the strains DEN-1 (7-2018), ZH-3 (9-2018), L-3 (6-2018), DEN-5 (10-2018) were sent to the Bioresource Centre – All-Russian Collection of Industrial Microorganisms (BRC VKPM) National Research Centre "Kurchatov Institute" - State Research Institute of Genetics. BRC VKPM NRC "Kurchatov Institute" – State Research Institute of Genetics conducted standard genetic studies.

Results and Discussion

Determining the taxonomic affiliation of L-3 (6-2018) strain
b) Analysis of the gene sequences coding 18S rRNA of the studied strain was performed using BLAST server [http://www.ncbi.nlm.nih.gov/blast].

Results

Primary screening on the GenBank database shows that the studied strain belongs to the following systematic group: *Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Metschnikowiaceae; Metschnikowia.*

Homology of at least 97% is considered to be the criterion for classifying the studied strain to a particular species.

The studied strain of the microorganism can be referred to a number of species. The method to compare nucleotide sequences coding 5,8 S rRNA gene and internal transcribed spacers ITS1 and ITS2 was used to determine taxonomic affiliation and phylo-

genetic affinity of similar species. In the course of the study the following results were obtained:

GATCTCGCTTATTGATATGCGCAGGTT CACCTACGGAMTCCTCCGCTTATTGATATG CGCASGWTCACCKACSRAWYCSKMCGYTTRW KRRTATRYATMGGTYACCCWMS CAAAWY CCTGG GGAAWACCCCGGGGCGCAATGTG CGTTCAAAGATTCAATGATTCACGTCTG CAAG TCATATTACGTATCGCAATTCGCTG CGTTCTTCATCGATGCGAGAACCAAGAG ATCCGTTGTTGAAAGTTTTTKRATTGWGTTA TTGAMRRAAAAGATTYAGWTGTTTTTYCYWA AAG GGGGWAAWA WKATT TTTWAWGAWY CYTCCSCARGGTCMCCYWMSGGAGGKGG

Phylogenetic analysis, performed by using closely related strains, shows that yeast species *Metschnikowia pulcherrima* is the closest to L-3 (6-2018) strain.

Identification of DEN-1 (7-2018) strain

b) Analysis of the gene sequences coding 18S rRNA.

The similarity of the nucleotide sequence of the gene coding 18S rDNA of the studied strain was analyzed using BLAST server [http://www.ncbi.nlm.nih.gov/blast].

Results

Primary screening on the GenBank database shows that the studied DEN-1 (7-2018) strain belongs to the following systematic group: *Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Metschnikowiaceae; Metschnikowia.*

Homology of at least 97% is considered to be the criterion for classifying the studied strain to a particular species. In this regard the studied strain belongs to several species.

The method to compare nucleotide sequences coding 5,8 S rRNA gene and internal transcribed spacers ITS1 and ITS2 was also used to determine phylogenetic affinity of similar species.

When sequencing the DNA section coding 5,8 S rRNA gene and internal transcribed spacers ITS1 and ITS2, the following sequence was obtained:

TCGGTCTCCGCTTATTGATATGCGCAGGTT CACCTACGGAWTCCTCCGCTTATTGATATG CGCAGGTTCACCTACSGAWTCCTMCGYT TAWTRRTATRYATCMGSTYACCCWCS CAAAAYCCTGGGGAATACCCCGGGGCG CATGTGCGTTCAAAGATTCAATGATTCACGT CTGCAAGTCATATTACGTATCGCATTCGCT

GCGTTCTTCATCGATGCGAGAACCAAGAGAT
CCGTTGTTGAAAGTTTTTTWATTGWGTGW
WTGAMRAWAA AKATTTG GAGTTT GTTTCTS
CMARAG WGTGW GAAWW ATTA TTA TGAAT
GATCCYCCCGYA CGCCCA CCGAAG GAA.

Phylogenetic analysis, performed by using closely related strains, shows that yeast species *Metschnikowia pulcherrima* is the closest to DEN-1 (7-2018) strain.

Identification of DEN-5 (10-2018) strain

b) Analysis of the gene sequences coding 18S rRNA.

The similarity of the nucleotide sequence of the gene coding 18S rDNA was analyzed using BLAST server [http://www.ncbi.nlm.nih.gov/blast].

Results

Primary screening on the GenBank database shows that the studied strain belongs to the following systematic group: *Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Pichiaceae; Pichia.*

Homology of at least 97% is considered to be the criterion for classifying the studied strain to a particular species. In this regard the studied strain belongs to several species.

The method to compare nucleotide sequences coding 5,8 S rRNA gene and internal transcribed spacers ITS1 and ITS2 was used to determine phylogenetic affinity of similar species.

When sequencing the DNA section coding 5,8 S rRNA gene and internal transcribed spacers ITS1 and ITS2, the following sequence was obtained:

TCGCATCTCCGCTTATTGATATGCGCAGGTTCA
CCTACGRAMTCCTCCGCTTATYGRTMKGCGC
AGGTTCACCWACRRAWTCCWSCTCKYW
CGGMCAGCTTCGCTCCCTTTCARGSRAGTCG
CAGCTCCAACG CTCTTTACACGTCGTCCGC
TCCGCTCCCCCRACTCTGSGCACGCGCAAGA
TGGAAACGACGCTCRACAGGCTGCCCCC
CGGAATGCCGAGGGGCGCAATGKGCGTT
CARRAACTCGATGATTCACGATGGSTGCRA
TTCACACTAGGTATCGCATTTCGCTRCGCKC
TTCAWCGATGCGAGAACCAAGAGATCCGWT
GTKGAAAGTTTTGTTTGTTTTTCGTARATT
TCTCTTGTCGACWATCTGSTATATTCCACATT
TTAGGTGTTGTTGTTTTCGTTCCGCTCACGCA
GWGTAGKACTAAATCACAGTWAWGATCCTT
CCGCAGGTTCACCTAMAR AAG.

Phylogenetic analysis, performed by using closely related strains, shows that yeast species

Pichia kudriavzevii is the closest to DEN-5 (10-2018) strain.

Identification of ZH-3 (9-2018) strain

(b) Analysis of the gene sequences coding 18S rRNA of the studied strain was performed using BLAST server [http://www.ncbi.nlm.nih.gov/blast].

Results

Primary screening on the GenBank database shows that the studied strain belongs to the following systematic group: *Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Pichiaceae; Pichia.*

Homology of at least 97% is considered to be the criterion for classifying the studied strain to one or another species. The analyzed strain can be referred to several species.

The method to compare nucleotide sequences coding 5,8 S rRNA gene and internal transcribed spacers ITS1 and ITS2 was also used to determine phylogenetic affinity of similar species.

When sequencing the DNA section coding 5,8 S rRNA gene and internal transcribed spacers ITS1 and ITS2, the following sequence was obtained:

CCGTATCCTCCKCTTATTGATRTGCGCAGGTTCA
CCTACGRARTCCTCCGCTTATTGATATRMTKC
ARGTTCACYTMSYSCWTCAYCCTCYTTTCGWA
TAAGGSTAGCCYGTTCTCAACTCTGCTTGCGC
AAGAAGGAACGACGCTSAGACGGCATGCCC
CATGGAATACCATGGGGCGCAATGTGCG
TTSAAGAACTCGATGATTCACGATGGCT
GCCATTCACAMTAGGTATCGCATTTCGCTG
CGCTCTTCATCGATGCGAGAACCAAGAGAT
CCCTTGTTGAAAGTTTTGKTWTGWTAARAC
TTAGTGACTGGTATTATTACGATTTTAGGT
GTTTGATGCGCTCACGCATGTGTATAAATA
TGATCACAGTTTGATCCTTCCGCAGGTTCAC
CTACGAAGAGGAC

Phylogenetic analysis, performed by using closely related strains, shows that yeast species *Pichia kluyveri* is the closest to ZH-3 (9-2018) strain

Conclusion

Figure Labels: Use 8 point Times New Roman for Figure labels. Use words rather than symbols or abbreviations whenwriting Figure axis labels to avoid confusing the reader. As an example, write the quantity "Magnetization", or "Magnetization,

M", not just "M". If including units in the label, present them within parentheses. Do not label axes only with units. In the example, write "Magnetization (A/m)" or "Magnetization {A[m(1)]}", not just "A/m". Do not label axes with a ratio of quantities and units. For example, write "Temperature (K)", not "Temperature/K".

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