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NOTE

Cloning of Genomic DNAs of *Trametes versicolor* Acting as Autonomously Replicating Sequences in *Saccharomyces cerevisiae*

Sora An[1], Kyoung Phil Park[1], Hyoung-Tae Choi[1], Kyu-Joong Kim[2], and Kyunghoon Kim[1]*

[1]*Division of Life Sciences, College of Natural Sciences, Kangwon National University, Chunchon 200-701, Korea*
[2]*Department of Biology, Kangnung National University, Kangnung 210-702, Korea*

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A genomic DNA library of the fungus *Trametes versicolor* was constructed in a yeast integration vector which contains the *URA3* gene of the budding yeast *Saccharomyces cerevisiae* and the gene responsible for hygromycin B resistance, and fragments acting as autonomously replicating sequences (ARSes) in the budding yeast were identified from the genomic DNA library. Sixteen recombinant plasmids from the library transformed the budding yeast *Saccharomyces cerevisiae* to Ura$^+$ at high frequencies. They were maintained stably under selective conditions, but were gradually lost from yeast cells at different rates under nonselective conditions, indicating that they contain eukaryotic origins of DNA replication and exist as extrachromosomal plasmids. Base sequences of four ARS DNAs among the 16 cloned fragments revealed that all of the four contain at least one 11 bp [(A/T)TTTA(T/C)(A/G)TTT(A/T)] consensus sequence of the budding yeast ARS.

Key words: ARS, *Trametes versicolor*, *Saccharomyces cerevisiae*, transformation, plasmid

Cellulose, hemicellulose, and lignin are major components of plant cell walls. While cellulose and hemicellulose are degraded by many microorganisms, lignin is one of the most recalcitrant biopolymers. For this reason lignin degradation is very important in the carbon cycle. White-rot fungi degrade lignin completely as well as cellulose and hemicellulose (Kirk and Farrell, 1987; Boominathan and Reddy, 1992; Reddy and D'Souza, 1994), whereas brown-rot fungi mineralize cellulose and hemicellulose efficiently but not lignin (Kirk and Farrell, 1987). Since *Trametes versicolor*, a white-rot fungus has a good ability to degrade many recalcitrant chemicals as well as lignin, many studies have been done on its lignin-degrading enzymes such as laccase and peroxidases along with the studies on its utilization in the treatment of environmentally persistent aromatics (Bumpus *et al.*, 1985; Eaton, 1985; Hammel *et al.*, 1992; Gold and Alic, 1993). Genes encoding laccase and peroxidases from *T. versicolor* have also been cloned (Johansson and Nyman, 1996; Ong *et al.*, 1997; Grey *et al.*, 1998; Cassland and Jönsson, 1999; Collins *et al.*, 1999). However, expressions and functions of the cloned genes are not well understood because shuttle vectors and the genetic transformation system for *T. versicolor* are not available yet.

Replication origins scattered throughout eukaryotic chromosomal DNA were first cloned as autonomously replicating sequences (ARSes) from the budding yeast *Saccharomyces cerevisiae* (Hsiao and Carbon, 1979; Stinchcomb *et al.*, 1979). A conserved nucleotide sequence motif, 5'-(A/T)TTTAPyPuTTT(A/T)-3', has been deduced as an essential ARS core consensus sequence of *S. cerevisiae* (Broach *et al.*, 1983; Kearsey, 1984; van Houten and Newlon, 1990). In this study, we have cloned several fragments from *T. versicolor* acting as ARSes in *S. cerevisiae* as an approach to the construction of appropriate shuttle vectors for *E. coli* and *T. versicolor*.

We first subcloned the *Bgl*II-*Bam*HI fragment encoding hygromycin B resistance of pAN7-1 (Punt *et al.*, 1987) into the *Bam*HI site of a yeast integrating plasmid (YIp) pRS306 (Sikorski and Hieter, 1989), and the resulting YIp was designated pKW105 (Fig. 1). With pKW105, we were able to transform a *ura3* budding yeast KY106 (*Mata leu2 ura3 trp1 lys2*) and a *T. versicolor* monokaryon 9522-1 (Kim *et al.*, 2002) to Ura$^+$ and hygromycin B resistance, respectively, by the restriction enzyme-mediated integration method (Leem

* To whom correspondence should be addressed.
(Tel) 82-33-250-8527; (Fax) 82-33-251-3990
(E-mail) kkim@kangwon.ac.kr

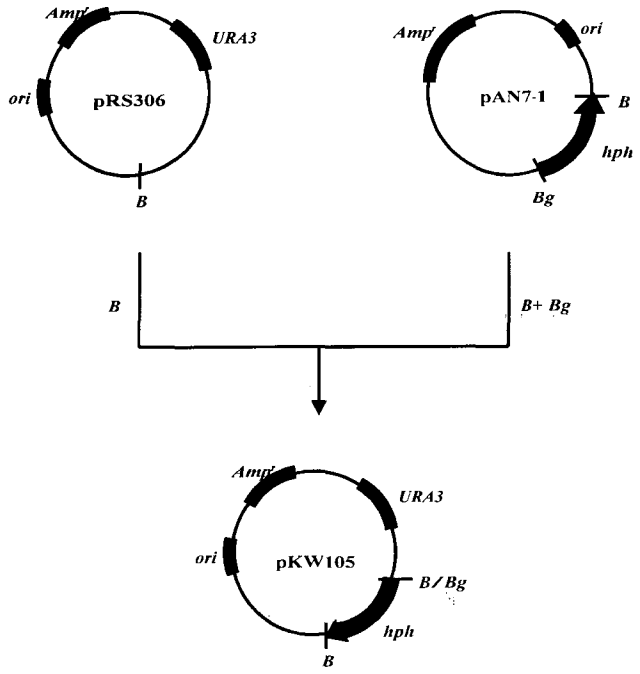

Fig. 1. Construction of a recombinant plasmid pKW105. Locations of genes for resistance to ampicillin (*Amp*^{r}) and hygromycin B (*hph*) are indicated. Locations of yeast *URA3* gene (*URA3*) and an origin of plasmid DNA replication (*ori*) in *E. coli* are also indicated. Abbreviations for restriction enzyme cleavage sites are: B, *Bam*HI; and Bg, *Bgl*II.

GATCGCCAGAGGTGTTGGTCGCCGCCCAAAGCCCTTCCCACATCGCCCTGCACAACCCGCCCGTGGTGAG
ATGCAGCGTGTTGAGAGAGCTAAACGCCATGAAGATCTATTTATATTTAATTTTAGTAATCTTTCACAA
GATTGAATATTTTTTTCAATATTTAAATATTCTTCATTATCTAAATCTTTTTTATCACGTATATATACTT
CTTTTTTCTTATTTTCATCAGATAATCTAAAATTTAAAGAGTCAATTAATTTTAATGCAATAAAACTAT
AAAAATCTTGAGGTATTGAATCAGAATCACCAGAAATTAAATTTAAATGTGATGCTAAAGATTCATCCC
CGATTAACAAACTTAAATGTTGATAACCATTACAAGTAGCATCTAATTGTACAGGAAAATAAGAAATATA
CGAAGTTTCATTAGAATTTAATGAATTATAATAATTATTAAATTCAAAACAGAAAGCTATAAATAATAA
TTTAGATTCAGCTTCTTTAATCAATTTACCATTTCTAAAATCTAAAATATCAGATAAATTATCATTAACC
CAATTTATTCTACTGTTATAAGATTTTTTATCTTATCCATTTCCATAACAATTAGCACCAAATATTTTT
AAAAAATGAATACTTTCGTTATCTCATTTTAAAATTCTATCCCCTTTACTAAATAATAATAAAGATTTAG
CATTAACTATACCTTGATAATTCAAATAATCAGCCATACAATAAATTCTACCTCTATTATCTATTCTAAC
AGGAATATAAAAATGAGGTACATTTTAAATATAGTGGCTAAAGATAAAATATTCATTTCTAATTGTTTTT
TACTTAAAAAACTATCTAATGTTTTTTTTTCATAAAAACTTAATTTTTTTTTTTATCTTCAAGAGGATG
TTTAAATACAAGATC

Fig. 2. Nucleotide sequence of a genomic DNA of *T. versicolor* acting as an ARS in *S. cerevisiae* (GenBank accession number AF506815). The boxed sequence matches the *S. cerevisiae* ARS core consensus. Sequences similar to the *S. cerevisiae* ARS core consensus are marked by thin lines (10/11 match) or dashed lines (9/11 or 8/11 match).

et al., 1999), confirming that pKW105 contains genes for ampicillin resistance, hygromycin B resistance, and *URA3*. Then a library of genomic DNA from *T. versicolor* was prepared by digesting chromosomal DNA of *T. versicolor* with *Sau*3AI, inserting the digest into a unique *Bam*HI site of pKW105, and transforming *E. coli* JM109 cells to ampicillin resistance with the recombinant DNA. The library DNA were isolated from a pool of the *E. coli* transformants and used to transform *S. cerevisiae* KY106 to Ura$^+$. We randomly screened 70 yeast transformants for the maintenance of the *URA3* marker under nonselective conditions. When the transformants were cultivated in YEPD (1% yeast extract, 2% peptone, 2% dextrose) liquid media for about 20 generations, more than 50% of the cells became Ura$^-$. This result indicates that all the transformants were derived by the establishment of extrachromosomal ARS$^+$ plasmids within the cells and the plasmids are unstable under nonselective conditions. Plasmid DNAs were isolated from randomly chosen 16 Ura$^+$ yeast transformants, used to retransform *E. coli* JM109 to ampicillin resistance, and the plasmid DNA were rescued from the *E. coli* transformants. All these DNAs were able to transform *S. cerevisiae* KY106 to Ura$^+$ at high frequencies comparable to those by other yeast replicative plasmids such as YCp50 and pRS306 (Sikorski and Hieter, 1989). For 4 clones out of the 16 ARS$^+$ clones, base sequences of *T. versicolor* DNAs acting as ARSes in *S. cerevisiae* were determined, and deposited in the GenBank (AF506812 to AF506815). Base sequences of the remaining 12 clones were not determined, because the inserts on the clones are bigger than 1 Kbp. The sequences of AF506812 and AF506815 turned out to be complementary, indicating that the two clones are derived from the insertion of an identical fragment into the pKW105 vector DNA in opposite orientations (Fig. 2). All of the four contained two to four copies of the 11 bp [(A/T)TTTA(T/C)(A/G)TTT(A/T)] consensus sequence of the budding yeast ARS (Fig. 2).

We tried to transform *T. versicolor* to hygromycin B resistance several times with the plasmid DNAs of the ARS$^+$ clones, but failed to get the transformants. Thus actual ARS sequences of *T. versicolor* might differ significantly from those of *S. cerevisiae*. It is also possible that the ARS$^+$ clones are too unstable to be maintained as extrachromosomal plasmids in *T. versicolor*. It might therefore be possible to get the transformants if a centromeric sequence of *T. versicolor* is added to the ARS$^+$ clones, because centromeres are essential for the correct partitioning of duplicated chromosome pairs during cell division.

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