Construction of a Sorbitol-Based Vector for Expression of Heterologous Proteins in *Saccharomyces cerevisiae*

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A new inducible yeast expression vector, pXS7, was constructed by using the promoter and terminator sequences from the *Saccharomyces cerevisiae* *SOR1* gene, which codes for the sorbitol dehydrogenase protein. We cloned the coding sequence of the *Saccharomyces* *YEF3* gene in this vector and demonstrated an increase in YEF3 protein levels when cells were grown in the presence of the sugar sorbitol.

The yeast *Saccharomyces cerevisiae* was one of the first organisms to be used for the production of heterologous proteins (2, 7). However, only a few inducible expression vectors have been reported so far (3, 4). We have previously demonstrated that *S. cerevisiae* produces a highly expressed sorbitol-inducible enzyme, sorbitol dehydrogenase, which is encoded by the *SOR1* gene (6). This report describes the construction and use of pXS7, which contains the transcriptional regulatory elements from *SOR1* for the production of proteins. Most strains of *S. cerevisiae* exhibit a long lag period for growth on sorbitol as a sole carbon source. Previously, we had isolated a mutant strain, 8000-8B LAG, that is able to grow rapidly on sorbitol and induce the production of the enzyme sorbitol dehydrogenase in the presence of the inducer sorbitol (6). We have used 8000-8B LAG to express proteins under sorbitol-inducible conditions from this new yeast vector, pXS7.

To construct the vector pXS7, a 3-kb *EcoRI* yeast genomic fragment containing the regulatory and coding regions of the *SOR1* gene (6) was engineered following insertion at the *EcoRI* site of the yeast vector pMW5 (a gift from B. D. Hall, University of Washington) (5). Most of the coding region of the *SOR1* gene was subsequently deleted, followed by the addition of adapter DNAs to modify the *SOR1* promoter and add a polylinker containing three unique cloning sites for insertion of heterologous DNA fragments between the promoter and terminator sequences of the *SOR1* gene (Fig. 1). The sequence at this region is shown in Fig. 1. The unique *Bam*HI and *Sal*I sites of the multicopy yeast vector pMW5 were first inactivated in order to make these sites unique in the multicloning linker.

To demonstrate the utility of the new vector, we have overexpressed the coding sequence of a yeast translation elongation factor gene, *YEF3*, by placing it under the control of the promoter and terminator regions of the *SOR1* gene in pXS7. An engineered 3.5-kb *Bam*HI/*Xho*I fragment containing the coding sequences for the *YEF3* gene encoding EF-3 (a gift of W. Fonzi, Georgetown University Medical Center) was inserted into the *Bam*HI/*Sal*I sites of pXS7 to yield plasmid pXS7EF-3. Strain 8000-8B LAG was transformed with pXS7 and pXS7EF-3 to produce strains YAB 161 and YAB 162, respectively (1). Tryptophan-prototrophic transformants were selected on synthetic defined (SD) medium lacking tryptophan. Strains were grown in 10 ml of SD medium lacking tryptophan and containing 2% glucose, 2% glucose plus 2% sorbitol, or

2% sorbitol for 20 h. Cells were harvested by centrifugation at $400 \times g$, and pellets were washed in 2 ml of yeast cracking buffer (50 mM Tris [pH 8], 2 mM EDTA, 2 mM β -mercaptoethanol, and 2 mM phenylmethylsulfonyl fluoride). Samples were centrifuged, and pellets were resuspended in yeast cracking buffer at 2×10^8 cells per 100 μ l in a microcentrifuge tube. Glass beads were added to the meniscus, and samples were frozen at -70°C , thawed slowly on ice, and vortexed 3 times for 30 s each time. Samples were refrozen, thawed, vortexed for an additional 30 s, and spun in a microcentrifuge for 1 min to remove beads and insoluble fraction.

Sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) analysis of cell extracts shows that there is an increase in expression of EF-3 (molecular weight, 116 kDa) only in cultures of YAB 162 containing the plasmid pXS7EF-3 grown in the presence of the inducer sorbitol (Fig. 2, lanes 4 and 6). The *SOR1* promoter appears to be partially induced in cultures of 8000-8B LAG grown in medium containing glucose and sorbitol (Fig. 2, lane 4), suggesting that the *SOR1* promoter is not 100% repressed by glucose. However, it is also possible that glucose depletion allows for derepression of the

FIG. 1. Construction of pXS7. Boldface letters, *SOR1* genomic DNA sequences. *SOR1* sequences from -14 to $+1029$ (6) have been replaced with the polylinker region (lowercase letters). Details of the construction of pXS7 are described in the text.

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FIG. 2. Coomassie blue-stained gel from SDS-PAGE analysis of EF-3 production. Lanes 1, 3, and 5 contain lysates from strain YAB 161 transformed with the pXS7 vector alone as controls. Lanes 2, 4, and 6 contain lysates from strain YAB 162, where the *YEF3* gene is controlled by the *SOR1* promoter. EF-3 protein can be seen as the heavily stained band at 116 kDa. Cultures were grown in SD medium lacking tryptophan and containing either 2% glucose (glu), 2% glucose plus 2% sorbitol (glu/sorb), or 2% sorbitol (sorb). Lane M contains prestained molecular-size markers.

promoter during the 20-h period in which the cultures are grown, and additional experiments would be required to elucidate this observation.

For a comparison of expression levels, the coding sequence of the *YEF3* gene was also expressed from the *GAL1* promoter in the 30-copy plasmid pYES2. Since the 8000-8B LAG strain does not grow on galactose, plasmids were transformed into

BJ5457 for expression studies. Cells were grown as described above except that strains containing the pYES2 constructs were grown in SD medium lacking uracil and containing 2% galactose. Lysates were run on SDS-PAGE gels and were treated with Sypro Red protein stain (Molecular Probes) for quantitation on the Storm 860 fluorescent imager (Molecular Dynamics). The levels of EF-3p produced from the *SOR1* promoter and from the *GAL1* promoter were estimated to be 4 and 3% of total protein, respectively (data not shown). We believe that the pXS7 expression plasmid will be a useful tool for the expression of proteins in *S. cerevisiae*.

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