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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

New Family of Pectinase Genes *PGU1b–PGU3b* of the Pectinolytic Yeast *Saccharomyces bayanus* var. *uvarum*

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Abstract—Using yeast genome databases and literature data, we have conducted a phylogenetic analysis of pectinase *PGU* genes from *Saccharomyces* strains assigned to the biological species *S. arboricola*, *S. bayanus* (var. *uvarum*), *S. cariocanus*, *S. cerevisiae*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, and hybrid taxon *S. pastorianus* (syn. *S. carlsbergensis*). Single *PGU* genes were observed in all *Saccharomyces* species, except *S. bayanus*. The superfamily of divergent *PGU* genes has been documented in *S. bayanus* var. *uvarum* for the first time. Chromosomal localization of new *PGU1b*, *PGU2b*, and *PGU3b* genes in the yeast *S. bayanus* var. *uvarum* has been determined by molecular karyotyping and Southern hybridization.

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Pectinolytic activity, which is largely determined by pectinase (endopolygalacturonase, EC 3.2.1.15), is an important trait of the yeast fermenting plant substrates with high-molecular-weight pectin. A single structural gene *PGU1* (=*PLG1*), encoding endopolygalacturonase, has been repeatedly sequenced from the yeast *Saccharomyces cerevisiae* strains of independent origin in different laboratories [1–8].

Using yeast genome databases and published data, we performed a phylogenetic analysis of pectinase genes *PGU* in 112 *Saccharomyces* strains belonging to biological species *S. arboricola*, *S. bayanus* (var. *uvarum*), *S. cariocanus*, *S. cerevisiae*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, and the hybrid taxon *S. pastorianus* (syn. *S. carlsbergensis*). Unlike other species, which have one *PGU* gene, the yeast *S. bayanus* var. *uvarum* was first shown to contain a superfamily of divergent species-specific *PGU* genes. This paper is devoted to the description of this phenomenon.

The main studied strains of *S. bayanus* var. *uvarum* and their origin are given in Table 1. The strains of other species are shown in Fig. 1. Acronyms of yeast culture collections: CBS—Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CLIB—Collection de Levure d'Intérét Biotechnologique, Tiverval-Grignon, France; MCYC—Departamento de Microbiologia, Escuela Tecnica Superior de Ingenieros Agronomos, Universidad Politecnica de Madrid, Spain; DBVPG—Dipartimento di Biologia Vegetale Universita di Perugia, Italy; IFO (=NBRC)—Institute for Fermentation, Osaka, Japan; NBRC (=IFO)—NITE Biological Resource Center, Osaka, Japan; NRRL—Northern Region Research Center, Peoria, United States; KCTC—Korean Collection for Type Cultures, Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, Taydzhon, South Korea. The search for homologues in GenBank (http://www.ncbi.nlm.nih.gov/genbank/), SGD (http://

Origin of the studied strains of the yeast *Saccharomyces bayanus* var. *uvarum*

Strain	Source and isolation locality
MCYC 623	caddis *Mesophylax adopersus*, Spain
17el	Grape juice, Bordeaux, France
TBVIc2.95	Fermenting pulp, Sauternes, France
YIIc2.93	Fermenting pulp, Sauternes, France
TBIIb13.92	Fermenting pulp, Sauternes, France
VS2.94	Fermenting pulp, Sancerre, France
PJS1.94	Fermenting pulp, Sancerre, France
PJS2.95	Fermenting pulp, Sancerre, France
PJP1.95	Fermenting pulp, Pouilly Fume, France
LC1.95	Fermenting pulp, Sancerre, France
DDI4.95	Fermenting pulp, Barsac, France
L 490	Wine, Tours, France

All yeasts were highly homozygous monospore homothallic cultures of museum strains. MCYC 623 = CBS 7001, 17el = CLIB 100, L490 = CBS 8713 = CLIB 110.

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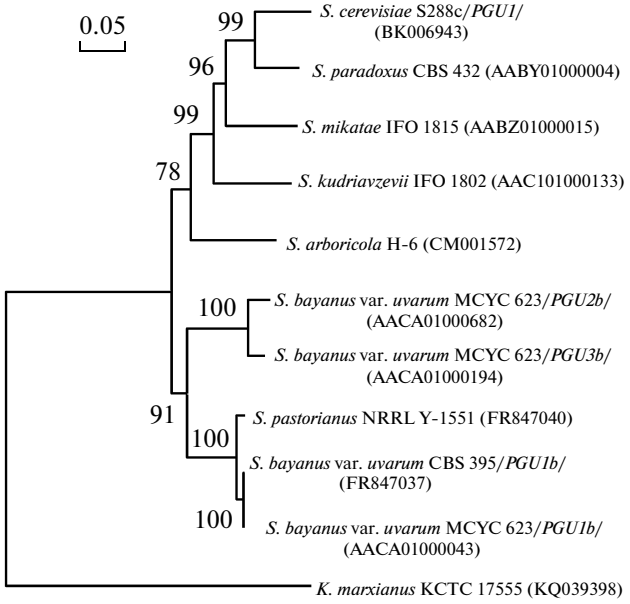

Fig. 1. Phylogenetic analysis of nucleotide sequences of *PGU* genes of *Saccharomyces* yeasts. The gene encoding endopolygalacturonase of the yeast *Kluyveromyces marxianus* was used as an outgroup. Bootstrap values > 70% are shown. The scale corresponds to 50 substitutions per 1000 nucleotide positions. *PGU* genes and accession numbers in GenBank are shown between slants and in parentheses, respectively.

www.yeastgenome.org/), and the Sanger Institute (http://www.sanger.ac.uk) databases with the reading frame (1086 bp) of the known nucleotide sequence of the *PGU1* gene (GenBank: BK006943) of the yeast *S. cerevisiae* S288c was performed using BLAST software. Note that the genome of the last yeast was sequenced and annotated (http://www.yeastgenome.org/). The *PGU* gene (GenBank: KQ039398) of the yeast *Kluyveromyces marxianus* KCTC17555 (CBS 6556) was used as an outgroup. Multiple alignment of the nucleotide sequences of structural genes *PGU* 1086 bp long was performed manually using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The phylogenetic tree was constructed by the neighbor-joining method in MEGA 6 software [9].

The conditions of intact chromosomal DNA preparations were described previously [10]. Electrophoresis of chromosomal DNA was performed in a CHEF-DR III device (Bio-Rad, United States) at 200 V in the following mode: 15 h at a field switching time of 60 s and 8 h at a field switching time of 90 s. 0.5X TBE cooled to 14°C was used as a buffer. Strain YNN 295 (Bio-Rad), the chromosome order and size of which are known, served as a karyotypic standard. After electrophoresis, the gel was stained with ethidium bromide, washed in distilled water, and photographed. Chromosomal DNA was transferred onto a nitrocellulose membrane using a Vacuum blotter (Bio-Rad). DNA was fixed on the membrane by annealing at 80°C for 2 h. A PCR-amplified fragment covering the major part of the coding region of the *PGU1b* gene of the *S. bayanus* strain MCYC 623 was used as a probe. *PGU* genes were amplified using the primers PGU13 (5'-CCACCAAACGCAATGATTT-3') and PGU14 (5'-ATGATGCACCTGAGCCAGAT-3'). PCR was performed in 30 μL of a buffer containing 2.5 mM $MgCl_2$, 0.1 mM of each dNTP, 50 pmol of each primer, 2.5 units of Taq-polymerase (Synthol, Russia), and 20–200 ng of DNA. The conditions of PCR were as follows: initial DNA denaturation at 94°C for 4 min; denaturation at 94°C for 60 s, primer annealing at 55°C for 60 s, and DNA synthesis at 72°C for 120 s (35 cycles); and final extention at 72°C for 10 min. A nonradioactive label was incorporated in accordance with Roche's instructions (Switzerland) using digoxigenin dig-II-dUTP. Hybridization and development of hybridization bands were also performed according to the manufacturer's instructions.

Figure 1 shows the results of phylogenetic analysis of *PGU* genes of only type and reference *Saccharomyces* strains. Complex situation was observed in the cluster containing *PGU* genes of the species *S. bayanus* and the hybrid taxon *S. pastorianus*. In the yeast *S. bayanus* (var. *uvarum*), we identified three divergent genes *PGU1b*, *PGU2b*, and *PGU3b* (according to our classification). A single *PGU1b* gene was identified in the type culture *S. bayanus* var. *uvarum* CBS 395 (GenBank: FR847037) as well as in the strain CLIB 113 (GenBank: FR847038). Using genetic and karyotypic analyses, we identified the last strain as *S. bayanus* var. *uvarum*. Three apparently species-specific divergent genes *PGU1b*, *PGU2b*, and *PGU3b* sharing 86.1–95.7% similarity were found by us in GenBank (accessions AACA01000043, AACA01000682, and AACA01000194, respectively) in the yeast MCYC 623 (Fig. 1). The affiliation of this strain to *S. bayanus* var. *uvarum* was also demonstrated by genetic and karyotypic analyses and DNA–DNA reassociation [10, 11]. The genome of the yeast MCYC 623 was sequenced (GenBank AACA01000015). This strain is a model object of genetic studies [11, 12]. The pectinolytic activity of strains CBS 395, CLIB 113, and MCYC 623, as well as *PGU1b*, *PGU2b*, and *PGU3b* genes remains to be established.

Molecular karyotyping and subsequent Southern hybridization with the *PGU1b* probe allowed preliminary mapping of *PGU1b–PGU3b* genes in chromosomes 1 and 4 and in the chromosome doublet 8/9 (Fig. 2). As expected, the *PGU1b* probe hybridized with *PGU2b* and *PGU3b* genes weaker than with the *PGU1b* gene and did not hybridize with the *PGU1* gene of the yeast *S. cerevisiae* YNN 295 and S288c (Fig. 2b, lanes *1*, *2*). These results clearly demonstrate that the *PGU1b PGU2b PGU3b* genotype is common to all studied strains of the yeast *S. bayanus* var. *uvarum*. The conservation of this genotype and the high pectinolytic activity of the yeast *S. bayanus* var. *uvarum* [13, 14] suggest that *PGU* genes of this yeast have dif-

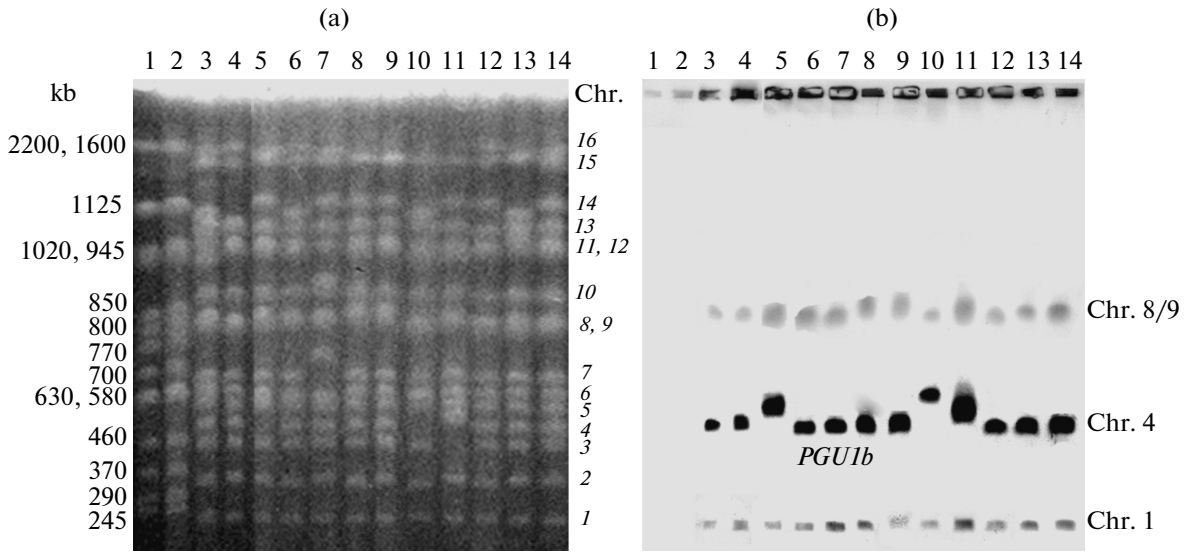

Fig. 2. (a) Pulsed-field gel electrophoresis and (b) Southern hybridization of chromosomal DNA of the yeast *Saccharomyces bayanus* var. *uvarum* with the *PGU1b* probe. Lanes: 3—MCYC 623, 4—17el, 5—TBVIc2.95, 6—YIIc2.93, 7—TBIIb13.92, 8—VS2.94, 9—PJS1.94, 10—PJS2.95, 11—PJP1.95, 12—LC1.95, 13—DDI4.95, 14—L 490. Reference strains of *S. cerevisiae*: 1—YNN 295, 2—S288c. The sizes of chromosomes of the standard strain *S. cerevisiae* YNN 295 are specified. Chromosomes of *S. bayanus* var. *uvarum* (chromosomes 1–16) are enumerated starting from the bottom.

ferent purpose. For example, a number of hydrolases that cleave pectin are known [15]. The activity and specialization of the discovered genes *PGU1b–PGU3b* and their precise chromosomal mapping are to be studied.

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