ISSN 0026-2617, Microbiology, 2019, Vol. 88, No. 1, pp. 39–45. © Pleiades Publishing, Ltd., 2019.
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EXPERIMENTAL ARTICLES

Phylogenetic Origin of the MAL and IMA α-Glucosidases of the International Genetic Line of *Saccharomyces cerevisiae* S288C

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Received July 18, 2018; Revised August 27, 2018; Accepted October 2, 2018

Abstract—Taking into account the accepted concept of the ancient whole genome duplication (WGD) in the yeast genus *Saccharomyces*, comparative analysis of the multiple α-glucosidases MAL and IMA of the genetic line *Saccharomyces cerevisiae* S288C and α-glucosidases of protoploid yeasts *Kluyveromyces* and *Lachancea*, which have not experienced genome duplication, was carried out. Only certain MAL and IMA isoforms of the latter two genera were shown to be in a close phylogenetic relationship to α-glucosidases MAL12, MAL32, and IMA1-IMA4 of *S. cerevisiae* S288C, while others were closer to the divergent IMA5. These results are consistent with the WGD concept, according to which the yeast *Saccharomyces*, *Kluyveromyces*, and *Lachancea* originated from the common protoploid ancestor and may therefore have common closely related α-glucosidases MAL and IMA. The identity of amino acid sequences of the IMA1-IMA4 isomaltoses of *S. cerevisiae* S288C to those of *L. dasiensis*, *L. fantastica*, *L. fermentati*, *L. lanzarotensis*, *L. meyersii*, *L. quebecensis*, and *L. thermotolerans* was 75–100%, while identity of the MAL maltoses of the same species was 75–99%. Importantly, the MAL and IMA α-glucosidases diverged independently in each genus, species, and even strain.

Keywords: Saccharomyces cerevisiae, the *MAL* and *IMA* genes, genome duplication, isomaltose, maltose, protoploid, phylogeny of α-glucosidases

DOI: 10.1134/S0026261719010065

The *Saccharomyes cerevisiae* genetic line S288C was the first eukaryotic organism for which the complete genome nucleotide sequence was determined (Goffeau et al., 1996), that is presently an international resource (*Saccharomyces* Genome Database, SGD htpp://www.yeast-genome.org). The genes present in the genome of this yeast are accepted as references for detection and investigation of the genes of various eukaryotic organisms.

Genetics of fermentation of α-glucoside maltose, which is important for the production of bread, kvass, beer, and technical and food alcohol, is of great fundamental and applied importance Thus, in eukaryotic microorganisms, the operon-like structure was initially found as maltose polymeric (multiple) *MAL* loci containing one regulatory gene and two structural ones, encoding maltose permease and maltose α-glucosidase (Needleman et al., 1984; Naumov and Yurkevich, 1985).

Biochemical and genetic analysis revealed two types of related α-glucosidases in *S. cerevisiae* (family GH13, international classification CAZy http://www.cazy.org). One type (maltose (EC 3.2.1.20) is responsible for hydrolysis and fermentation of α-1,4-glucosides (maltose and turanose), while the second one (isomaltose/α-methylglucosidase, EC 3.2.1.10) is responsible for hydrolysis and fermentation of α-1,6-glucosides (α-methylglucoside and isomaltose) (Naumov and Naumoff, 2012; Deng et al., 2014). Such substrates as sucrose and paranitrophenyl-α-D-glucopyranoside are common for both enzymes. The international project (Goffeau et al., 1996) on sequencing and annotation of the genome of *S. cerevisiae* S288C genetic line revealed, apart from the known maltose genes *MAL12* and *MAL32*, a new closely related family of isomaltose genes *IMA1–IMA5* (Naumoff and Naumov, 2010; Brown et al., 2010; Teste et al., 2010). Identity between amino acid sequences of the known maltoses MAL12, MAL32, MAL62 and isomaltoses IMA1–IMA4 does not exceed 99 and 92%, respectively, while they share 71% identical amino acid residues between each other. The IMA5 isomaltose has only 60–66% amino acid residues identical to those of both types of α-glucosidases.

The literature data on the evolution of α-glucosidases deposited to international genetic databases are

† Deceased.

incomplete (Naumoff and Naumov, 2010; Naumoff, 2010; Brown et al., 2010; Teste et al., 2010). The goal of the present work was to determine the origin of the IMA and MAL α-glucosidases in *S. cerevisiae* S288C.

MATERIALS AND METHODS

The studied yeast strains and their origin are listed in Table 1. Search for homologous α-glucosidases (maltoses and isomaltoses) in the studied yeast species was carried out in the GenBank (http://www.ncbi.nlm.nih.gov/genbank/) database using BLAST. Complete amino acid sequence of the MAL12 maltose (GenBank accession no. YGR292W) from *S. cerevisiae* S288C (589 amino acid residues) was used as a quary. Partial protein sequences were not used.

Genetic relations between the studied species were established using phylogenetic analysis of the nucleotide sequences of the 26S rDNA D1/D2 domain (600 bp). Multiple alignment of the nucleotide and amino acid sequences was carried out manually using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Phylogenetic trees were constructed using the Neighbor-Joining method implemented in MEGA 6 (Tamura et al., 2013).

RESULTS AND DISCUSSION

Detection of multiple related IMA and MAL α-glucosidases made it possible to investigate their phylogenetics. Total copy numbers for these genes in the genomes of various yeast species were as follows: *S. cerevisiae*, 8; *Lachancea* (*Saccharomyces*) *kluyveri*, 5; *Scheffersemyces* (*Pichia*) *stipitis*, 5; *Lachancea* (*Kluyveromyces*) *thermotolerans,* 4; *Kluyveromyces lactis*, 3; *Debaryomyces hansenii*, 2; *Candida albicans*, 2; and *Schizosaccharomyces pombe*, 1 (Brown et al., 2010; Naumoff, 2010; Teste et al., 2010). Five maltose genes were found in *S. cerevisiae* strains in different numbers and combinations: *MAL12*, *MAL22*, *MAL32*, *MAL42*, and *MAL62* (Needleman et al., 1984). Phylogenetic relations between *L. thermotolerans* and *L. kluyveri* α-glucosidases, which are the most important and the ones closest to those of *S. cerevisiae*, have not been studied. It was shown only that one and two α-glucosidases of *L. thermotolerans* and *L. kluyveri*, respectively, contained the Val216-Gly-Ser tripeptide in their substrate-specific diagnostic site (Teste et al., 2010). The latter is typical of isomaltoses, while maltoses possess Thr-Ala-Gly (Yamamoto et al., 2004; Naumoff and Naumov, 2010). Site-directed mutagenesis confirmed the role of the Val216 residue for the substrate specificity of yeast isomaltose (Yamamoto et al., 2004).

The phylogenetic tree constructed based on amino acid sequences of the MAL and IMA α-glucosidases is shown on Fig. 1. The known maltoses MAL12 (GenBank no. YGR292W) and MAL32 (YBR299W) and isomaltoses IMA1–IMA5 (YGR287C, YOL157C, YIL172C, YJL221C, and YJL216C, respectively) of *S. cerevisiae* S288C, as well as α-glucosidases of their close and distant relatives, were used for comparative analysis. The diagnostic sites Val216-Gly-Ser and Thr-Ala-Gly allowed us to differentiate between isomaltoses and maltoses of all studied yeasts, apart from the divergent maltoses present in *Schi. pombe* (NP_595063.1) and *D. hansenii* (DEHA2E00528p). Phylogenetic analysis of amino acid sequences of α-glucosidases revealed existence of several separated clusters (Fig. 1). maltoses of the distantly related to *S. cerevisiae* yeast genera (*Schi. pombe*, *D. hansenii*, and *Schef. stipitis*) occupied an isolated position on the tree. Importantly, *D. hansenii* possessed two divergent maltoses with 46% identity. The similarity between amino acid sequences of the DEHA2A13882p maltose and *Schef. stipitis* maltoses was 70–72%, while the DEHA2E00528p maltose was more closely related to the enzyme of phylogenetically unrelated yeasts *Schi. pombe*.

The α-glucosidases of *S. cerevisiae*, *K. lactis*, *K. dobzhanskii*, and ten *Lachancea* species formed a separate cluster with 100% reliability. A well-separated cluster (98% bootstrap support) containing two subclusters with high (100%) support was found among the α-glucosidases of these yeasts. The first subcluster comprised the IMA1–IMA4 isomaltoses of *S. cerevisiae* S288C and the isomaltoses of *L. dasiensis*, *L. fantastica*, *L. fermentati*, *L. lanzarotensis*, *L. meyersii*, *L. quebecensis*, and *L. thermotolerans*, which exhibited 75–100% identity. The second subcluster contained the MAL maltoses of these seven species and of *L. mirantina*, which had 75–99% identity. The similarity of these isomaltoses and maltoses was lower, 68–72%.

The heterogeneous group with 55% bootstrap support contained, apart from the MAL maltoses and IMA isomaltoses of *K. lactis*, *K. dobzhanskii*, *L. dasiensis*, *L. kluyveri*, *L. lanzarotensis*, *L. meyersii*, *L. mirantina*, *L. nothofagi*, *L. quebecensis*, and *L. thermotolerans*, also the IMA5 isomaltose of *S. cerevisiae* S288C. In general, identity of the α-glucosidases of this group was significantly lower than in the first cluster (57–88%). The highest similarity was found for *L. kluyveri* isomaltoses (SAKL0A00154p, SAKL0C00176p) and maltoses (SAKL0A05698p, SAKL0A05698p, and SAKL0C02112p): 88 and 73–79%, respectively. Comparable similarity (70–72%) was also observed for the IMA5 isomaltose of *S. cerevisiae* and two *L. kluyveri* isomaltoses. The LADA_0F00320g1_1 isomaltoses of *L. dasiensis*, *L. nothofagi* (LANO_0G18272g1_1), and *L. mirantina* (LAMI_0F07580g1_1) were the most divergent ones, with 73–83% identity of amino acid sequences and 57–64% similarity to other α-glucosidases of this cluster. Importantly, in the Val216-Gly-Ser diagnostic triplet of the first two isomaltoses Ser was replaced by Gly: Val216-Gly-Gly, while in LAMI_0F07580g1_1

Table 1. Origin of the studied yeast strains

Strain	Species	Source of isolation	α-Glucosidases (IMA, MAL)	Protein name or GenBank accession no.
		Saccharomyces		
S288C	*S. cerevisiae*	Genetic line	IMA1	YGR287C
			IMA2	YOL157C
			IMA3	YIL172C
			IMA4	YJL221C
			IMA5	YJL216C
			MAL32	YBR299W
			MAL12	YGR292W
		Kluyveromyces		
NRRL Y-1140	*K. lactis*	Cream, United States	MAL	KLLA0D00231p
			MAL	KLLA0B00242g
CBS 2104	*K. dobzhanskii*	*Drosophila pseudoobscura*, United States	MAL	CDO93416.1
		Lachancea		
CBS 10888	*L. dassiensis*	Leaves of the fern Leaves of the *Angiopterisly godiifolia* fern, Taiwan	IMA	LADA_0G16754g1_1
			IMA	LADA_0F00320g1_1
			MAL	LADA_0G16688g1_1
			MAL	LADA_0H04346g1_1
CBS 6924	*L. fantastica*	Soil, South Africa (Pretoria)	IMA	LAFA_0F00782g1_1
			MAL	LAFA_0E00188g1_1
			MAL	LAFA_0D17568g1_1
CBS 6772	*L. fermentati*	Soft drinks, South Korea	IMA	LAFE_0G01134g1_1
			IMA	LAFE_0A00254g1_1
			MAL	LAFE_0G01090g1_1
CBS 3082	*L. kluyveri*	*Drosophila pinicola,* United States	IMA	SAKL0A00154p
			IMA	SAKL0C00176p
			MAL	SAKL0A05698p
			MAL	SAKL0A05654p
			MAL	SAKL0C02112p
CBS 6340	*L. thermotolerans*	Confectionery, canned food Mirabel, Pastry, Mirabel preserves, Russia	IMA	KLTH0B00308p
			MAL	KLTH0E17006p
			MAL	KLTH0G19470p
			MAL	KLTH0H05324p
CBS 8951	*L. meyersii*	Seawater (mangrovesmangrove swamp), Bahamas	IMA	LAME_0A00232g1_1
			MAL	LAME_0D11342g1_1
			MAL	LAME_0C08394g1_1
CBS 11611	*L. nothofagi*	*Nothofagus* excudate, Argentina	IMA	LANO_0G18272g1_1
			MAL	LANO_0F01354g1_1
CBS 11717	*L. mirantina*	Kashasa (“Brazilian rum”), Brazil	IMA	LAMI_0F07580g1_1
			MAL	LAMI_0B00122g1_1
			MAL	LAMI_0F07646g1_1

Table 1. (Contd.)

Strain	Species	Source of isolation	α-Glucosidases (IMA, MAL)	Protein name or GenBank accession no.
CBS 12615	*L. lanzarotensis*	Grapes, Canaries, Spain	IMA	LALA0_S01e00672g
			MAL	LALA0_S01e19174g
			MAL	LALA0_S13e02894g
CBS 14138	*L. quebecensis*	Maple bark, Canada	IMA	LAQU0S17e02674g1_1
			MAL	LAQU0S06e05864g1_1
			MAL	LAQU0S02e00122g1_1
			MAL	LAQU0S02e11166g1_1
Debaryomyces				
CBS 767	*D. hansenii*	Unknown	MAL	DEHA2A13882p
			MAL	DEHA2E00528p
Scheffersomyces				
CBS 6054	*Schef. stipitis*	Fruit tree, insect larvae, France	IMA	ABN65252.1
			MAL	ABN67312.1
			MAL	ABN67767.1
			MAL	ABN66628.1
			MAL	ABN64883.1
Schizosaccharomyces				
CBS 7264	*Schi. pombe*	Grape juice, Sweden	MAL	NP_595063.1

Designations: CBS, The Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; NRRL, USDA-ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, United States. T indicates type cultures.

Gly was replaced by Ala: Val216-Ala-Gly. It should be noted that all known *L. fantastica* α-glucosidases fell into the first cluster, while the α-glucosidases of *L. kluyveri*, *L. nothofagi*, *K. lactis*, and *K. dobzhanskii* occurred only in the second cluster. The α-glucosidases of the remaining eight studied *Lachancea* species were distributed between two clusters.

The modern classification of ascomycetous yeasts is based on phylogenetic analysis of a number of molecular markers, primarily of the D1/D2 domain of the 26SrRNA gene (~600 bp). The extensive GenBank database of the D1/D2 sequences is widely used for determination of the taxonomic position of new strains. The phylogenetic tree of the nucleotide sequences of the 26S rDNA D1/D2 domains of the studied yeasts is shown on Fig. 2. The cluster comprising *S. cerevisiae* S288C and ten *Lachancea* species, *K. lactis* NRRL Y-1140, and *K. dobzhanskii* CBS 2104 is revealed with 100% support. Their distant relatives, *Schi. pombe*, *D. hansenii*, and *Schef. stipitis*, occupy an isolated position on the phylogenetic tree. Good agreement between topologies of the trees constructed using the α-glucosidase amino acid sequences and the D1/D2 nucleotide sequences (Figs. 1 and 2) should be noted.

Natural interspecies transfer of the *PGU* pectinase genes from *S. cerevisiae* to *S. bayanus*, and from *S. paradoxus* to *S. cerevisiae* was shown previously (Naumov et al., 2016), as well as of the *SUC*, *MAL*, and *RTM* genes and Y'-sequence from *S. cerevisiae* to *S. bayanus* (Naumova et al., 2005, 2011). Transfer of the *PGU* genes between related species *Galactomyces citriaurantii*, *Geotrichum klebahii*, and *Galactomyces candidus* was also observed (Shalamitskiy and Naumov, 2017). The possibility of interspecies gene transfer is probably caused by existence of the common system of mating types within one genus, which allows member-species to hybridize in any combination (Naumov, 2015; Naumov et al., 2009). In the case studied in the present work, this mechanism of transfer of the *MAL* and *IMA* genes between members of different genera should be ruled out. Species of different genera cannot hybridize, and their genomes cannot recombine. Only pheromone intergeneric interaction of yeasts of different genera is known, e.g., between *S. cerevisiae* and *L. kluyveri* (McCullough and Herskowitz, 1979; Naumov and Pishkur, 1999).

Thus, common origin of different yeast genera is the only plausible explanation for occurrence of closely related α-glucosidases in members of these genera. While the concept of complete genome duplication in the course of evolution of some yeast species, including the *Saccharomyces*, species is presently well-founded, while it did not occur in the species of the

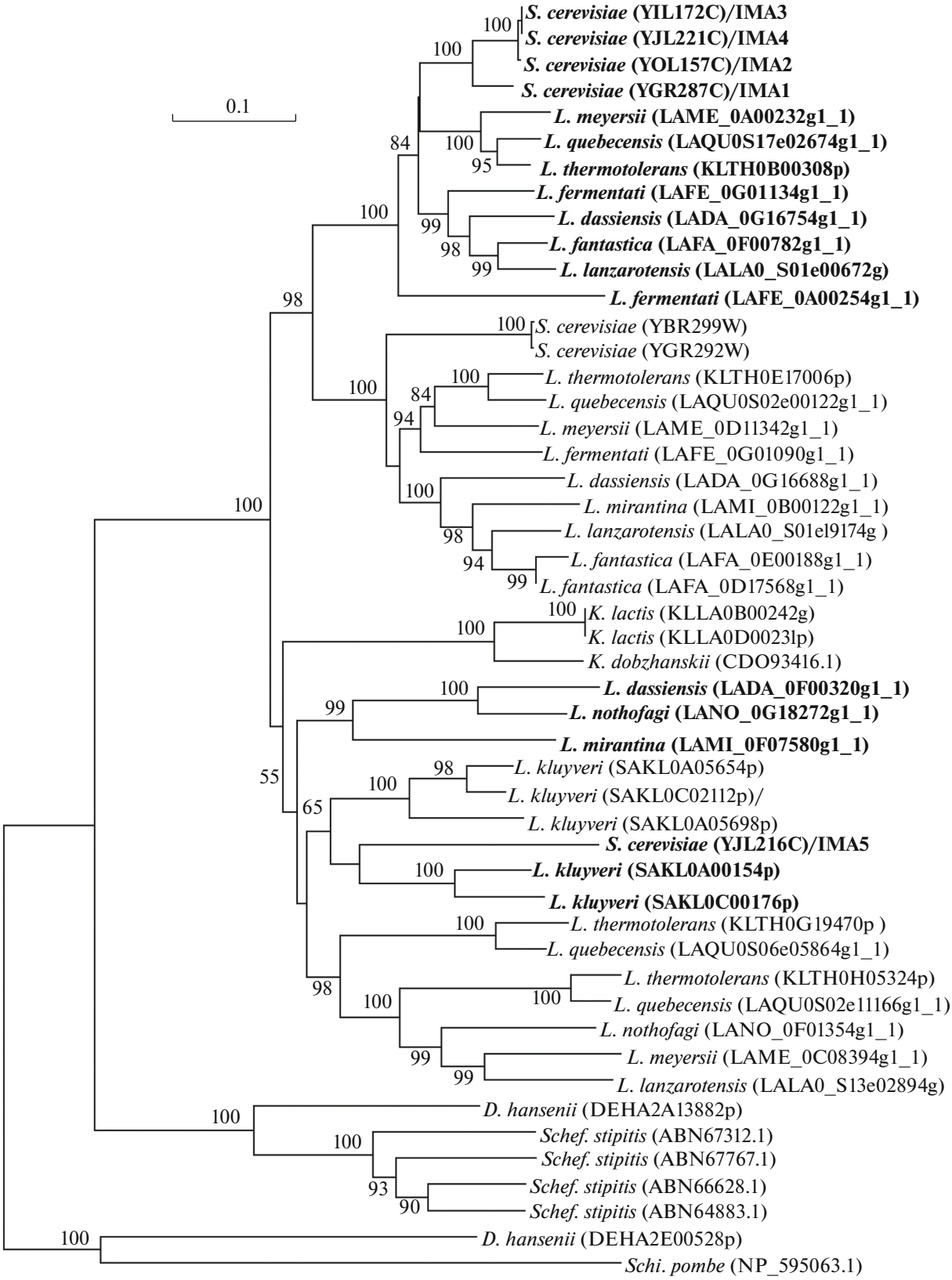

Fig. 1. Phylogenetic tree of α-glucosidases of *Saccharomyces cerevisiae* S288C and related *Lachancea* (*L.*) and *Kluyveromyces* (*K.*) species. The bootstrap values exceeding 50% are shown. The scale corresponds to 100 amino acid substitutions per 1000 residues. The GenBank accession nos. of α-glucosidases are shown in parentheses. Isomaltoses, unlike maltoses, are shown in bold. maltoses of *Debaryomyces hansenii* and *Schizosaccharomyces pombe* were used as an outgroup.

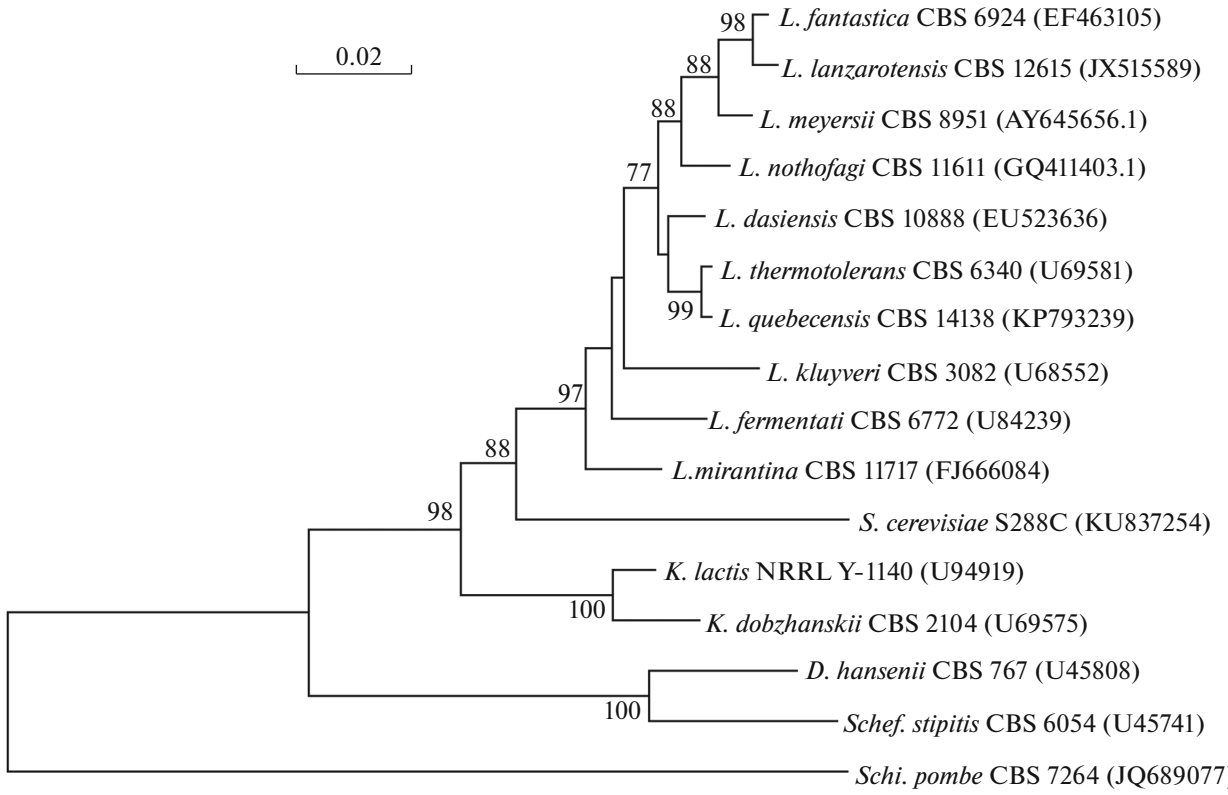

Fig. 2. Phylogenetic tree of the nucleotide sequences of the 26S rDNA D1/D2 domain of *Saccharomyces cerevisiae* S288C and related *Lachancea* (*L.*) and *Kluyveromyces* (*K.*) species. The bootstrap values exceeding 70% are shown. The scale corresponds to 20 substitutions per 1000 nucleotides. The GenBank accession nos. of the D1/D2-sequences are shown in parentheses. *Schizosaccharomyces pombe* was used as an outgroup.

protoploid genera *Lachancea* (viz. *L. kluyveri*, *L. dasiensis*, *L. fantastica*, *L. fermentati* и *L. thermotolerans*) and *Kluyveromyces* (viz. *K. lactis*) (Kellis et al., 2004; Scannell et al., 2007; Souciet et al., 2009; Dujon and Louis, 2017). Complete duplication of the eight ancestral chromosomes in *S. cerevisiae* occurred after their divergence with *Lachancea* (Souciet et al., 2009; Dujon and Louis, 2017). The haploid chromosome number is 16 in *Saccharomyces* and 8 in *Lachancea* species. Our results indicate that isomaltoses and maltoses were formed in the common protoploid ancestor of the genera *Saccharomyces*, *Lachancea*, and *Kluyveromyces*, i.e. prior to their divergence and prior to complete duplication of the *Saccharomyces* genome. Divergence of α-glucosidases with both IMA and MAL activities occurred subsequently within each genus, species, and even strain.

The *S. cerevisiae* isogenic genetic lines created and identified previously (Mortimer and Johnston, 1986; Naumov et al., 1994) still provide research on genetics, genomics, and evolution of the *Saccharomyces* yeasts and act as a basis for investigation of other ascomycetous yeasts.

ACKNOWLEDGMENTS

The authors are grateful to D.G. Naumoff for his help in this work. The work was supported by the Russian Foundation for Basic Research, project no. 17-04-00309.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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Translated by P. Sigalevich