FEMS Yeast Research 5 (2005) 663–668

FEMS Yeast Research

www.fems-microbiology.org

Endo-polygalacturonase in *Saccharomyces* wine yeasts: effect of carbon source on enzyme production

Florentina Radoi [a,b], Masao Kishida [a,*], Haruhiko Kawasaki [a]

[a] *Department of Applied Biochemistry, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan*

[b] *Department of Microbiology, Faculty of Biotechnology, University of Agricultural Sciences and Veterinary Medicine, Bucharest, 59 Marasti, Sector 1, 71329 Bucharest, Romania*

Received 17 June 2004; received in revised form 4 September 2004; accepted 7 September 2004

First published online 2 November 2004

Abstract

Eight wine yeast strains of *Saccharomyces* sp. were tested for polygalacturonase (PGase) activity, after cultivation on various carbon sources. No strain showed any activity when grown on glucose, while five strains produced PGase in the presence of galactose and polygalacturonate. These data suggest that the PGase of wine strains is repressed by glucose and induced by galactose and polygalacturonate. The existence of the PGase gene in the wine strains and its similarity with that of the laboratory strains was proved by Southern hybridization and PCR amplification. The promoter region of the PGase gene in the wine strains was slightly different from that of the laboratory strains. This possibly explains the different pattern of gene expression in wine and laboratory strains. The PGase of wine strains produced di- or tri-galacturonic acid from polygalacturonic acid, different from the fungal PGase.

Keywords: *Saccharomyces cerevisiae*; Wine yeast; Polygalacturonase; Catabolite repression

1. Introduction

Pectinolytic enzymes are widely used in the beverage industry to clarify fruit juices and wine. Endopolygalacturonase (PGase: EC 3.2.1.15), which degrades the pectic substrate (polygalacturonic acid) by splitting 1,4-α-glycosidic bonds, is a main component of industrial pectinolytic enzymes [1]. This enzyme plays important roles in the food industry, especially in wine making. Most strains of *Saccharomyces cerevisiae*, usually used in wine making, do not show the capacity to degrade pectic substrates. A few wild strains have been reported to have the ability to degrade pectin in wine fermentation [2–4]. Recently, by chemical mutagenesis a mutant producing PGases has been derived from a laboratory strain of *S. cerevisiae* [5], and the PGase has been characterized biochemically [6]. Cloning and sequencing of the PGase gene from the mutant and the parent revealed that both genes were completely identical in the PGase-coding region and 5′-upstream region, whereas the PGase gene was expressed in the mutant but not in the parent [7,8]. On the other hand, the expression of the PGase gene reported in the SCPP strains of *Saccharomyces bayanus* is upregulated by the presence of pectin and surprisingly is enhanced under conditions of filament formation [9]. These reports suggest that the regulation of the PGase gene in *Saccharomyces* is complicated.

It has been expected that pectinolytic wine yeasts can improve liquefaction, clarification and filterability of grape must, releasing more color and flavor

* Corresponding author. Tel.: +81 72 2549455; fax: +81 72 2549921.
E-mail address: masyksd@biochem.osakafu-u.ac.jp (M. Kishida).

1567-1356/$22.00
doi:10.1016/j.femsyr.2004.09.006

compounds entrapped in the grape skins, thereby making a positive contribution to the wine bouquet [10]. Here, we have studied the pectinolytic activity of commercial wine yeasts and the influence of various carbon sources on the PGase production by the yeasts to elucidate the regulatory mechanism of PGase gene expression, and also have constructed mutants from some wine yeasts constitutively producing PGase to improve wine making.

2. Materials and methods

2.1. Media, strains and culture conditions

Yeast strains used are shown in Table 1. Media used were YPD (2% glucose, 1% yeast extract and 1% peptone), and YPGal (2% galactose, 1% yeast extract and 1% peptone). In some experiments, the 2% glucose in YPD was replaced with 1% glucose and 1% galactose in YP(D:Gal), 1% glucose and 1% sodium galacturonate in YP(D:MGA), and 1% glucose and 1% sodium polygalacturonate in YP(D:PGA). Halo-formation plates were prepared by upper-layering 1% sodium polygalacturonate with 1.5% agar on every plate medium. The yeast strains were cultured at 30 °C. The *Escherichia coli* strain used was DH-5α (Toyobo Biochemicals, Osaka, Japan).

2.2. Preparation of enzyme solution

Every medium was inoculated with 10^6 cells ml^{-1} preinoculum of every yeast strain. After cultivation for 72 h at 30 °C the supernatants were dialyzed overnight against 0.01-M sodium-acetate buffer (pH 5.0) to be used as crude enzymes.

Table 1
Yeast strains used

Strain	Property	Source
Saccharomyces cerevisiae		
KW1	Wine yeast	NRIB[a]
KW3	Wine yeast	NRIB
KW4	Wine yeast	NRIB
OC2	Wine yeast	IBRC[b]
L2226	Wine yeast	IBRC
UvaFerm	Wine yeast	IBRC
UvaFerm CEG	Wine yeast	IBRC
S288C (IFO 1136)	Laboratory strain	IFO[c]
DKD-5DH	Laboratory strain	[11]
SMF3	$PGase^+$ mutant from DKD-5DH	[6]
Saccharomycesi bayanus		
EC1118	Wine yeast	IBRC

[a] National Research Institute of Brewing (Higashi Hiroshima, Japan).
[b] Iwate Biotechnology Research Center (Kitakami, Japan).
[c] Institute of Fermentation Osaka (Osaka, Japan).

2.3. Assay of pectinolytic activity

Pectin- and pectate-lyase activities were assayed using methyl-esterified polygalacturonate and polygalacturonic acid (free acid), respectively, as substrates, by measuring the increase of the optical density at 235 nm [12].

Two methods were employed for determining PGase activity. For a rapid assay the yeasts were cultivated on halo-formation plates for 3–4 days at 30 °C. PGase-positive strains were detected by showing a halo on the plate upon application of 6-N HCl.

PGase activity was assayed by measuring the amount of reducing sugar released, using the Somogyi–Nelson method [6], as follows. The reaction mixture was composed of 0.1% sodium polygalacturonate and crude enzyme solution (0.2% volume of the total reaction mixture) in 0.02-M sodium acetate buffer (pH 5.5). The reaction was performed at 37 °C for 60 min. One unit of PGase activity was defined as the activity that liberates reducing groups corresponding to 1 μmol of D-galacturonic acid under the above-mentioned conditions.

2.4. DNA techniques

Extraction of yeast genomic DNA was according to Wach et al. [13]. The polymerase chain reaction (PCR) was performed with a Takara Thermal Cycler (Takara Bio, Kusatsu, Japan) in 35 cycles of 60 s at 94 °C for denaturation, 30 s at 48 °C for annealing, and 90 s at 72 °C for DNA-polymerase reaction. Primers, synthesized from the nucleotide sequences based on the N-terminus, the C-terminus and the 1536-bases upstream from the initiation codon of the *S. cerevisiae* PGase gene, *PSM1*/*PGU1*, were, respectively, as follows: PGSM-N1, CCTAGATCTATGATTTCTGCTAATTCATTA; PGSM-C2, CTGCGGATCCTTAACAGCTTGCACCAGATC; PGSM-P3, ACAAGTCGACTTGTCCTGCC. The PCR products were purified with Purification kit (Amersham Pharmacia Biotech, Buckinghamshire, England) and cloned into pGEM-T Easy Vectors (Promega, Madison, WI, USA). Southern analysis against genomic DNA transferred onto Hybond-N+ membrane (Amersham Pharmacia Biotech) was done using ECL labeling and Detection kit (Amersham Pharmacia Biotech). The *PSM1* gene [8] was used as the probe of Southern analysis. Protocols of kits were according to supplier. Other DNA techniques were done as described by Sambrook et al. [14].

2.5. Thin-layer chromatography

Degradation products from polygalacturonate by PGase from mutants were analyzed by thin-layer chromatography. An *endo*-polygalacturonase of *Aspergillus*