Construction of an Effective Host-Vector System for the Yeast 
Saccharomyces exiguis Yp74L-3

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An effective host-vector system specific to the yeast Saccharomyces exiguis Yp74L-3 was constructed to promote the molecular genetic analyses for the yeast. To obtain a stable reversionless host strain, we constructed an S. exiguis strain carrying leu2::SCURA3 by disrupting the S. exiguis LEU2 gene with the S. cerevisiae URA3 gene. A vector plasmid unique to S. exiguis was subsequently developed by inserting both the LEU2 gene and an ARS cloned from S. exiguis into an Escherichia coli phagemid, pUC119. The vector constructed, pTH119 was able to transform the S. exiguis leu2::SCURA3 strain to Leu † efficiently. The stability of the vector in the S. exiguis host cells resembled that of a YRp-type vector in S. cerevisiae.

Key words: Saccharomyces exiguis; LEU2 gene; autonomously replicating sequence (ARS); host-vector system; Saccharomyces cerevisiae

Saccharomyces yeasts isolated from natural habitats are widely known to have homothallic life cycles. In order to develop genetic manipulation systems in these yeasts, we have successfully obtained heterothallic haploid strains from a homothallic yeast, Saccharomyces exiguis Yp74L-3, by introducing a mutation into a homothallism-controlling gene. Using these heterothallic haploid strains, S. exiguis Yp74L-3 was found to be a biologically distinct species from the well-characterized yeast S. cerevisiae. Although the structures of the α pheromones of these two yeasts are different, both α pheromones can stimulate a-type cells in either yeast. To clarify the species differentiation mechanism, we did comparative studies of the two yeasts focusing on sexual interactions. In addition, electrophoretic karyotyping and hybridization analyses with several genes cloned from S. cerevisiae as probes indicated distinct differences between the genomes of these two species.

In S. cerevisiae, four typical types of vectors, YIp, YEp, YRp, and YCp, have been developed, and a large number of strains carrying various kinds of auxotrophic mutations are available for host cells. Using these host-vector systems, a number of S. cerevisiae genes related to sex differentiation were cloned and intensively investigated at the molecular level. However, a useful host-vector system in S. exiguis has not yet been established.

Although the YEp13 plasmid containing the LEU2 gene of S. cerevisiae was found to convert the leu2 mutant of S. exiguis to the Leu † phenotype, the transformation event occurred at an extremely low frequency. Recently, we have cloned and sequenced the LEU2 gene and two ARSs from the S. exiguis genome, suggesting that S. exiguis recognizes an ARS distinct from the ARS of S. cerevisiae.

In this study, we constructed a reversionless host strain by disrupting the LEU2 gene of S. exiguis with the S. cerevisiae URA3 gene. Subsequently, we constructed an efficient vector plasmid by inserting both the LEU2 gene and an ARS essential region from S. exiguis into a convenient phagemid, pUC119. The combination of a reversionless host strain of S. exiguis and the newly developed vector plasmid would be a powerful host-vector system to promote a comparative study of the genes involved in sex differentiations between S. exiguis and S. cerevisiae.

Materials and Methods

Yeast strains, media, culture conditions, and classical genetic analysis. THE32 (MATα ura4) and THE24 (MATα ura4) are S. exiguis heterothallic haploid strains derived from a homothallic diploid strain, Yp74L-3. The media used to grow yeast cells were YEPD as complete medium, and SD as minimal medium. Appropriate nutrients were added to SD as described by Kaiser et al. A sporation medium was used to obtain tetrad spores from diploid cells. Cultivation in liquid media was done with vigorous shaking. For solid media, 2% agar powder was added to liquid media. The temperature of cultivation was 26°C throughout. Conventional techniques described for S. cerevisiae were used for the genetic analysis on S. exiguis.

Plasmids and molecular genetic procedures. The E. coli phagemid pUC119 and S. cerevisiae integration vector YIp5 were used. Molecular genetic manipulations were done as described by Sambrook et al. Specific DNA regions were amplified by PCR. Hybridization analysis was done with a DNA probe prepared from S. cerevisiae.
Yeast transformation and plasmid stability assay.
Yeast transformation was done as described by Ito et al.\textsuperscript{15} with slight modification. Plasmid stability in transformed cells was measured by the method of Kaiser et al.\textsuperscript{15}

Results and Discussion
Construction of a reversionless host strain in \textit{S. exigus}
To construct a reversionless auxotrophic host strain of \textit{S. exigus}, we planned to disrupt the \textit{LEU2} gene on the chromosome of \textit{S. exigus}. To this end, we used the \textit{URA3} gene cloned from \textit{S. cerevisiae}\textsuperscript{15} and the \textit{LEU2} gene cloned from \textit{S. exigus}.\textsuperscript{10} The \textit{URA3} gene of \textit{S. cerevisiae} complements the \textit{ura4} mutation of \textit{S. exigus}.\textsuperscript{11}

Figure 1 shows the strategy used to disrupt the \textit{LEU2} gene of \textit{S. exigus} on a plasmid. First, an approximately 3.4-kbp \textit{PvuII}-\textit{SpI} fragment, which contained the \textit{S. cerevisiae URA3} gene (denoted \textit{ScURA3}) and the tetracycline-resistance gene, was liberated from the \textit{YIp} plasmid. This fragment was then inserted into the \textit{StuI} site of the \textit{S. exigus LEU2} gene (denoted \textit{SeLEU2}) on the \textit{pUC119} plasmid, resulting in a disruption of the \textit{SeLEU2} gene, \textit{leu2::ScURA3}.

We then replaced the \textit{LEU2} gene on the \textit{S. exigus} chromosome with the \textit{leu2::ScURA3} construct on the plasmid. The fragment from the \textit{VspI} site on the \textit{pUC119} to the \textit{XbaI} site in the \textit{SeLEU2} was liberated as a linear DNA fragment and used for the transformation of the \textit{S. exigus} \textit{THE32} strain (\textit{MATa ura4}). Three hundreds and forty colonies with the Ura\textsuperscript{+} and Leu\textsuperscript{+} phenotype were obtained following the transformation experiment using 30 \textmu g of a linear DNA.

Next, we examined whether the phenotype was caused by the integration of the \textit{ScURA3} into the \textit{S. exigus LEU2} locus. The genomic DNA from one of the Ura\textsuperscript{+} Leu\textsuperscript{-} strains, \textit{THE41}, was amplified by PCR using the primer sets which sandwich the \textit{StuI} site in the \textit{SeLEU2} (Fig. 1), where the fragment containing the \textit{ScURA3} might be integrated. Figure 2 shows that an approximately 3.4-kbp fragment containing the \textit{S. cerevisiae URA3} gene was integrated into the \textit{S. exigus LEU2} locus in the \textit{THE41} strain. This result indicates that the \textit{SeLEU2} was definitively disrupted by the insertion of the \textit{ScURA3}, leading to the Ura\textsuperscript{+} Leu\textsuperscript{-} phenotype in the \textit{THE41} strain.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Disruption of the \textit{S. exigus LEU2} Gene on a Plasmid Using the \textit{S. cerevisiae URA3} Gene.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Integration of the \textit{S. cerevisiae URA3} Gene into the \textit{LEU2} Locus of \textit{S. exigus}.}
\end{figure}

The genomic DNAs from an original strain (THE32) and a Ura\textsuperscript{+} Leu\textsuperscript{-} transformant (THE41) derived from THE32 were amplified by PCR using primers P1 and P2 mentioned in Fig. 1, and electrophoresed in an agarose gel (lanes 1 and 2, respectively). These DNAs were transferred to a nylon membrane and probed with the \textit{URA3} gene of \textit{S. cerevisiae} (lanes 3 and 4 correspond to lanes 1 and 2, respectively). Symbol M stands for the \lambda \textit{HindIII} marker. The numbers on the right side represent approximate sizes (bps) of the PCR products.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Segregation of Leucine and Uracil Auxotrophies in Tetrads from the \textit{THE41} Strain Crossed with a Tester Strain.}
\end{figure}

The \textit{S. exigus} \textit{THE41} strain was crossed with the \textit{S. exigus} \textit{THE24} strain (\textit{MATa ura4}) and its tetrads were dissected. Symbols A, B, C, and D represent names of segregants from one ascus. Lanes 1 and 2 represent cell growth on the leucine-deficient and uracil-deficient plates, respectively.
Fig. 4. Construction of S. exiguus-Specific Vector Plasmid.
The SeLEU2-containing SalI-SalI fragment from the S. exiguus genome (hatched box, see also Fig. 1) was blunted with Klenow fragment and inserted into the pUC119 plasmid, which had been cleaved with AatII and blunted with T4 DNA polymerase. The region containing an ARS essential sequence of S. exiguus (closed box, denoted Se307ARS)\(^\text{10}\) was amplified by PCR using a specific primer set that contained the BsrGI recognition site at each end (open plus closed boxes). This fragment was subsequently inserted into the BsrGI site upstream of SeLEU2. The constructed vector plasmid denoted pTH119 contained both the LEU2 gene and an ARS from the S. exiguus genome, and comprised 5437-bp.

Disruption of the LEU2 gene of the THE41 strain was confirmed by the fact that the leucine auxotrophy of the THE41 strain was not complemented by crossing with an S. exiguus MATa leu2 strain (data not shown). The THE41 strain was crossed with the S. exiguus THE42 strain (MATa ura4) and its tetrads were analyzed. Figure 3 shows a typical result obtained from one ascus of 10 ascii examined. The Leu\(^+\) phenotype was completely cosegregated with the Ura\(^+\) phenotype in all ascii. According to the observations mentioned above, the genotype of the S. exiguus THE41 strain should be expressed by MATa leu2::ScURA3 ura4. No Leu\(^+\) cell spontaneously appeared from the THE41 strain even upon examining more than 10\(^6\) cells, thereby indicating that the THE41 strain is a useful reversionless host strain suitable for the S. exiguus transformation system.

Construction of a vector plasmid specific to S. exiguus
We subsequently constructed an effective vector plasmid containing the LEU2 gene and an ARS derived from the S. exiguus genome. In addition to the S. exiguus LEU2 gene (SeLEU2), one of the ARS essential regions (denoted Se307ARS) has been recently cloned and sequenced.\(^\text{10}\) We introduced these two elements of S. exiguus into a convenient phagemid, pUC119 (Fig. 4). The constructed plasmid containing both SeLEU2 and Se307ARS was denoted pTH119.

Transformation of the THE41 host strain with pTH119 vector plasmid
We examined the characteristics of the plasmid pTH119 on the transformation of the S. exiguus THE41 strain. The plasmid pTH119 was able to produce approximately 4000 Leu\(^+\) transformants per 1 \(\mu\)g of DNA from the THE41 strain. The plasmid pTH119 was retained in approximately 5% of cells when grown in a nonselective medium for 6 generations, indicating the instability of the plasmid in the THE41 cells. The behavior observed in the experiment resembles that of the YRp-type vectors containing the S. cerevisiae ARS1 in the S. cerevisiae host cells.

The combination of the S. exiguus THE41 strain and the pTH119 vector plasmid was shown to be an effective host-vector system of S. exiguus. Future research will focus on comparing the genes involved in sex differentiation from S. exiguus and S. cerevisiae at the molecular level, using the host-vector system established in this study. These analyses will contribute to the understanding of species differentiation in Saccharomyces yeasts from the evolutionary viewpoint.

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References


