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# Transcriptional Activation of *FLR1* Gene during *Saccharomyces cerevisiae* Adaptation to Growth with Benomyl: Role of Yap1p and Pdr3p

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**The adaptation of *Saccharomyces cerevisiae* to growth in the presence of the antimitotic fungicide benomyl involves the dramatic activation of *FLR1* transcription, taking place during benomyl-induced latency following sudden exposure to the fungicide. *FLR1* gene encodes a plasma membrane transporter of the major facilitator superfamily (MFS) conferring resistance to multiple drugs, in particular to benomyl. *FLR1* activation is completely abolished in a mutant devoided of *YAP1* gene being exerted by Yap1p either directly or via Pdr3p. *YAP1* gene was proved to be a determinant of benomyl resistance; the duration of the adaptation period preceding cell division under benomyl stress was longer for the Δ*yap1* population, presumably due to the abolishment of *FLR1* activation during latency. Although benomyl resistance mediated by Yap1p is reduced in a *FLR1* deletion mutant, results also indicate that Yap1p may have other target genes that confer benomyl resistance in yeast.** © 2001 Academic Press

***Key Words: Saccharomyces cerevisiae;* benomyl; *YAP1* gene; *FLR1* gene; MFS-MDR transporters; *PDR3* gene.**

Following widespread use of the systemic benzimidazole fungicide benomyl in agriculture and horticulture, benomyl resistant strains of many fungal pathogens have emerged, reducing the usefulness of this fungicide to provide crop protection against a wide range of diseases (1). *FLR1* gene (ORF YBR008c), encoding a member of the multidrug-resistance (MDR) 12-spanner family 1 of the major facilitator superfamily (MFS) (2, 3), confers resistance to this antimitotic fungicide in the non-pathogenic model system *Saccharomyces cerevisiae.* Flr1 is a plasma membrane protein [4 and our group (N. Brôco and I. Sá-Correia), unpublished results], presumably a multidrug efflux pump energized by the transmembrane proton gradient. This transporter is a multidrug determinant, also required for yeast resistance to fluconazole, 4-nitroquinoline *N*-oxide (4-NQO), cycloheximide, methotrexate, cerulenin and diazaborine (4–7).

The physiological adaptation of yeast cells, grown in the absence of any exogenous stress factor, during the period of latency induced by the presence of a specific growth inhibitor, is critical to their eventual recovery and entrance into exponential stressed growth. Nevertheless there is a lack of information concerning the yeast responses that may occur during this period of adaptation. In the present work, we have examined the time-dependent alteration of *FLR1*-mRNA levels during the period of adaptation to growth with benomyl, following the sudden exposure of unadapted yeast cells to this fungicide. Results confirmed previous indications, obtained by using a *FLR1-lacZ* fusion, suggesting that the adaptation of yeast cells to growth in the presence of benomyl involves the dramatic activation of *FLR1* expression. They further indicate that *FLR1* regulation occurs at the transcriptional level. We also show results indicating that the Yap1p is essential to *FLR1* transcriptional activation induced by benomyl. This same observation was just published by another laboratory (8). The transcriptional activator Yap1p belongs to the basic leucine zipper (bZIP) family and is involved in the oxidative stress response in yeast through the transcriptional activation of a number of stress genes under oxidative stress conditions (9–11). The role of Yap1p on *FLR1* regulation is consistent with the presence of three Yap1p-response elements in *FLR1* promoter and with previous indications revealing that the abundance of *FLR1*-mRNA increases in *S.*

Abbreviations used: MDR, multiple-drug-resistance; MFS, major facilitator superfamily; SFH, short flanking homology; PCR, polymerase chain reaction; LFH, long flanking homology; 4-NQO, 4-nitroquinoline N-oxide; ORF, open reading frame.

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**TABLE 1**

*Saccharomyces cerevisiae* Strains and Plasmids Used in This Study

| Name | Genotype/description | Reference |
|---|---|---|
| Strains | | |
| W303-ISC02b | *MAT*a, *ura3-1, leu2-3, 112, his3-11,15,15, trp1-1, ade1-2, can 1-100* | (6) |
| W303-ISC02a | *MAT*a, *flr1Δ::KanMX4, ura3-1, leu2-3,112, his3-11, 15, 15, trp1-1, ade1-2, can 1-100* | (6) |
| FY1679-28C | *MAT*a, *ura3-52, leu2-Δ1, trp1-Δ63, his3Δ200, GAL2*$^+$ | (36) |
| FY1679-28C/ TDEC | *MAT*a, *pdr1-Δ2::TRP1,pdr3Δ::HIS3, ura3-52, leu2-Δ1, trp1-Δ63, his3Δ200, GAL2*$^+$ | (37) |
| FY1679-28C/ EC | *MAT*a, *pdr1-Δ2::TRP1, PDR3, ura3-52, leu2-Δ1, trp1-Δ63, his3Δ200, GAL2*$^+$ | (37) |
| EC60 | *MAT*a, *PDR1 (from IL125-2B), pdr3Δ::HIS3, ura3-52, leu2-Δ1, trp1-Δ63, his3Δ 200, GAL2*$^+$ | (38) |
| FY1679-28C. Δ*yap1* | *MAT*a, *yap1Δ::loxp-KanMX4-loxp, ura3-52, leu2-Δ1, trp1-Δ63, his3Δ200, GAL2*$^+$ | This work |
| FY1679-28C. Δ*flr1* | *MAT*a, *flr1Δ::KanMX4, ura3-52, leu2-Δ1, trp1-Δ63, his3Δ200, GAL2*$^+$ | This work |
| Plasmids | | |
| pFL38 | Centromeric cloning vector | (25) |
| pUG6 | Plasmid containing the *loxP-KanMX4-loxP* module | (23) |
| *FLR1-lacZ* | *FLR1-lacZ* gene fusion into plasmid YcpAJ152 | (6) |
| pYORC_YBR008c | *FLR1* LFH-replacement cassette into pUG7 | (6) |
| pYCG_*YBR008c* | *FLR1* gene into pFL38 | (6) |
| pYORC_*YAP1* | *YAP1* LFH-replacement cassette into pFL38 | This work |
| pYCG_*YAP1* | *YAP1* gene into pFL38 | This work |

*cerevisiae* overproducing Yap1p (12) or *Candida albicans* Cap1, a protein highly homologous to Yap1p (5). Yeast response to oxidative stress appears to involve a post-translational modification of Yap1p leading to increased binding of the protein to DNA (13, 14). By mediating the transcription activation of a number of stress genes or genes encoding multidrug transporters, Yap1p confers pleiotropic drug resistance when overexpressed *in vivo* (4, 5, 9, 15–18). In particular, resistance to fluconazole, cycloheximide, 4-NQO and cerulenin mediated by Yap1p is strongly reduced in a *FLR1* deletion mutant (5, 7). In the present work we extend to benomyl the range of growth inhibitory compounds to which *YAP1* confers resistance and show results indicating that Yap1p may have other target genes that confer benomyl resistance in yeast, besides *FLR1* gene. In a previous study we found that benomyl-induced activation of *FLR1* gene expression, monitored by a *FLR1-lacZ* fusion, is partially reduced in yeast cells expressing *YAP1* gene but devoided of *PDR3* gene (6). Pdr3p is a transcriptional regulator of the $Zn_2Cys_6$ family involved in the control of multiple or pleiotropic drug resistance (PDR) in yeast (19). In the present work we also re-examined the role of *PDR3* on *FLR1* expression during benomyl-induced latency.

## MATERIALS AND METHODS

*Strains, media and plasmids.* *Saccharomyces cerevisiae* strains and plasmids used in this study are listed in Table 1. The minimal growth medium MM2 used for yeast growth contained (per liter): 1.7 g yeast nitrogen base without amino acids or $NH_4^+$ (Difco), 20 g glucose, 2.65 g $(NH_4)_2SO_4$, 80 mg adenine, 10 mg histidine, 10 mg leucine, 20 mg tryptophan and 20 mg uracil. Selective minimal medium MM2-U (MM2 lacking uracil) was used for plasmid maintenance. Transformation of yeast cells was performed by the method of Gietz *et al.* (20).

*Escherichia coli* strain XL1 blue was used as plasmid host. The bacteria was grown in LB (Sigma) medium and cloning procedures were carried out by standard methods (21).

*YAP1 and FLR1 gene disruptions in S. cerevisiae FY1679_28c.* Disruption of *YAP1* in FY1679_28c was performed using a disruption cassette consisting of a dominant marker *loxP-KanMX4-loxp* flanked by short flanking homology (SFH) regions to the target ORF (22). This was prepared by DNA amplification by polymerase chain reaction (PCR) using pUG6 plasmid (23) as template and the following primers: 5′-ATGAGTGTGTCTACCGCCAAGAGGTCGCTGGATGTCGTTTCTCCGGGGCGGCCGCTTCGTACGCTGCAGGTCGAC-3′ and 5′-TTAGTTCATATGCTTATTCAAAGCTAATTGAACGTCTTCTGCATTGCGGCCGCGCATAGGCCACTAGTGGATCTG-3′. These primers included, at the 5′ end, 40 nucleotides homologous to the flanking region of *YAP1* gene followed by the *Not*I site and, at the 3′ end, 28 nucleotides homologous to pUG6 (sequences underlined). The PCR product thus obtained was used to transform FY1679_28c strain and transformants were selected on YPD plates with 200 mg/l geneticin. The correct replacement of the gene by the deletion cassette was verified by two independent PCR reactions, as described before (6).

*FLR1* gene disruption in strain W303 was carried out before (6). *FLR1* disruption in FY1679_28c was carried out in this work following an identical procedure, going over the long flanking homology (LFH) deletion cassette plasmid inserted into pUG7 prepared before (6). This construction, pYORC_*YBR008c*/pYORC_*FLR1* (Table I), was prepared to be used for *FLR1* inactivation in any *S. cerevisiae* strain.

*YAP1 gene cloning.* *YAP1* gene was cloned by the gap-repair technique (24). A LFH-deletion cassette was obtained by PCR using as template chromosomal DNA isolated from the FY1679_28c.Δ*yap1*, and the following two primers: 5′-CTTTTTACCAATATCATCAC-3′ and 5′-ATAGAAAGCGTTGGAAATTC-3′. They were designed to be located at 930 bp and 390 bp upstream and downstream the start and the stop codons of *YAP1* gene, respectively. This LFH deletion

cassette was cloned into pFL38 plasmid (25) generating plasmid pYORC_*YAP1* which was used to clone the YAP1 gene by gap repair, generating plasmid pYCG_*YAP1*.

*Drug susceptibility assays.* The susceptibility to benomyl of deletion mutants or recombinants was in general compared by spot assays. The cell suspensions used to inoculate the agar plates were exponential cells grown in MM2 liquid media, until the standardized culture $OD_{600} = 0.2 \pm 0.02$ was reached, followed by dilution to a standardized $OD_{600nm} = 0.05 \pm 0.005$. These cell suspensions and diluted (1:2; 1:4) suspensions were applied as spots (4 $\mu$l) onto the surface of the agarized MM2 medium or, whenever necessary, in selective agarized medium MM2-U, both supplemented with adequate benomyl concentrations. Plates were incubated at 30°C for 3 to 8 days, depending on the severity of growth inhibition. Susceptibility assays were also carried out in MM2 medium at 30°C with orbital agitation at 250 rpm. Growth curves were followed by measuring culture $OD_{600nm}$. The concentration of viable cells during yeast cultivation was assessed as the number of colony forming units (CFU) onto minimal medium agar plates incubated at 30°C for 3 days.

*Effect of benomyl on FLR1 gene expression–FLR1-mRNA quantification.* RNA extraction from yeast cells was performed according to the hot phenol method (26). Northern blot hybridizations were carried out according to Peden *et al.* (27) and the total RNA in each sample used for Northern blotting was approximately constant [20$\mu$g (by $OD_{260\ nm}$)]. The specific DNA probe was prepared by PCR amplification using the primers 5′-CCGGCATGCAGAAGGTAGAAGAGTTACGG-3′ and 5′-GACGGCCATAGCGTGCAGTT-3′. The probe was the 1.12 kb *Bam*HI restriction fragment of the resulting PCR product; it contained the promoter region and the 5′ end of *FLR1* coding region, which is the most divergent sequence between MFS-MDR homologues encoding genes. The RNA extracted from Δ*flr1* cells was used to prepare a control experiment to confirm the specificity of *FLR1* mRNA detection. The 1.1 kb *Bam*HI-*Hind*III fragment of *S. cerevisiae ACT1* gene in plasmid pUC19-*ACT1* (28), was also used as probe to prepare an internal control. Membranes were exposed to hyperfilm-$\beta$max (Amersham) films and incubated with an intensifying screen at −70°C for approximately 1 or 2 days to obtain *ACT1* or *FLR1* signals, respectively. The relative intensities of the hybridization signals in the autoradiograms were quantified by densitometry (UVP gel documentation system, GDS2000).

*β-galactosidase assays. FLR1* expression was also assessed based on $\beta$-galactosidase ($\beta$-gal) activity, using the *FLR1–lacZ* fusion in a plasmid, prepared before (6). Yeast strains were transformed with the plasmid fusion or the cloning vector and cells were growth in benomyl supplemented and unsupplemented selective medium. Assays were based on the method of Miller as previously described (29) and $\beta$-gal units (U) were defined as the increase in $A_{420\ nm}\ min^{-1}\ OD_{600\ nm}{}^{-1} \times 1000$.

## RESULTS AND DISCUSSION

### *Activation of FLR1 Transcription during Yeast Adaptation to Growth under Benomyl Stress*

When cells of *Saccharomyces cerevisiae* W303-ISC02b grown in the absence of benomyl, were used to inoculate the same growth medium supplemented with 4 mg/l of benomyl, cell viability slightly decreased, as assessed by the number of colony forming units (Fig. 1A). The recovery of exponential growth under benomyl stress was possible after an initial period of adaptation of approximately 7 h. Growth recovery was also observed for a population devoided of *FLR1* gene but after a longer period of adaptation of approximately 15 h (Fig. 1A). Interestingly, the elimination of another

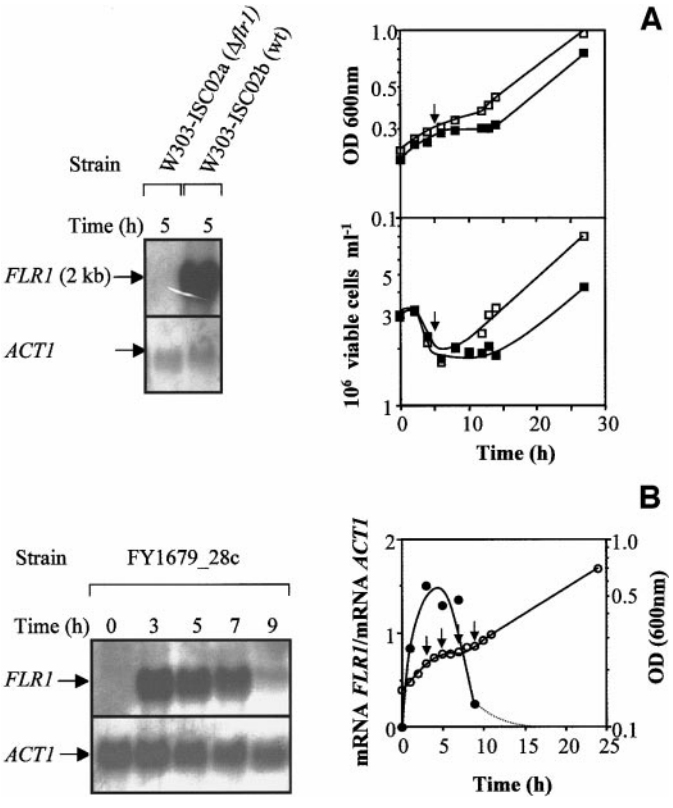


**FIG. 1.** Results from Northern blot hybridization of total RNA probed with *FLR1* and *ACT1* genes. Total RNA was extracted from cells of *S. cerevisiae* W303-ISC02b (wild-type) and W303-ISC02a (Δ*flr1*) (A) or FY1679_28c (B) grown with 4 or 2.5 mg/l of benomyl, respectively. The growth curves, based on culture optical density at 600 nm or concentration of viable cells of strains W303-ISC02b (wild-type) (□), W303-ISC02a (Δ*flr1*) (■) and FY1679_28c (○) are also shown; the arrows indicate the culture samples used to compare mRNA levels in the autoradiograms shown. Relative values of mRNA *FLR1*/mRNA *ACT1* (●) shown were estimated by densitometry of autoradiograms from two independent Northern experiments.

MFS-MDR transporter encoded by *AZR1* gene (ORF YGR224w) and involved in acetic acid resistance, also reduces the duration of acetic-acid-induced latency being the growth kinetics of adapted cells under acetic acid stress apparently independent of *AZR1* gene expression (30). Although *FLR1*-mRNA levels were undetectable in yeast cells grown in the absence of benomyl and used as the inoculum, after 5 h of adaptation to benomyl, a 2 kb hybridization band, corresponding to *FLR1*-mRNA, was easily observed, being this same transcript absent from cells of the deletion mutant Δ*flr1* cultivated under identical conditions, with the fungicide (Fig. 1A). The dependency of *FLR1*-transcription level on the time of incubation with benomyl was examined using a different strain, *S. cerevisiae* FY1679_28c. This strain was used in a previous study (6) and because it is more susceptible to benomyl than W303-ISC02b, a lower concentration of the fungicide (2.5 mg/l) was used to impose an identical level

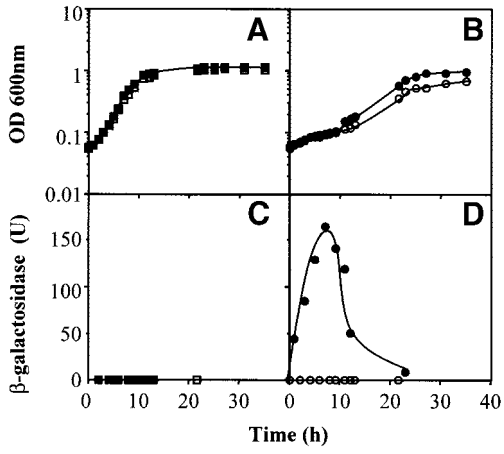


**FIG. 2.** Growth curves (A and B) and β-galactosidase activity (C and D) of cells of *S. cerevisiae* FY1679_28c (■, ●) and FY1679_28c. Δ*yap1* (□, ○) harboring a *FLR1-lacZ* fusion plasmid, during unstressed (■, □) or (1.5 mg/l) benomyl-stressed cultivations (●, ○).

of growth inhibition under identical cultivation conditions. Results revealed that *FLR1*-mRNA levels reached high values during the period of adaptation to the fungicide but were drastically reduced as soon as cells started duplication under benomyl stress (Fig. 1B). During exponential growth, either in the presence or absence of benomyl, FLR1-mRNA levels were barely detectable (Fig. 1B and results not shown). The same pattern of *FLR1*-mRNA level modification during benomyl stressed cultivation was found, independently of the duration of the initial period of adaptation to benomyl (results not shown). The duration of this lag period was shorter or longer depending on the higher or lower initial concentration of unadapted cells, respectively. We propose that *FLR1* transcription activation is among a number of induced responses to environmental challenge that may allow the yeast cell to cope with the deleterious effect of benomyl and, eventually, to adapt to growth under high benomyl stress. Although the physiological adaptation during latency induced by the presence of a specific inhibitor is critical to the eventual growth recovery, there is a lack of information concerning the yeast responses that may occur during this period. The dramatic activation of *FLR1* gene by benomyl and the stimulation of plasma membrane $H^+$-ATPase activity induced by octanoic acid (31) or copper (32) are among the few examples.

### *The Strong Transcriptional Activation of FLR1 Gene during Yeast Adaptation to Benomyl Is Fully Dependent on YAP1 Gene*

*YAP1* gene disruption in FY1679_28c completely abolished the activation of *FLR1* gene expression taking place during the period of yeast adaptation to benomyl. Gene expression was monitored based on β-galactosidase activity from a *FLR1-lacZ* fusion plasmid construction (Fig. 2). Interestingly, the susceptibility to benomyl of the growth of the mutant devoided of *YAP1* gene was higher than wild-type susceptibility while no growth phenotype was detected using the same basal medium lacking the fungicide (Figs. 2A and 2B). Results from Northern blot experiments confirmed the lack of detectable levels of *FLR1*-mRNA during benomyl-induced latency of the strain devoided of *YAP1* gene, similarly to results obtained with unstressed cells of both the wild-type and the Δ*yap1* mutant (Fig. 3). Our results are therefore in agreement with those just published by Nguyên *et al.* (8). The growth and viability curves of the two unadapted populations during cultivation under benomyl stress confirmed results registered in Fig. 2 indicating that *YAP1* gene is a determinant of resistance to the fungicide (Fig. 3). Specifically, they indicate that the duration of the period of adaptation preceding cell division under benomyl stress is longer for the Δ*yap1* mutant population. Altogether, results suggest that the higher susceptibility to benomyl of the population devoided of *YAP1* gene may be at least partially due to the abolishment of *FLR1* transcriptional activation taking place in the wild-type population during latency induced by the fungicide.

### *Role of FLR1 and YAP1 Expression on Benomyl Resistance in Yeast*

To compare the effects of the expression of *FLR1* and *YAP1* genes on yeast resistance to benomyl, *FLR1* gene was eliminated in FY1679_28c where *YAP1* had been

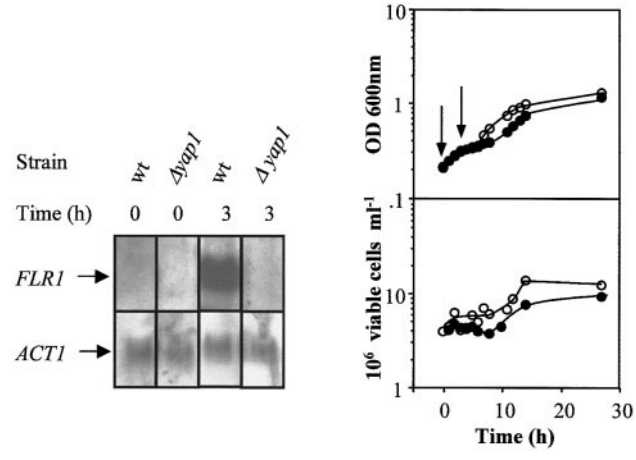


**FIG. 3.** Results from Northern blot hybridization of total RNA probed with *FLR1* and *ACT1* genes. Total RNA was extracted from cells of *S. cerevisiae* FY1679_28c (wild-type) and FY1679_28c. Δ*yap1*, harvested during benomyl (2.5 mg/l)-stressed cultivation, at time 0 or after 3 h of cultivation. Growth curves, based on culture optical density at 600 nm and on the concentration of viable cells of *S. cerevisiae* FY1679_28c (○) and FY1679_28c. Δ*yap1* (●) are also shown; the arrows indicate the culture samples used to compare mRNA levels in the autoradiogram shown.

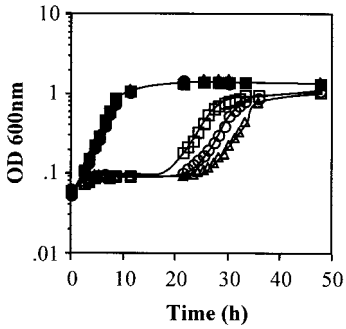


**FIG. 4.** Growth curves of *S. cerevisiae* strains FY1679_28c (wild-type) (■, □), and the Δ*flr1* (●, ○) and Δ*yap1* (▲, △) deletion mutants in minimal medium either supplemented with 4 mg/l of benomyl (□, ○, △) or unsupplemented (■, ●, ▲).

eliminated. Results indicate that Yap1p may have other target genes that confer benomyl resistance in yeast since the lack of *YAP1* expression affected more significantly the growth curve under benomyl stress than *FLR1* elimination did (Fig. 4). However, they also indicate that yeast resistance towards an identical benomyl deleterious effect, mediated by *YAP1* expression, is reduced in the *FLR1* deletion mutant (Fig. 5). Altogether, our observations strongly suggest that benomyl resistance mediated by *FLR1* gene involves Yap1-dependent-transcriptional activation of *FLR1* gene taking place during cell adaptation to the fungicide.

### *Effect of PDR3 Deletion on Benomyl Induced FLR1-Transcriptional Activation*

Based on the indications obtained that Yap1p is essential to benomyl-induced activation of *FLR1* gene transcription, we reexamined the effect of Pdr1p and Pdr3p on this regulation. The elimination in the wild-type strain FY1679_28c, expressing Yap1p, of *PDR1* gene did not affect the level of *FLR1* transcription observed after 1 hour of benomyl stressed cultivation while the elimination of *PDR3* or of both *PDR3* and

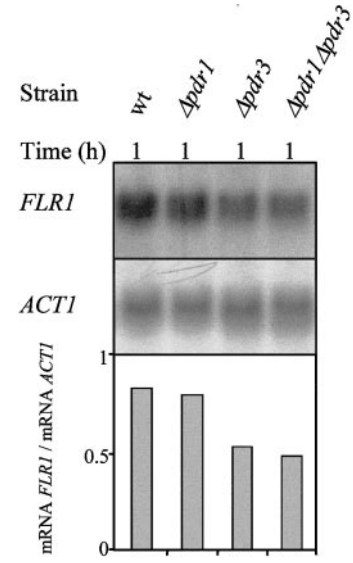


**FIG. 6.** Results from Northern blot hybridization of total RNA probed with *FLR1* and *ACT1* genes. Total RNA was extracted from cells of *S. cerevisiae* FY1679_28c (wild-type, wt) or the Δ*pdr1*, Δ*pdr3*, and Δ*pdr1*Δ*pdr3* deletion mutants, after 1 h of cultivation in the presence of benomyl (2.5 mg/l), using the growth conditions leading to the growth curve registered in Fig. 7A. Relative values of the mRNA *FLR1*/mRNA *ACT1* quantified by densitometry of this autoradiogram are also shown.

*PDR1* genes, reduced this level by 35–40% (Fig. 6). Although the qualitative effect of *PDR3* elimination confirmed previous results obtained using a *FLR1-lacZ* fusion, the percentage of reduction of *FLR1* activation was nevertheless significantly below the value estimated before (85%) (6). We considered the hypothesis that this considerable difference could be due to the fact that, to facilitate the quantification of mRNA during benomyl-induced latency, we have used a concentration of inoculum above the value used in the previous study. Specifically, an initial culture $OD_{600nm}$ of 0.2 was used in the present work instead of 0.05. This 4-fold increase of the inoculum size led to a less inhibited growth curve in the presence of 2.5 mg/l of beno-

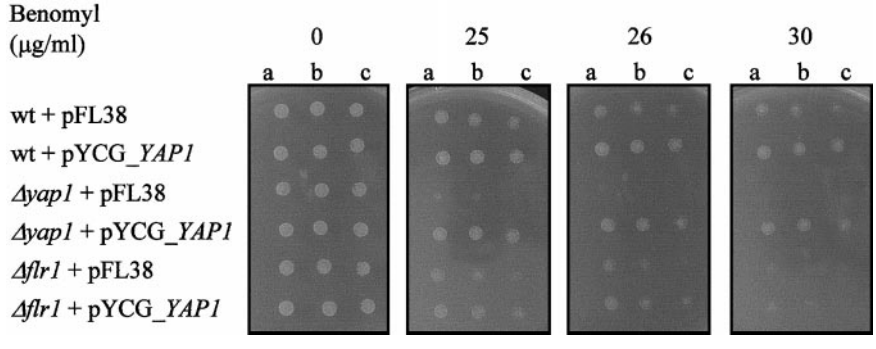


**FIG. 5.** Comparison of the susceptibility to benomyl at the indicated concentrations, of strains FY1679_28c (wild-type, wt) and the Δ*yap1* and Δ*flr1* deletion mutants harboring the recombinant plasmid pYCG_*YAP1*, with *YAP1* gene into pFL38, or the cloning vector. The cell suspensions used to prepare the spots in (b) and (c) were 1/2 and 1/4 dilutions of the cell suspensions used in (a), respectively.

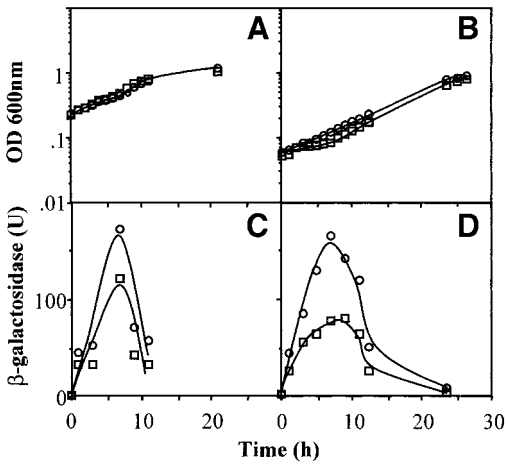


**FIG. 7.** Growth curves (A and B) and β-galactosidase activity (C and D) of cells of *S. cerevisiae* FY1679_28c (wild-type) (○) and the Δ*pdr3* mutant (□) harboring a *FLR1-lacZ* fusion plasmid, during benomyl stressed cultivations with 2.5 mg/l (A and C) or 1.5 mg/l (B and D), of benomyl and initial culture $OD_{600nm}$ of 0.2 (A and C).

myl compared with the growth curve obtained before, even though we had then used a lower concentration of fungicide (1.5 mg/l) (6). With this in mind, we compared the effect of *PDR3* disruption in *FLR1* expression during cultivation under these two distinct conditions (Fig. 7). Results of β-galactosidase activity indeed indicated that the level of reduction of *FLR1* activation in a strain devoided of *PDR3* gene, depends on the level of growth inhibition by benomyl (Fig. 7). They also confirm the more severe reduction of benomyl-induced-activation of *FLR1* expression observed before when different cultivation conditions led to a higher susceptibility to benomyl (Figs. 7B and 7D). They also confirm the less significant reduction of *FLR1*-mRNA activation under benomyl stress in a strain devoided of *PDR3,* as estimated in the present work, when, despite the increase of the concentration of fungicide used, a higher inoculum size led to a less inhibited growth (Figs. 7A and 7C). It is therefore possible that under less appropriated conditions, Nguyên *et al.* (8) have failed to detect Pdr3p effect on benomyl-induced *FLR1* activation. Indeed, the effect is only clearly detected during the extended period of latency of a severely inhibited cultivation. The effect of the inoculum size here registered has been described for many microorganisms and antimicrobial agents. In particular, the inhibitory effect of sorbic acid in *Zygosaccharomyces bailli* was considered to be caused by diversity in the population of yeast cells with higher probability of sorbic acid resistance cells being present in large inocula. (33). This concept is consistent with the observation that an inoculum cell population grown under unstressed conditions exhibit a significant heterogeneity concerning the intracellular pH ($pH_i$) (31).

Considering the presence in the *FLR1* promoter of a PDRE motif together with three Yap1p-response elements (5, 6, 34), *FLR1* regulation may be exerted by Yap1p either directly or via the Pdr3p. This hypothetical model was suggested to us by the observation that *YAP1* deletion completely abolished *FLR1* transcription activation induced by benomyl and that *PDR3* deletion partially reduced this activation in the wild-type strain expressing *YAP1.* The possible link between these transcriptional regulators was proposed before (35) to explain the dependence of Yap1p-mediated resistance to specific drugs on the presence of a functional *PDR3* gene.

## ACKNOWLEDGMENTS

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