

Saccharomyces cerevisiae Σ 1278b Has Novel Genes of the *N*-Acetyltransferase Gene Superfamily Required for L-Proline Analogue Resistance

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We discovered on the chromosome of *Saccharomyces cerevisiae* Σ 1278b novel genes involved in L-proline analogue L-azetidine-2-carboxylic acid resistance which are not present in the standard laboratory strains. The 5.4 kb-DNA fragment was cloned from the genomic library of the L-azetidine-2-carboxylic acid-resistant mutant derived from a cross between *S. cerevisiae* strains S288C and Σ 1278b. The nucleotide sequence of a 4.5-kb segment exhibited no identity with the sequence in the genome project involving strain S288C. Deletion analysis indicated that one open reading frame encoding a predicted protein of 229 amino acids is indispensable for L-azetidine-2-carboxylic acid resistance. The protein sequence was found to be a member of the *N*-acetyltransferase superfamily. Genomic Southern analysis and gene disruption showed that two copies of the novel gene with one amino acid change at position 85 required for L-azetidine-2-carboxylic acid resistance were present on chromosomes X and XIV of Σ 1278b background strains. When this novel *MPR1* or *MPR2* gene (sigma 1278b gene for L-proline analogue resistance) was introduced into the other *S. cerevisiae* strains, all of the recombinants were resistant to L-azetidine-2-carboxylic acid, indicating that both *MPR1* and *MPR2* are expressed and have a global function in *S. cerevisiae*.

We previously investigated the cryoprotective effect of amino acids on freezing stress in the yeast *Saccharomyces cerevisiae* and found that proline, known as an osmoprotectant (6), has a cryoprotective activity nearly equal to that of glycerol or trehalose (37). In bacteria, it was found that feedback inhibition of glutamate kinase acted as the primary mechanism for the control of proline biosynthesis from glutamate (34). Proline-overproducing mutants of *Escherichia coli* (7), *Salmonella enterica* serovar Typhimurium (5), and *Serratia marcescens* (36) had mutations which resulted in desensitization of the feedback inhibition of glutamate kinase (25) and which did not lead to the production of proline oxidase (5). *S. cerevisiae* synthesizes proline from glutamate via the intermediates γ -glutamyl phosphate, γ -glutamyl semialdehyde, and Δ^1 -pyrroline-5-carboxylate by almost the same pathway as found in bacteria, but the rate-limiting step has not been determined (39). In general, the microorganisms that overproduce various amino acids have been obtained by isolating mutants resistant to analogues of corresponding amino acids (43). We therefore isolated L-proline analogue L-azetidine-2-carboxylic acid (AZC)-resistant mutants derived from an L-proline-nonutilizing strain of *S. cerevisiae* (37). Some of the AZC-resistant mutants were found to accumulate a larger amount of proline and showed a prominent increase in cell viability compared to the parent after freezing in the medium.

Recently, we showed that the strain with a disruption of the *PUT1* gene, which encodes proline oxidase, accumulated higher levels of proline in the cells and conferred higher resistance to water stress conditions relative to wild-type strains (38). Our results indicated that the intracellular proline level and stress

resistance of *S. cerevisiae* are directly correlated and that the increased flux in the metabolic pathway of proline is effective for constructing new freeze-tolerance yeasts. Therefore, it is of great interest to clarify the mechanism of the proline accumulation and the freeze tolerance in the AZC-resistant mutants.

In this work, we isolated the gene involved in AZC resistance from the genomic library of the mutant. We describe the unexpected discovery of an additional DNA fragment with novel genes *MPR1* and *MPR2* (sigma 1278b gene for L-proline analogue resistance) in *S. cerevisiae* Σ 1278b and the partial characterization of the genes, which were present only in strains with the Σ 1278b background.

MATERIALS AND METHODS

Strains and vectors. The *S. cerevisiae* strains used in this study are described in Table 1. Strain MB329-17C was derived from a cross between S288C and Σ 1278b (40). An AZC-resistant mutant strain, FH506, with higher levels of intracellular proline was isolated from strain MB329-17C after ethyl methanesulfonate mutagenesis (37). Strain CKY263 was used to induce expression of the *MPR1* gene under control of the *GAL1* gene promoter. Strain XU-1 is a haploid derived from sake yeast strain K-9 (16). *E. coli* strain JM109 [*recA1* Δ (*lac-proAB*) *endA1* *gvrA96 thi-1 hsdR17 relA1 supE44*(F' *traD36 proAB⁺ lacI^q Z Δ M15*)] was used for subcloning of the *MPR1* gene.

Two *S. cerevisiae*-*E. coli* shuttle vectors, pYES2 (Invitrogen, San Diego, Calif.) and pRS406 (Stratagene, La Jolla, Calif.), both of which contain the bacterial ampicillin resistance gene and the *S. cerevisiae* *URA3* gene, were used for the cloning and for chromosomal integration, respectively, of the *MPR1* gene. Plasmids YEp24 (4) harboring the *URA3* gene and pRS404 (Stratagene) (33) harboring the *TRP1* gene were used for disruptions of the *MPR1* and *MPR2* genes.

Culture media. The media used for growth of *S. cerevisiae* were SD (2% glucose, 0.67% Bacto Yeast Nitrogen Base without amino acids [Difco Laboratories, Detroit, Mich.]) and YPD (2% glucose, 1% Bacto Yeast Extract, 1% Bacto Peptone). SD medium contains ammonium sulfate (0.1%) as the nitrogen source. When appropriate, required supplements were added to the media for auxotrophic strains. Yeast strains were also cultured on SD agar plates containing AZC (Sigma Chemical Co., St. Louis, Mo.). The *E. coli* recombinant strains were grown in Luria-Bertani (LB) medium (31) containing ampicillin (50 μ g/ml). If necessary, 2% agar was added to solidify the medium.

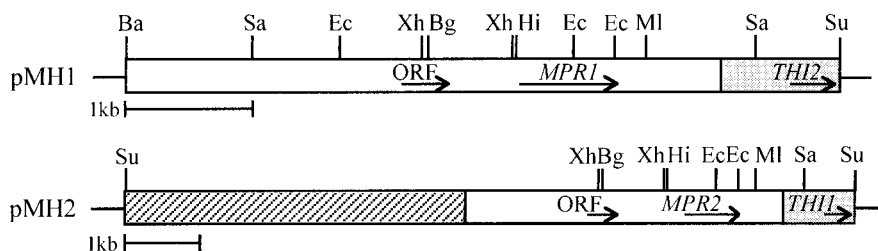
Cloning of the *MPR1* and *MPR2* genes. The enzymes used for DNA manipulations were obtained from Takara Shuzo (Kyoto, Japan). Conventional tech-

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TABLE 1. Yeast strains used in this study

Strain	Genotype	Background and/or source
Σ1278b	α wild-type <i>MPR1 MPR2 AZC^r</i>	M. Brandriss
MB329-17C	α <i>ura3-52 trp1 put1-54 MPR1 MPR2 AZC^r</i>	S288C and Σ1278b, M. Brandriss
FH506	α <i>ura3-52 trp1 put1-54 MPR1 MPR2 AZC^r</i>	MB329-17C, this study
FH506D1	α <i>ura3-52 trp1 put1-54 mpr1::URA3 MPR2 AZC^r</i>	This study
FH506D2	α <i>ura3-52 trp1 put1-54 MPR1 mpr2::URA3 AZC^r</i>	This study
FH506D12	α <i>ura3-52 trp1 put1-54 mpr1::URA3 mpr2::TRP1</i>	This study
S288C	α wild-type <i>mal1 gal2</i>	C. Kaiser
CKY2	a <i>ura3-52 his4-619 gal2</i>	S288C, C. Kaiser
CKY263	a <i>leu2-3,112 ura3-52 GAL</i>	S288C, C. Kaiser
XU-1	a <i>ura3</i>	Japanese sake strain K-9, Y. Kubo

A



B

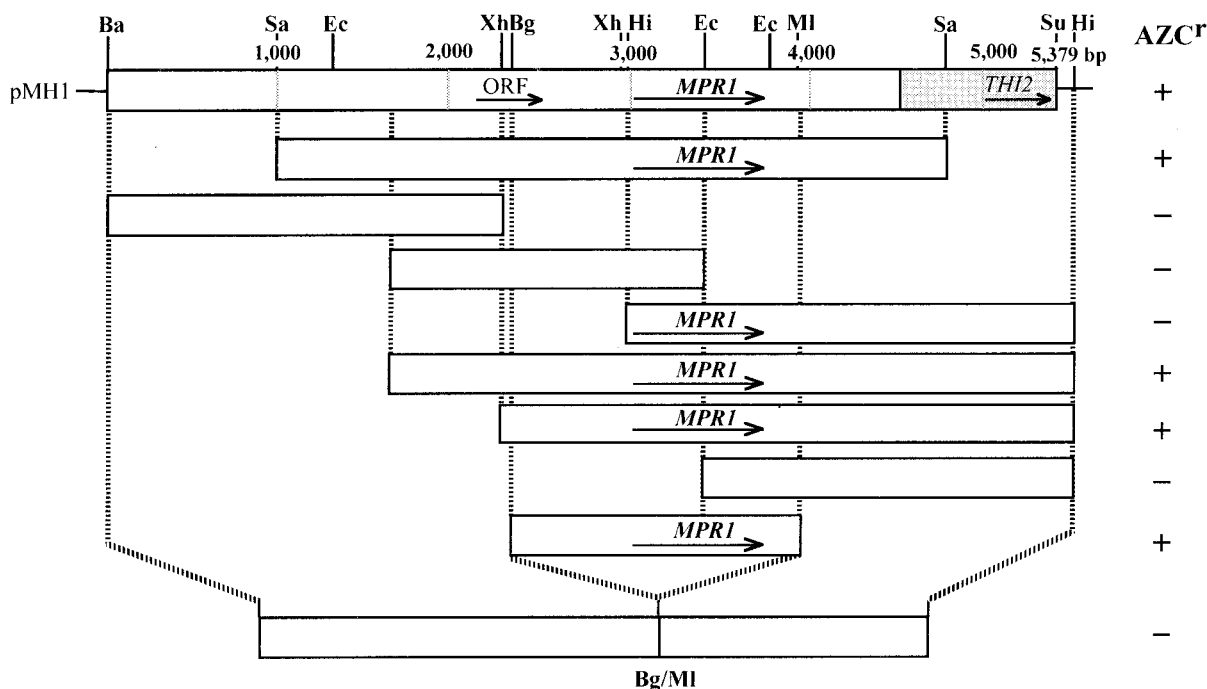


FIG. 1. Restriction map of the cloned DNA fragment and deletion analysis to identify the region required for AZC resistance. The predicted size and transcriptional orientation of each deduced open reading frame (ORF) is shown by an arrow. Restriction enzymes: Ba, *Bam*HI; Bg, *Bgl*II; Ec, *Eco*RI; Hi, *Hind*III; MI, *Mlu*I; Sa, *Sac*I; Su, *Sau*3AI; Xh, *Xho*I. (A) Restriction map of the cloned DNA fragment in pMH1 and pMH2. Two plasmids had the overlapping 5.4-kb *Sau*3AI insert (open box). The region in each plasmid matching the sequence on chromosome XIV (pMH1) or X (pMH2) of *S. cerevisiae* S288C is indicated by a shaded box. The hatched box in pMH2 represents the unknown, partially sequenced 4.6-kb fragment. (B) Analysis of *MPR1* deletion mutants. Each DNA fragment was subcloned into pYES2, and the resultant plasmids were introduced into strain CKY2. AZC resistance of the Ura⁺ transformants was examined on SD agar plates containing AZC (0.3 mg/ml) after incubation at 30°C for 3 days. +, growth; -, no growth.

niques (29) were used for *S. cerevisiae* genomic DNA preparation and transformation. Genomic DNA was prepared from the AZC-resistant mutant FH506 and partially digested with *Sau3AI*. *Sau3AI* fragments larger than 5 kb were ligated into the unique *Bam*HI site of pYES2. The genomic library containing over 10,000 independent *E. coli* clones was transformed into strain MB329-17C, and *Ura*⁺ colonies were replica plated onto SD agar plates containing 3 mg of AZC per ml. Two AZC-resistant colonies were isolated, and the AZC resistance was the plasmid-dependent phenotype. Two plasmids (pMH1 and pMH2) had different but overlapping 5.4-kb inserts based on restriction digestions and DNA sequence analysis. The nucleotide sequence of the cloned DNA fragment was confirmed with a model 377 DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, Calif.) by the dideoxy-chain termination method. Plasmid pMH3 was constructed by cloning the 3.7-kb *Sac*I-*Sac*I fragment from pMH1 into the *Sac*I site of pRS406. The linearized pMH3 cut with *Stu*I in the *URA3* gene of pRS406 was introduced for integration of the *MPR1* gene to the *URA3* locus of the recipient strain. To place the open reading frame of the *MPR1* gene under control of the *GAL1* gene promoter, the 930-bp *Hind*III-*Mlu*I fragment from pMH1 was ligated to the large fragment of pYES2 digested with *Hind*III and *Mlu*I.

Disruptions of the *MPR1* and *MPR2* genes. Plasmid pMPRIU or pMPRIT was constructed by deleting the 1.6-kb *Bgl*III-*Mlu*I fragment containing the *MPR1* gene from plasmid pMH1 and inserting the 1.2-kb *Hind*III fragment containing the *URA3* gene of plasmid YEP24 or the 1.6-kb *Aat*II-*Nae*I fragment containing the *TRP1* gene of plasmid pRS404, respectively, by blunt-end ligation. For *MPR1* or *MPR2* gene disruption, the 3.3-kb *Sac*I-*Sac*I fragment containing *mpr1::URA3* of pMPRIU was integrated into the *MPR1* or *MPR2* locus in strain FH506 by transformation. The *Ura*⁺ phenotype was selected, and the gene disruption was verified either by Southern blotting or PCR. Subsequent disruption of the *MPR2* or *MPR1* gene was performed in a similar manner by using the 3.7-kb *Sac*I-*Sac*I fragment containing *mpr1::TRP1* of pMPRIT. The nucleotide sequences of the *MPR1* and *MPR2* genes were confirmed by analyzing the genomic PCR products of the disruptant. The primers for DNA sequencing were 5'-TTGATATTTAG TGAAGCGCA-3', 5'-TTAGCTGAATCCGAGTTGATAGC-3', 5'-GCTCG AGAAGCTTCGAATGC-3', 5'-GCCAACCTTCTGACCTCTATG-3', and 5'-CGACCGCTCGTTATTCGTTCTT-3'.

Southern blot analysis. Southern blot analysis was carried out using ECL (enhanced chemiluminescence) direct nucleic acid labeling and detection systems (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom). As a DNA probe, the DNA fragments of the *PRO1* gene, which encodes γ -glutamyl kinase, and of *MPR1* were prepared by PCR. For the *PRO1* gene, the primers were designed based on the nucleotide sequence determined by Li and Brandriss (20). The forward primer was 5'-CGGAATTCGGCTCTTCATCGCTAGT-3', and the reverse primer was 5'-CGGGATCCGGTCACTGTGCAAACCT-3'. For the *MPR1* gene, the forward primer was 5'-TAGCTGAATCCGAGTTGATAGC-3', and the reverse primer was 5'-GTGCAATGCATCAACCGGTTCC-3'. Unique amplified bands of 1,161 bp for the *PRO1* gene and 1,636 bp for the *MPR1* gene were purified from agarose gel, and their nucleotide sequences were confirmed. The DNA fragments were then denatured and labeled with horseradish peroxidase according to the protocol recommended by the supplier.

Pulsed-field gel electrophoresis. Stationary-phase cultures were obtained by growing cells at 30°C for 24 h in 10 ml of YPD medium. The harvested cells were resuspended in cold 50 mM EDTA (pH 8.0), and cell concentrations were determined. The agarose-embedded yeast DNA was prepared by using a CHEF Yeast Genomic DNA Plug kit (Bio-Rad, Hercules, Calif.). The plugs were washed in 0.5× TBE buffer (45 mM Tris-borate [pH 8.3], 1 mM EDTA) before loading. The genomic DNAs were separated in 1.0% low-melting-point preparative-grade agarose (Bio-Rad) on a CHEF-DR III apparatus (Bio-Rad). Pulsed-field gel electrophoresis was carried out for 40 h at 14°C in 0.5× TBE buffer with a switching interval of 75 s and a voltage gradient of 6 V/cm.

Computer analysis of DNA and amino acid sequences. Sequence data for the 5.4-kb *Sau3AI* fragment were analyzed by a computer using the program DNASIS (version 3.6; Hitachi Software Engineering, Tokyo, Japan). Based on the DNA sequence, protein homology searches were performed via the World Wide Web by using the BLAST search engine at the National Center for Biotechnology Information (1).

Nucleotide sequence accession number. The nucleotide sequence of the cloned 5.4-kb DNA fragment including the *MPR1* gene found in plasmid pMH1 has been submitted to DDBJ/EMBL/GenBank databases under accession no. AB031349.

RESULTS

Identification of the gene involved in AZC resistance. The genomic DNA library of the AZC-resistant mutant FH506 was constructed in a high-copy-number plasmid pYES2. In two *Ura*⁺ transformants which showed the AZC-resistant phenotype, two plasmids (pMH1 and pMH2) were isolated and had overlapping 5.4-kb inserts (Fig. 1A). It is worth noting that the nucleotide sequence of an approximately 4.5-kb fragment in the cloned DNA exhibited no sequence identity with the ge-

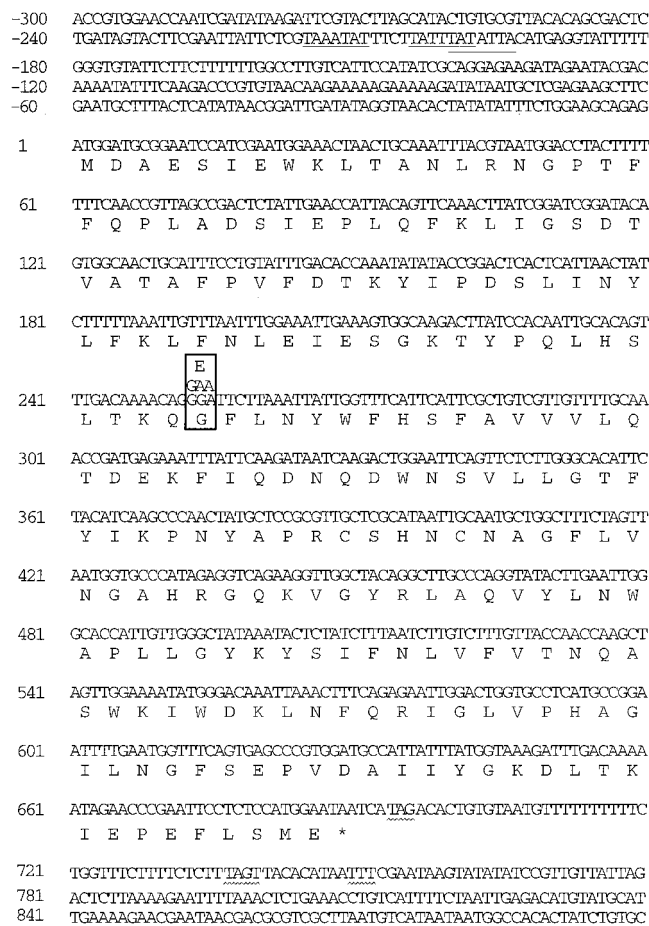


FIG. 2. Nucleotide and predicted amino acid sequences of the *MPR1* gene. +1 and the asterisk refer to the putative translational initiation site and the termination codon, respectively. One base change which leads to Gly and Glu at position 85 in *MPR1* and *MPR2*, respectively, is boxed. Matches to known consensus sequences are marked as follows: TATA box (underline) and transcription termination (wavy underline).

nome sequence of *S. cerevisiae* S288C, while the 0.9-kb fragment from the 3' end completely matched those of chromosomes IV (accession no. SCYDL244W), VI (accession no. YSCCHRVIN), X (accession no. SCYJR156C), and XIV (accession no. SCDNANO). Plasmid pMH2 had an unknown 4.6-kb fragment, which is in part sequenced, in addition to the overlapping 5.4 kb (Fig. 1A). The novel 5.4-kb fragment had a G+C content of 35.9%, which is almost equivalent to that of the total DNA (39.5%) in *S. cerevisiae* S288C. Computer-assisted analysis of the sequenced region confirmed two possible open reading frames encoding a >10-kDa protein besides the *THI2* gene, which encodes the transcriptional activator of thiamine biosynthetic genes (Fig. 1A). Deletion analysis indicated that the essential region for AZC resistance lay within the 1.6-kb *Bgl*III-*Mlu*I fragment containing the open reading frame (Fig. 1B). Thus, we concluded that the region is required for AZC resistance and named it the *MPR1* gene.

Nucleotide sequence analysis of the *MPR1* gene. The sequence of 1,200 nucleotides containing the *MPR1* gene and its 5' and 3' flanking regions is shown in Fig. 2. Sequence analysis revealed one open reading frame from positions +1 to +690, capable of encoding a polypeptide of 229 amino acids with a molecular mass of 26.2 kDa. The first ATG codon is sur-

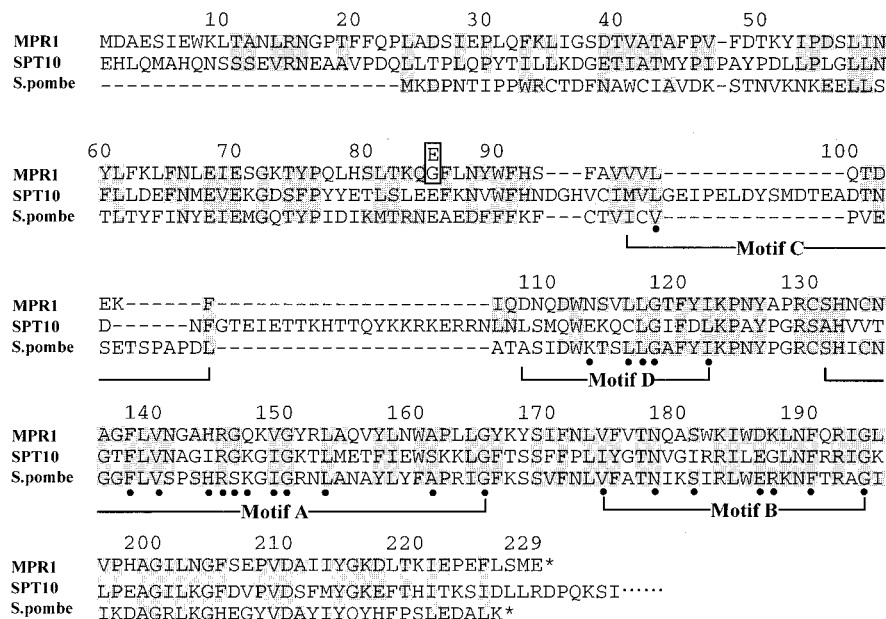


FIG. 3. Amino acid sequence deduced from the nucleotide sequence of the *MPR1* gene (*MPR1*) and its alignment with *S. cerevisiae* Spt10p (SPT10) and the *S. pombe* hypothetical 23.8-kDa protein (*S. pombe*). Numbers above the sequences refer to the *MPR1* gene. An amino acid residue at position 85 in the *MPR1* gene product (Gly for the *MPR1* gene product and Glu for the *MPR2* gene product) is boxed. Amino acids with identity or similarity are shown in shaded boxes. A horizontal line indicates the absence of the corresponding amino acid residue at this position. Filled dots under the sequences indicate the highly conserved positions in consensus motifs (A to D) of the *N*-acetyltransferase superfamily (23). The GenBank accession numbers for *S. cerevisiae* Spt10p and the *S. pombe* hypothetical 23.8-kDa protein are L24435 and Z67999, respectively.

rounded by purines at positions -3 (G) and $+4$ (G), bases that have been proposed to play a role in translation (18). Three sequences with some relationship to the TATA box, the consensus sequence TATA(A/T)A(A/T) (3), were found in the upstream region: TAAATAT at -216 , TATTTAT at -205 , and TATATTA at -201 relative to the start site of the *MPR1* open reading frame. All three were located upstream of the *Hind*III site at -67 . When the open reading frame was placed under control of the galactose-inducible *GAL1* promoter in pYES2, the recombinant strain CKY263 showed the AZC-resistant phenotype on SD agar plates containing 2% galactose instead of glucose as the source of carbon, indicating that the open reading frame would encode a polypeptide involved in AZC resistance (data not shown). In the 3' untranslated sequence, a tripartite terminator, 5'-TAG...TAGT...TTT-3' (44), was found in the region between nucleotides $+694$ and $+752$.

Deduced amino acid sequence of the *MPR1* gene product. By comparison of the amino acid sequence of the predicted *MPR1* protein to entries in the protein databases (SwissProt, PIR, and PRF), the protein sequence was found to be a member of the *N*-acetyltransferase superfamily (24). In particular, the sequence was homologous to the amino-terminal sequence of the *S. cerevisiae* SPT10 (*SUD1*)-encoded protein with 640 amino acids, a negative transcriptional regulator (23, 42). Within the overlapping region of 229 amino acids, 33% of the amino acids were identical, with 50% considered to be similar (Fig. 3). Also, the sequence showed 32% identity to the fission yeast *Schizosaccharomyces pombe* hypothetical 23.8-kDa protein (24) (Fig. 3).

Chromosomal location of the *MPR1* and *MPR2* genes. To confirm the origin of the *MPR1* gene in *Saccharomyces* species, total DNA was isolated from each strain (S288C and Σ 1278b background) and used for genomic Southern hybridization

(Fig. 4A). When the 1,161-bp fragment within the *PRO1* gene was used as a probe, a 5,284-bp fragment containing the entire *PRO1* gene was detected in all strains tested (Fig. 4B). When the *MPR1* gene was used as a probe, three bands corresponding to the 1,702-, 1,635-, and 335-bp fragments were observed only in the Σ 1278b background strains (lanes 1 to 3).

To further elucidate the location of the *MPR1* gene, pulsed-field gel electrophoresis (Fig. 5A) and subsequent Southern analysis using the *MPR1* gene (1.6-kb *Bgl*II-*Mlu*I fragment including the putative coding region and the 5' and 3' noncoding regions) (Fig. 5B) were carried out. The most striking result was that each of the Σ 1278b strains had two copies of the gene, one on chromosome X and the other on chromosome XIV, although the electrophoretic karyotypes varied considerably between the strains tested. Therefore, the two genes at the different locations required for AZC resistance were given different names: *MPR1* for the gene on chromosome XIV and *MPR2* for the gene on chromosome X. Taken together with the DNA sequencing data, these findings suggested that the *MPR1* and *MPR2* genes were located on the left arm of chromosome XIV and the right arm of chromosome X, respectively, approximately 15 kb from the telomere in either case (Fig. 5C). These results indicate that the *MPR1* and *MPR2* genes are present only in strains with Σ 1278b background.

Structural comparison of the *MPR1* and *MPR2* genes. We cloned and sequenced the 1.6-kb *Bgl*II-*Mlu*I fragments containing the *MPR1* and *MPR2* genes from each disruptant. Comparison of the *MPR1* and *MPR2* nucleotide sequences revealed that both sequences matched perfectly except for only one base at position 254, leading to Gly and Glu at position 85 in *MPR1* and *MPR2*, respectively (Fig. 2). Therefore, the novel DNA fragments containing the *MPR1* gene on chromosome XIV and the *MPR2* gene on chromosome X were ascertained to be

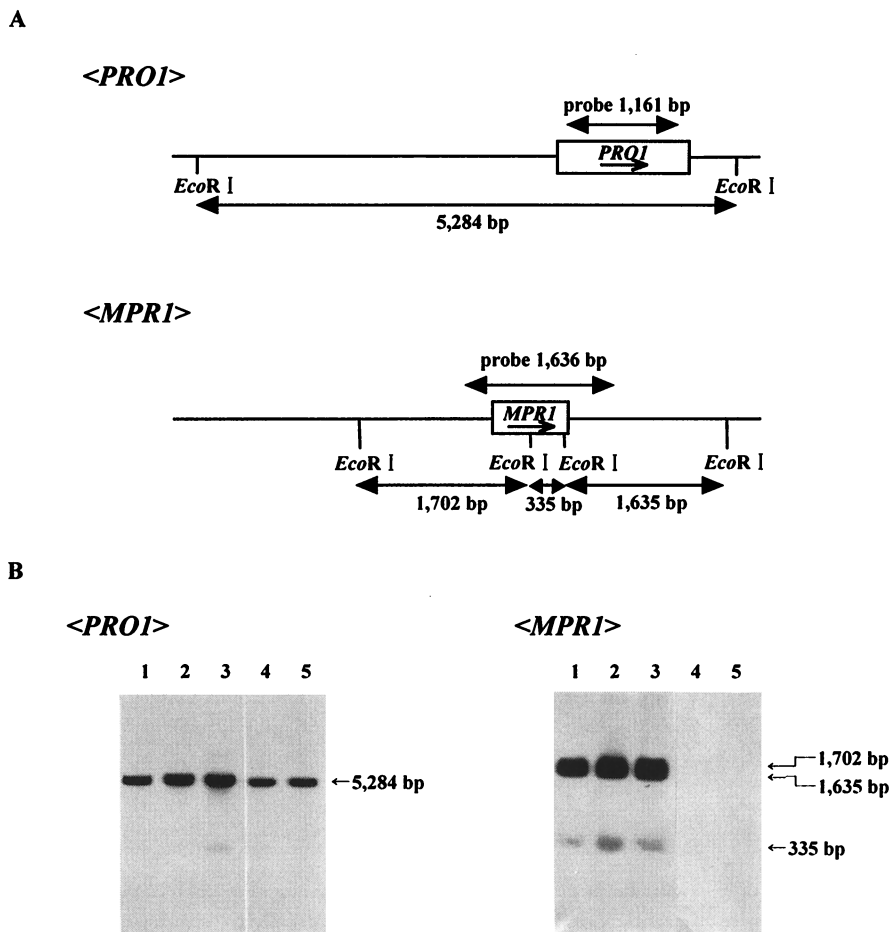


FIG. 4. Southern blot analysis of genomic DNAs from *S. cerevisiae* strains. (A) Construction used to identify the *PRO1* or *MPR1* gene. Locations of the *EcoRI* sites are marked. The *PRO1* and *MPR1* genes are indicated by shaded boxes; arrows show the direction of transcription. (B) Southern hybridization. Five micrograms of yeast genomic DNA from each strain was digested with *EcoRI*, electrophoresed on an 0.8% agarose gel, transferred onto a nylon membrane, and hybridized with a 1,161-bp fragment for the *PRO1* gene (left) and 1,636 bp fragment for the *MPR1* gene (right). Lane 1, Σ 1278b; lane 2, FH506; lane 3, MB329-17C; lane 4, S288C; lane 5, XU-1. An *Eco*T141-*Bgl*III digest of λ DNA was used as the DNA size standard.

cloned into pYES2, resulting in pMH1 and pMH2, respectively (Fig. 1A).

In addition, the 1.6-kb *Bgl*III-*Mlu*I fragments containing the *MPR1* and the *MPR2* genes from each disruptant of the parent strain MB329-17C and the wild-type strain Σ 1278b were sequenced. It should be noted that no mutations in the *MPR1* and *MPR2* genes were found in the three strains FH506, MB329-17C, and Σ 1278b (data not shown).

Expression of the *MPR1* and *MPR2* genes in other *S. cerevisiae* strains. We examined the growth of various *S. cerevisiae* strains on SD agar plates containing AZC. The strains with Σ 1278b background (Σ 1278b, FH506, and MB329-17C) showed greater AZC resistance than the other strains (S288C, CKY2, and XU-1) (Fig. 6A). The sensitivities to heat shock and to osmotic stresses in Σ 1278b background strains were similar to those of S288C strains (data not shown). *MPR1* *MPR2* double disruptants failed to grow on AZC-containing plates, whereas *MPR1* or *MPR2* single disruptants remained AZC resistant (Fig. 6A). These results demonstrate that the *MPR1* or *MPR2* gene is required for resistance of Σ 1278b background strains to L-proline analogues.

The episomal plasmid pMH1 harboring the *MPR1* gene was introduced into the other laboratory or sake yeast strains, and AZC resistance of the *Ura*⁺ transformants was examined. All

of the recombinants were capable of growing on SD agar plates containing AZC, thereby acquiring the AZC-resistant phenotype (Fig. 6B), but did not show increases in proline content and cell viability after freezing to the host cells (data not shown). In the case of integration of the *MPR1* gene to the *URA3* locus of each strain by plasmid pMH3, a similar finding was obtained but the transformants showed AZC resistance slightly lower than that of pMH1 (Fig. 6B). Expression of the *MPR2* gene found in plasmid pMH2 also conferred AZC resistance to the other strains (data not shown). These results indicate that both the *MPR1* and *MPR2* genes present in the Σ 1278b strain are expressed in other *S. cerevisiae* strains, where they play global roles involved in L-proline analogue resistance.

DISCUSSION

In this paper, we describe novel genes involved in L-proline analogue resistance in the chromosome of *S. cerevisiae* Σ 1278b. Previous works have reported only the observation of a point mutation or a deletion of a few bases in comparisons of some genes between Σ 1278b and S288C strains. For example, the *FLO8* gene encodes a nuclear protein required for diploid filamentous growth, haploid invasive growth, and flocculation (17). Strain S288C has a “naturally occurring mutation” con-

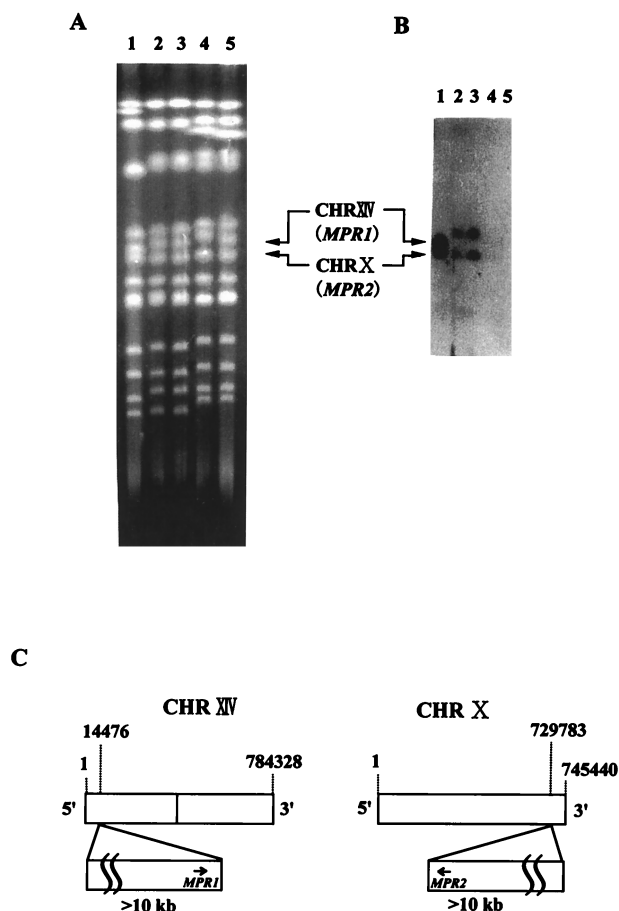


FIG. 5. Chromosomal locations of the *MPR1* and *MPR2* genes in *S. cerevisiae* strains. (A) Pulsed-field gel electrophoresis of genomic DNAs from *S. cerevisiae* strains. Electrophoresis was carried out as described in Materials and Methods, and the gel was stained with ethidium bromide. CHR, chromosome. (B) Southern hybridization. After electrophoresis, each genomic DNA was transferred onto a nylon membrane and hybridized with a 1,636-bp fragment for the *MPR1* gene labeled using the ECL direct nucleic acid labeling system. Lane 1, Σ 1278b; lane 2, FH506; lane 3, MB329-17C; lane 4, S288C; lane 5, XU-1. (C) Schematic map of the *MPR1* gene on chromosome XIV (left) and the *MPR2* gene on chromosome X (right) of strain Σ 1278b. Predicted transcriptional orientation of *MPR1* or *MPR2* is shown by an arrow.

sisting of a single base change and resulting in a stop codon in the coding sequence of the *FLO8* gene; the mutation prevents both pseudohyphal development and haploid invasion (21). *AQY2*, encoding an aquaporin water channel protein, has an 11-bp deletion causing a frameshift in strain S288C (2). Further, the null mutant of the *SEMI* gene is viable but is temperature sensitive in a Σ 1278b background and not in an S288C background (15). Likewise, it is probable that the gene is essential in one background but not in the other. Some genes, especially those dealing with amino acid permeation, might exist only in strain Σ 1278b because the strain seems to have unique genetic features for the specific transport systems of various amino acids (11). For instance, the general amino acid permease is largely inactive when cells are grown with ammonia or glutamate as the nitrogen source, but it is highly active when cells are grown with a poor nitrogen source such as proline or urea (41). To our knowledge, the present study is the first to report the discovery of a novel gene that is present in strain Σ 1278b but not in other laboratory strains (S288C, etc.).

Unfortunately, our isolated clones did not cover the whole

length of the unknown DNA fragment containing the *MPR1* and *MPR2* genes, which was not found in the genome project using strain S288C. The >10-kb fragments are inserted at the far right and left ends of chromosomes X and XIV, respectively (Fig. 5). In this study, only a junction at one end was determined, and accordingly we are now extending the sequence so that it will rejoin the complete genome sequence. In standard laboratory strains, chromosome length polymorphism is thought mainly to originate from movement of Ty elements in and out of chromosomes and from Ty-associated duplications or deletions (12). Moreover, the chromosome size variation observed in yeast strains suggests that more drastic chromosomal rearrangements might also occur. It is known that repeated sequences such as Ty elements or solo long terminal repeats are able to promote chromosomal translocations by ectopic recombination (26). If Ty-mediated rearrangements were responsible, one would consider that the novel fragment might reside at the ends of chromosomes, but no repeated sequence involved in Ty elements was found at the end of cloned DNA fragments, suggesting that these DNA fragments are in subtelomeric locations. Much more research on genome evolution is needed to elucidate the local genomic structure and origin of the gene.

When nucleotide sequences were compared, one amino acid change at position 85 was found between the *MPR1* and *MPR2* genes. This change might be regarded as significant to function, because Glu85 is conserved in Spt10p and the *S. pombe* hypothetical 23.8-kDa protein. The notable finding is that no mutation occurred in both *MPR1* and *MPR2* of strain FH506 compared to the parent strain MB329-17C and the wild-type strain Σ 1278b, indicating that the *MPR1* and *MPR2* genes in the AZC-resistant mutant FH506 were both wild type, not mutant. The question arises as to why strain FH506 showed AZC resistance higher than that of strain MB329-17C. The higher AZC resistance of FH506 was dominant to the parent strain and the characteristic segregated 2:2 in tetrads, suggesting that the phenotype is due to a single nuclear mutation (data not shown). Expression of the *MPR1* and *MPR2* genes isolated from strain FH506 conferred AZC resistance to other *S. cerevisiae* strains but did not cause increases in proline content and cell viability after freezing of the host cells (data not shown). As we reported previously (37), strain FH506 showed a prominent increase in cellular proline content and cell viability after freezing in the medium compared to MB329-17C. Therefore, strain FH506 seems to have a mutation in the gene involved in proline biosynthesis or degradation, leading it to accumulate intracellular proline and to be more AZC resistant than strain MB329-17C, although the possibility that the mutation occurred in the cloned DNA fragment outside the 1.6-kb *Bgl*II-*Mlu*I fragment containing the *MPR1* or *MPR2* gene cannot be ruled out. The search for a mutated gene on FH506 is in progress.

At present, the detailed function of *MPR1* or *MPR2* is not clear yet. AZC, an L-proline analogue, is known to be taken up into cells and incorporated into proteins instead of proline (22) and to induce the synthesis of abnormal proteins, although probably without a prominent and immediate blockade of protein synthesis. Proline is transported into cells via two transporters, the general amino acid permease (encoded by *GAP1*) (11) and the proline-specific permease (encoded by *PUT4*) (19), which respond at the transcriptional and posttranscriptional levels to nitrogen repression (35). Recent reports have shown that the *SEC13* gene, encoding an essential component for the secretory pathway, is responsible for targeting certain amino acid permeases to the plasma membrane, and the *sec13-1* mutant was more resistant to toxic amino acid ana-

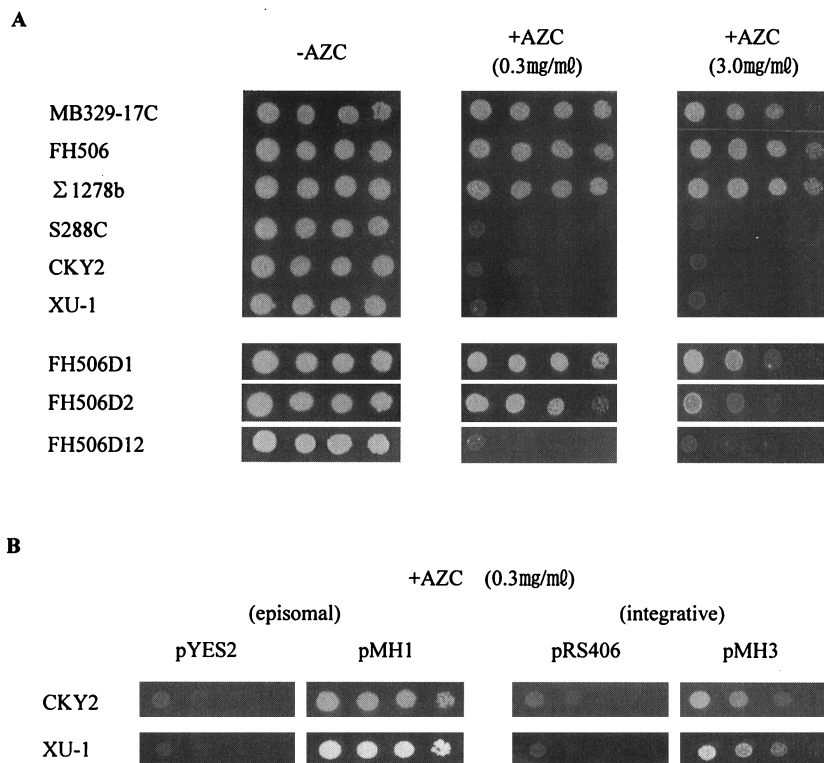


FIG. 6. Growth phenotype of *S. cerevisiae* strains on minimal medium containing AZC. Approximately 10^6 cells of each strain and serial dilutions of 10^{-1} to 10^{-3} (from left to right) were spotted onto SD plates with appropriate amino acids in the absence (-AZC) and presence (+AZC) of AZC. Plates were incubated at 30°C for 3 days. (A) Function of the *MPR1* and *MPR2* genes in $\Sigma 1278b$ background strains. The *MPR1* and *MPR2* disruptants derived from strain FH506 are represented by FH506D1 and FH506D2, respectively. The *MPR1 MPR2* double disruptant is represented by FH506D12. (B) Function of the *MPR1* gene in the other *S. cerevisiae* strains. Plasmids pMH1 and pMH3 were constructed from vectors pYES2 and pRS406, respectively.

logues such as AZC and 4-aza-DL-leucine (27). The mutant defects on amino acid uptake were specific for *GAP1* and *PUT4* gene products, and these permeases were unable to be exported to the cell surface (28).

Nucleotide sequencing of the *MPR1* and *MPR2* genes revealed that the putative protein sequence belongs to the *N*-acetyltransferase superfamily. Neuwald and Landsman (24) reported that GCN5-related histone acetyltransferases belong to a far more extensive superfamily of both known and putative *N*-acetyltransferases and that this superfamily is characterized by four conserved regions spanning over 100 residues. Several other members of this superfamily are also associated with gene regulation. These include the *S. cerevisiae SPT10*-encoded protein and two bacterial proteins, PaiA, which negatively controls sporulation and degradative enzymes in *Bacillus subtilis* (14), and FlaG, which regulates synthesis and assembly of flagellin proteins in *Caulobacter crescentus* (32). Spt10p, which has not been shown to contain histone acetyltransferase activity, is required for transcription of particular histone genes (8) and influences the transcription of a variety of other unlinked genes, including *PUT1* and *PUT2* (23, 42). We confirmed that the *SPT10* gene is present in strain $\Sigma 1278b$ (data not shown), although the nucleotide sequence and the transcript have not yet been analyzed. Because we assume that the *MPR1* or *MPR2* gene functions as an Spt10p-like transcriptional regulator, we will identify the target gene(s) by using the AZC-sensitive mutant derived from the AZC-resistant S288C recombinant carrying the *MPR1* gene on pYES2. Preliminary experiments revealed that *GDH1* (encoding NADP-specific glutamate dehydrogenase) and *RSP5* (encoding ubiquitin-protein ligase)

might be the candidates (data not shown). Both gene products are known to repress *GAP1* and *PUT4* or inactivate Gap1p and Put4p in the presence of ammonia (11, 13). We are now investigating the *MPR1* and *MPR2* function based on the hypothesis that both genes repress or inactivate the *GAP1* and *PUT4* genes by regulating expression of the *GDH1* and *RSP5* genes, leading to the L-proline analogue resistance phenotype. Also, the amino acid sequence of the predicted *MPR1* and *MPR2* gene products is homologous with the *S. pombe* hypothetical 23.8-kDa protein. It should be of interest to investigate the *S. pombe* gene at both transcriptional and translational levels.

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