ISSN 0026-2617, Microbiology, 2012, Vol. 81, No. 3, pp. 276–280. © Pleiades Publishing, Ltd., 2012.
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REVIEWS

Molecular Genetic Differentiation of Yeast α-Glucosidases: Maltase and Isomaltase

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Received August 22, 2011

Abstract—The review is dedicated to the molecular genetics of yeast α-glucosidases: the maltase and isomaltase isozymes. Comparative analysis of the genome sequence of the yeast *Saccharomyces cerevisiae* S288C using the isomaltase gene of *Saccharomyces cerevisiae* ATCC56960 revealed a new family of polymeric isomaltase genes *IMA1–IMA5* located in the telomeric regions of chromosomes VII, XV, IX, X, and X, respectively. The isomaltase overexpression and substrate specificity are discussed.

Keywords: *Saccharomyces cerevisiae*, phylogeny of α-glucosidases, maltase, isomaltase, *IMA* genes.

DOI: 10.1134/S0026261712030101

Biochemical and genotypic analyses have shown that *S. cerevisiae* has two types of α-glucosidases. One type (maltase, EC 3.2.1.20) is responsible for hydrolysis and fermentation of α-1,4-glucosides (maltose, turanose); the second type (isomaltase/α-methylglucosidase, EC 3.2.1.10), for hydrolysis and fermentation of α-1,6-glucosides (α-methylglucoside, isomaltose) [1–14]. Both enzymes also use the common substrates sucrose and *p*-nitrophenyl-α-D-glucopyranoside. The α-glucosidase determinants belong to the system of fermentation genes of the corresponding sugars (*MAL* and *MGL* genes). We will dwell on them in greater detail.

GENETIC CONTROL

***MAL* genes.** Maltose fermentation in the yeast *S. cerevisiae* is controlled by at least five polymeric, not-closely-linked telomeric loci: *MAL1–MAL4* and *MAL6* [15–17]. Each locus consists of three closely mapped complementary genes: *GEN1*, the maltose permease gene; *GEN2*, the α-glucosidase (maltase) gene; and *GEN3*, the regulatory *MAL* activator gene. For example, the composition of the two loci *MAL1* and *MAL6* is *MAL11, MAL12, MAL13* and *MAL61, MAL62, MAL63*, respectively. The first and second numbers in these symbols indicate the locus and the gene, respectively. The maltose genes may produce the effect of inter- and intralocus complementations in both the *cis*- and *trans*-positions.

***MGL* genes.** The tetrad analysis based on the material of different genetic lines of *S. cerevisiae* showed that α-methylglucoside fermentation is determined by the following gene pairs: *MGL1 MGL2, MGL3 MGL2, MGL4 MAL1, MGL4 MGL1, MGL3 MAL4*$_c$, *MGL1 MAL1* (where *MAL4*$_c$ is a constitutive mutation in the regulatory gene and locus *MAL1* is represented by three genes: *MAL11 MAL12 MAL13*) [18, 19]. Any one of these pairs suffices for α-methylglucoside fermentation. It was also shown that the mutations leading to constitutive maltase synthesis in the loci *MAL1, MAL2, MAL3*, and *MGL6* also result in α-methylglucosidase formation and α-methylglucoside fermentation [20]. The functions of the genes *MGL* and *MAL* in α-methylglucoside fermentation have not been studied to a sufficient degree. It is considered that the *MGL2* gene is responsible for the transport of α-methylglucoside into the cell [21, 22], whereas the second gene, *MGL1* (or *MGL3*), is a regulatory gene. Prior to sequencing of the *S. cerevisiae* genome the α-methylglucosidase gene(s) remained unknown. The complementation analysis of *S. cerevisiae* strains of various origin showed that the system(s) of *MGL* genes is by far more complex and embraces at least five complementary genes: *MGLa, MGLb, MGLc, MGLd,* and *MGLe* [23–25]. At present, the name isomaltase is used instead of α-methylglucosidase, since α-methylglucoside is a synthetic substrate and isomaltose is a natural compound.

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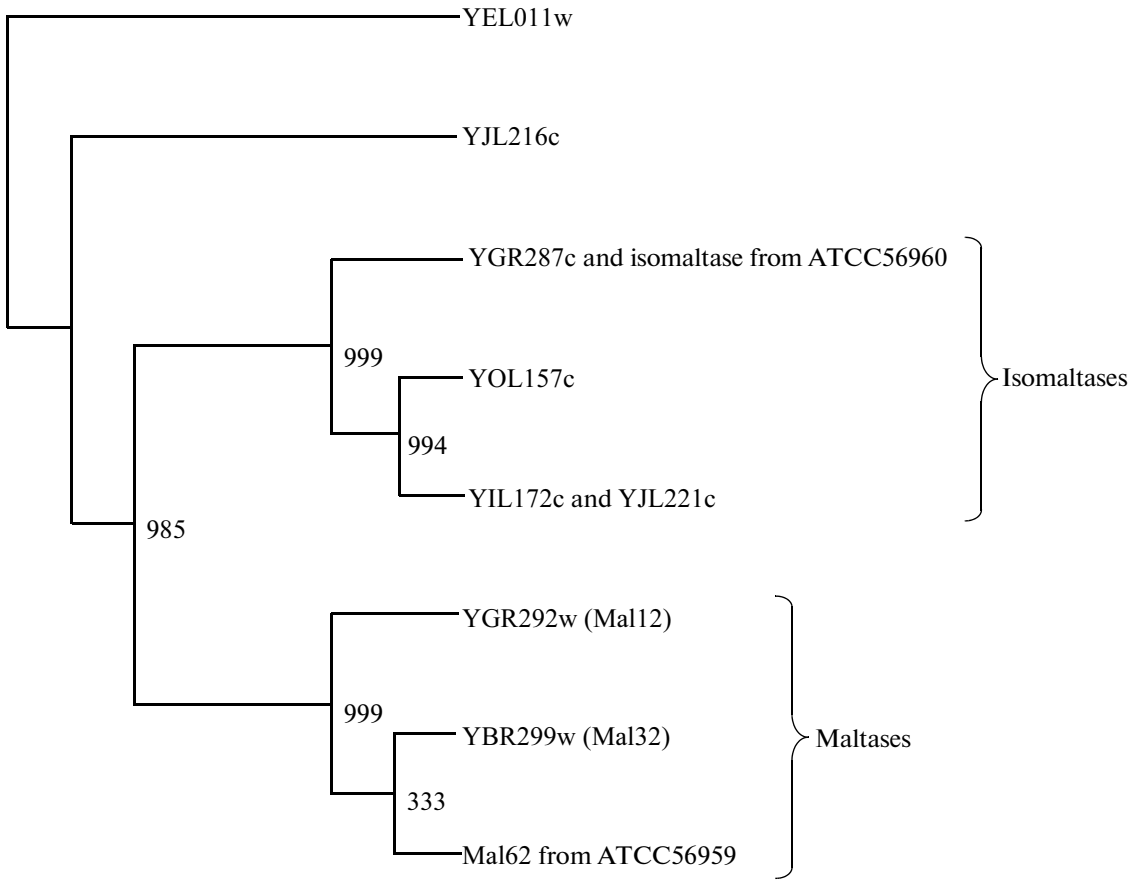

Phylogenetic tree of the GH13 family of α-glucosidases from strain *Saccharomyces cerevisiae* S288C constructed using the maximal parsimony method with the PHYLIP software package (http://evolution.gs.washington.edu/phylip.html). Two proteins from other *S. cerevisiae* strains, maltase from strain ATCC56959 and isomaltase from strain ATCC56960, were also used. The protein YEL011w was chosen as an outgroup. The statistical reliability of the tree nodes was assessed using the bootstrap analysis: the number of verifying pseudoreplicas out of 1000 is indicated next to each node. The clusters corresponding to isomaltases and maltases are marked with curly brackets on the right [28, 29].

PHYLOGENETIC ANALYSIS

The work [26] on cloning the maltase and isomaltase genes from different *S. cerevisiae* strains played a leading role in identification of the isomaltase gene. The international project on sequencing and annotation the genome of the *S. cerevisiae* S288C genetic line [27] offered new possibilities for studying the α-glucosidase genes. Our bioinformatic analysis of the S288C genome [28, 29] revealed a new family of isomaltase *IMA* genes, for the first time. In this strain, we revealed the genes *IMA1–IMA4* and *IMA?/IMA5*, together with the known maltase genes *MAL12* and *MAL32* [17, 30].

GH13 family. Glycoside hydrolases (glycosidases and carbohydrases) are a vast group of enzymes (EC 3.2.1) catalyzing the cleavage of the *O*-glycoside bond. Based on the homology of amino acid sequences, their catalytic domains are grouped in the CAZy international classification [31, 32] into more than one hundred families (GH1–GH130). Sequencing of the genome of *S. cerevisiae* S288C [27] showed that 46 glycosidases belonging to 17 families were encoded in it [31, 33]. The enzymes with α-glucosidase activities are present in two families: GH13 and GH31. The proteins from these two families were also revealed in other yeast species [31, 33].

In the CAZy classification [31], some of the GH13 proteins are combined to form 36 subfamilies (GH13_1–GH13_36). Analysis of some of the unclassified proteins of this family revealed 10 additional subfamilies [33, 34]. Of nine known *S. cerevisiae* S288C proteins containing the GH13 domains, two belong to subfamilies: amylo-(1,4 $\longrightarrow$ 1,6)-transglucosidase (EC 2.4.1.18) of the subfamily GH13_8 (gene *YEL011w*, chromosome V) and amylo-α-1,6-glucosidase (EC 3.2.1.33) of the subfamily GH13_25 (gene *YPR184w*, chromosome XVI). The remaining seven proteins are evolutionarily very close to each other (see below) and therefore may be regarded as the representatives of the independent subfamily GL3C0220 (according to the Génolevures database [35]). Among these proteins are maltases Mal12 (gene

Identification of a new family of isomaltase *IMA1–IMA5* genes [28, 29]

Open reading frame	Chromosome and telomere*	Proposed name of the gene
YGR287c	VII, R	*IMA1*
YOL157c	XV, L	*IMA2*
YIL172c	IX, L	*IMA3*
YJL221c	X, L	*IMA4*
YJL216c	X, L	*IMA?/IMA5*

Notes: * L and R designate the left and right chromosomal telomeres, respectively.

YGR292w, chromosome VII) and Mal32 (gene *YBR299w/YBR2117*, chromosome II), isomaltase encoded by the *YGR287c* gene (chromosome VII), as well as four biochemically uncharacterized proteins encoded by the genes *YIL172c* (chromosome IX), *YJL216c* (chromosome X), *YJL221c* (chromosome X), and *YOL157c* (chromosome XV). α-1,3-Glucosidase (EC 3.2.1.84) belongs to the family GH31; its gene *YBR229c* is located in *S. cerevisiae* S288C chromosome II.

It should be noted that comparative study [26] of the amino acid sequences of the yeast maltase (from strain *S. cerevisiae* ATCC56959) and isomaltase (from strain *S. cerevisiae* ATCC56960) with α-glucosidases from other organisms revealed diagnostically valuable amino acid residues: Val in isomaltases and Thr in maltases, in the conservative site. Site-directed mutagenesis confirmed the significance of this Val residue, as well as of the subsequent Gly and Ser residues, for the substrate specificity of yeast isomaltase. Replacement of these three amino acid residues with Thr, Ala, and Gly (like in natural maltase) made it possible to change the substrate specificity of this enzyme [26].

In order to construct the phylogenetic tree of the *S. cerevisiae* S288C α-glucosidases, we carried out multiple and pairwise comparisons of all the 10 catalytic domains of the families GH13 and GH31 of this organism [28, 29]. For the domains encoded by the genes *YBR229c* (the family GH31) and *YPR184w* (the subfamily GH13_25), we only succeeded in obtaining short local alignments with the remaining eight domains. It is not surprising, because the subfamily GH13_25 is one of most divergent (along with GH13_33), and it was proposed to be regarded as an independent family of glycoside hydrolases [32, 34]. The proteins YIL172c and YJL221c turned out to be identical and were considered by us as one when the sequences were compared. The remaining seven domains were used for multiple alignment and construction of the phylogenetic tree (figure). The maltase Mal62 GH13 domain from strain *S. cerevisiae* ATCC56959 was also added to the analysis. The *YEL011w*-encoded GH13-domain was used as the outgroup. The phylogenetic analysis demonstrated the existence of two clearly isolated α-glucosidase clusters (with more than 99% bootstrap support). One of them includes three maltases; the other, isomaltase and its three close paralogs. In the diagnostic site, all the three maltases contain the tripeptide Thr-Ala-Gly, four proteins from the isomaltase cluster contain Val-Gly-Ser. The level of identity of amino acid sequences within these clusters is not lower than 99 and 92%, respectively, while the proteins of different clusters have about 71% of identical amino acid residues. The data obtained make it possible to consider all the four proteins of the isomaltase cluster as isomaltases and the genes encoding them, as the representatives of one *IMA* family (table). The protein YJL216c shares a mere 60–66% of identical amino acid residues with the representatives of both clusters and is an external group in relation to them in the phylogenetic tree. However, in the diagnostic site, this protein has the Val-Gly-Ser tripeptide, which probably indicates the presence of the isomaltase rather than maltase activity.

It should be noted the existence of two groups of S288C α-glucosidase genes differing in length was first reported in [30]. However, at that time, nothing was known about the differences in the substrate specificity of the enzymes encoded by these two groups of genes. Only the results of the study of isomaltase from strain ATCC56960 [26] made it possible to reveal [28, 29] the new family of the isomaltase genes *IMA1–IMA4* in the genome of *S. cerevisiae* S288C. The nomenclature of the *IMA1–IMA4* genes is adopted in the International *Saccharomyces* Genome Database [36]. After our article [28] was submitted for publication in 2009, two more papers [37, 38], in which the *IMA* genes were revealed, were submitted in 2010. In the study [37], the *IMA* genes were treated as divergent *MAL* genes, whereas in [38], our nomenclature of *IMA1–IMA4* genes was used, with our fifth *IMA*? gene designated as *IMA5* (table).

EXPRESSION OF THE *IMA* GENES

The function of the *IMA* genes was studied thoroughly by Teste et al. [38]. The cloning and expression of the *IMA* genes on the yeast plasmid made it possible to determine the activity of the corresponding enzymes on different substrates: *p*-nitrophenyl-α-D-glucopyranoside, maltose, maltotriose, α-methylglucoside, and isomaltose. The cloned gene of maltase Mal12 with overexpression was used for comparison. The enzymatic activity was determined in unpurified extracts. Overexpression of the *MAL12* confirmed that it encoded α-1,4-glucosidase hydrolyzing maltose and maltotriose, but not α-methylglucoside and isomaltose, whereas the overexpression of *IMA1* and *IMA2* resulted in the synthesis of α-1,6-glucosidase hydrolyzing α-methylglucoside and isomaltose. The product of the *IMA5* gene hydrolyzed isomaltose and maltose, but not α-methylglucoside. As for *IMA3* (100%

identical sequence with the *IMA4* gene), its product had a weak activity but a broad substrate specificity, and hydrolyzed sugars both with α-1,4- and α-1,6-glucoside bonds.

The assimilation tests were performed using the derivatives of strain CEN.PK113-7D, which is capable of growth on the synthetic medium containing 1% isomaltose or 1% α-methylglucoside. Fermentation of these sugars was studied in Durham tubes. Deletion of the *IMA1* gene in this strain resulted in the complete absence of growth on media with isomaltose and α-methylglucoside. Deletions of any other of the four *IMA* genes did not lead to the absence of growth on these substrates as long as the *IMA1* gene was present. In order to verify the functions of the *IMA2, IMA3*, and *IMA5* genes, each of them was overexpressed on the plasmids in mutant *ima1*Δ. The genes *IMA1* and *IMA2* on the plasmid ensured good growth on isomaltose. Growth was worse in the presence of the *IMA3* and *IMA5* genes. The addition of each *IMA* gene, except for *IMA5*, also restored growth on α-methylglucoside. It was found that for expression of the *IMA* genes, the presence of the *AGT1* gene responsible for isomaltose and α-methylglucoside transport into the cell was required [39]. Growth on isomaltose may depend on the maltose activator gene *MAL23p* [38]. The expression of *IMA1* and *IMA5* genes is induced by maltose, isomaltose, and α-methylglucoside.

The differentiation of the α-glucosidase *MAL* and *IMA* genes in the yeast *S. cerevisiae* opens up wide possibilities for studying the evolution of these genes in the species of the genus *Saccharomyces* and in closely related genera. The work in this direction has already been started [33, 37, 38].

ACKNOWLEDGMENTS

We are grateful to E.S. Naumova for her interest in our work. This study was supported by the Russian Foundation for Basic Research, project 09-04-00664.

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