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The Yeast *Saccharomyces kluyveri* as a Recipient Eukaryote in Transkingdom Conjugation: Behavior of Transmitted Plasmids in Transconjugants

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The prokaryote *Escherichia coli* successfully conjugated with the eukaryote *Saccharomyces kluyveri*, which is relatively distant from the species *S. cerevisiae*. To achieve this transkingdom conjugation, we constructed three types of conjugative plasmids, namely integrative, replicative, and centromere vectors, for *S. cerevisiae*. By transfer of any of the three plasmids from *E. coli*, an *S. kluyveri* Ura$^-$ mutant was converted to the Ura$^+$ phenotype. This phenotype was easily lost under nonselective conditions. Southern analysis of the transconjugants clearly indicated the presence of the plasmids in many different structures and sizes.

The four different types of plasmid vectors for *Saccharomyces cerevisiae* are distinguishable by their mechanisms of replication in the transformant. These are (i) integrative vectors (YIp, autonomously replicating sequence [ARS]-less integrative plasmids), (ii) replicating vectors (YRp, ARS-containing plasmids), (iii) centromere vectors (YCp, CEN-containing plasmids), and (iv) episomal vectors (YEp, 2μ *ori* plasmids). These plasmids frequently do not work in other yeasts because of the species specificity of their chromosomal elements.

Saccharomyces kluyveri is a heterothallic budding yeast with two mating types (**a** and α). It is a relatively distant species from *S. cerevisiae* according to restriction fragment length polymorphism analysis of mitochondrial DNAs (11) and sequence analysis of α pheromones (14). Recently, the transkingdom conjugation between *Escherichia coli* and *S. cerevisiae* has been established (5, 6, 8–10). A preliminary report on conjugation between *E. coli* and the fission yeast *Schizosaccharomyces pombe* has also appeared (12). Because of the evolutionary and ecological interest of these studies, we attempted to carry out transkingdom conjugation between *E. coli* and *S. kluyveri*. These crosses allowed us to compare and contrast *S. kluyveri* and *S. cerevisiae* as recipients of different plasmid types transferred from *E. coli* with respect to two criteria: the efficiency of conjugation and subsequent plasmid stability.

E. coli HB101, which harbors conjugative and helper plasmids, was used as a donor. *S. kluyveri* D1-15 (4), was used as a recipient for the transkingdom conjugation (Table 1). Plasmids pAY201, pAY205, and pAY211 were constructed on the basis of pKT230, which was derived from RSF1010 (1) (Fig. 1 and Table 1). Plasmid pAY201, which carries *URA3* and tetracycline resistance genes from YIp5 (13), was originally designed as an integrative plasmid for *S. cerevisiae*. Plasmid pAY205 was constructed by the insertion of a *TRP1/ARS1* fragment from *S. cerevisiae* (13) at the *Eco*RI site of pAY201. Plasmid pAY211 has been constructed by the insertion of *S. cerevisiae CEN4* from YCpG-11 (7) at the *Eco*RI site of pAY205. Plasmid pRK2013 (3) containing mobilization genes was used as a helper plasmid.

The conjugation procedure has been described elsewhere (9, 10). The results of transkingdom conjugation between *E. coli* HB101 and the *ura3* mutant of *S. kluyveri* D1-15 are summarized in Table 2. We have observed that the conjugation frequency varies by more than 100 times and is particularly dependent on the recipient strains and mating conditions. By carefully choosing such conditions, we could control the conjugation frequency within a factor of 10. As shown in Table 2, the Ura$^+$ phenotype was conjugatively transmitted to *S. kluyveri* from *E. coli* which harbors one of the three kinds of pAY shuttle plasmids (YIp, YRp, or YCp for *S. cerevisiae*). However, the conjugation frequency was about 10^2 to 10^3 times lower than that obtained using *S. cerevisiae* YNN281 as the recipient. Ura$^+$ transconjugants did not appear in the absence of helper (cross numbers 3, 6 and 9), and essentially no Ura$^+$ revertants appeared in *S. kluyveri* (cross number 11). These results clearly indicate that three kinds of pAY plasmids were successfully transmitted from *E. coli* to *S. kluyveri* by transkingdom conjugation. Moreover, the frequency of conjugation caused by ARS-containing plasmid pAY205 was about 10 times higher than that caused by ARS-less plasmid pAY201. This suggests that *ARS1* from *S. cerevisiae* can work even in relatively distant species such as *S. kluyveri*, although its function is not as strong as it is in *S. cerevisiae*.

Previously, we found that the *URA3* gene introduced by transformation or conjugation can yield an "abortant microcolony" in *S. cerevisiae* (10). The abortant colonies were

TABLE 1. Plasmids and strains

Plasmid or strain	Relevant characteristics	Reference or source
YIp5	*URA3*, Tc^r	13
pKT230	*oriV-Q oriT-Q mob-Q*, Km^r	1
pRK2013	*oriV-C oriT-P mob-P tra-P*, Km^r	3
pAY201	*oriV-Q oriT-Q mob-Q URA3*, Km^r, Tc^r	10
pAY205	*oriV-Q oriT-Q mob-Q URA3 TRP1 ARS1*, Km^r, Tc^r	10
pAY211	*oriV-Q oriT-Q mob-Q URA3 TRP1 ARS1* CEN4 Km^r, Tc^r	This study
E. coli HB101	F$^-$ *recA13 pro* Sm^r	2
S. kluyveri D1-15	*MAT***a** *ura3 his*	4
S. cerevisiae YNN281	*MAT***a** *trp1-Δ ura3-52 ade2-1 lys2-801 his3-Δ200*	YGSC[a]

[a] YGSC, Yeast Genetic Stock Center, Berkeley, Calif.

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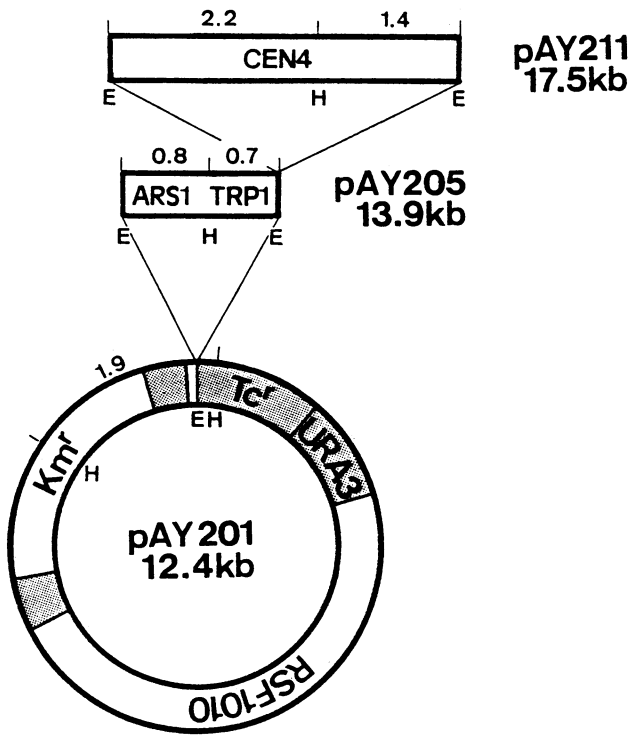

FIG. 1. Construction of *E. coli*-yeast conjugative shuttle pAY vectors. pAY205 was constructed by the insertion of an *ARS1/TRP1* fragment into pAY201. pAY211 was constructed by the insertion of a *CEN4* fragment into pAY205. E and H indicate *Eco*RI and *Hin*dIII cleavage sites, respectively. The shaded region indicates homologous region with YIp5 probe.

essentially formed because of transient expression of the *URA3* gene without chromosomal integration. According to Fujimura (4), the *URA3* gene of *S. cerevisiae*, when introduced by transformation, never recombines with the *S. kluyveri* chromosome because of its lack of homologous DNA sequence. Therefore, integration is highly repressed in *S. kluyveri*. Can conjugatively transmitted *URA3* make recipient *S. kluyveri* grow abortively? Abortant colonies of *S. kluyveri* did not appear unlike in *S. cerevisiae*. *S. kluyveri* transconjugants harboring pAY201 formed only normal colonies instead of abortant microcolonies. Furthermore, pAY201, as well as pAY205 and pAY211, were quickly cured under nonselective conditions (data not shown). This indicates that pAY201 exists as an autonomously replicating plasmid in spite of its lack of ARS. In other words, pAY201 itself may have some potential portion(s) of ARS activity which functions in *S. kluyveri*. Fujimura (4) has also obtained data indicating that a sequence(s) analogous to *S. kluyveri* ARS is expected to exist in YIp5, which consists of the main portion of pAY201 (Fig. 1).

To obtain more direct evidence for the presence of pAY plasmids in *S. kluyveri* transconjugants, total DNAs extracted from transconjugants were subjected to Southern hybridization after electrophoresis. *URA3* and tetracycline resistance genes from YIp5, which were a common component of pAY plasmids, were used as hybridization probes. We showed previously that there is no homology between genomic DNA of *S. kluyveri* and YIp5. DNA extracted from transconjugants formed by transfer of pAY201, pAY205, or pAY211 was digested with *Hin*dIII. The resultant digestion products were fractionated by agarose gel electrophoresis and analyzed by Southern hybridization using ^{32}P-labeled YIp5 probe. As shown in Fig. 2, pAY plasmids transmitted from *E. coli* by conjugation are clearly present in *S. kluyveri* transconjugants. To our surprise, almost all plasmids were structurally altered during conjugation, in contrast to conjugation into *S. cerevisiae* (Fig. 2A, lanes 2 through 6; 2B, lanes 1, 3, and 4; and 2C, lanes 2 through 6). Due to the homologous sequence among the original pAY plasmid and the YIp5 probe (Fig. 1), the hybridization signals are expected to appear at 10.5 and 1.9 kb in the case of *Hin*dIII fragments of pAY201, whereas 10.5- and 2.7-kb fragments are expected in the case of *Hin*dIII digestion of pAY205 and pAY211. As for the alteration ratio, the yield of altered plasmids for pAY201, pAY205, and pAY211 was 6 of 12, 3 of 6, and 6 of 6 transconjugants analyzed, respectively. In order to investigate the mechanism of this structural alteration, *E. coli* cells were transformed to kanamycin or tetracycline resistance by using plasmid DNA isolated from yeast transconjugants. Subsequently, the pAY plasmids extracted from *E. coli* transformants were digested with *Hin*dIII and then analyzed by agarose gel electrophoresis. As shown in Fig. 3, the conjugatively transmitted pAY plasmids were altered in their structures, regardless of plasmid type, compared with the original plasmids. When Fig. 2 and Fig. 3 are compared, some plasmid profiles cannot be reconciled (see, for example, Fig.

TABLE 2. Conjugation of *E. coli* and *S. kluyveri* via pAY plasmids

No.	Cross			No. of transconjugants per recipient
	Donor	Helper	Recipient	
1	HB101(pAY201)	HB101(pRK2013)	YNN281[a]	0.5×10^{-7}
				0.9×10^{-4} (abortant)[b]
2	HB101(pAY201)	HB101(pRK2013)	D1-15	1.0×10^{-7}
3	HB101(pAY201)		D1-15	ND[c]
4	HB101(pAY205)	HB101(pRK2013)	YNN281[a]	1.1×10^{-4}
5	HB101(pAY205)	HB101(pRK2013)	D1-15	0.8×10^{-6}
6	HB101(pAY205)		D1-15	ND
7	HB101(pAY211)	HB101(pRK2013)	YNN281[a]	0.6×10^{-4}
8	HB101(pAY211)	HB101(pRK2013)	D1-15	2.9×10^{-7}
9	HB101(pAY211)		D1-15	ND
10		HB101(pRK2013)	D1-15	ND
11			D1-15	ND

[a] Recipient is *S. cerevisiae* as a control (see Table 1). All others are *S. kluyveri*.
[b] Abortant microcolony. All others are normal colonies.
[c] ND, not detected ($\ll 1 \times 10^{-8}$).

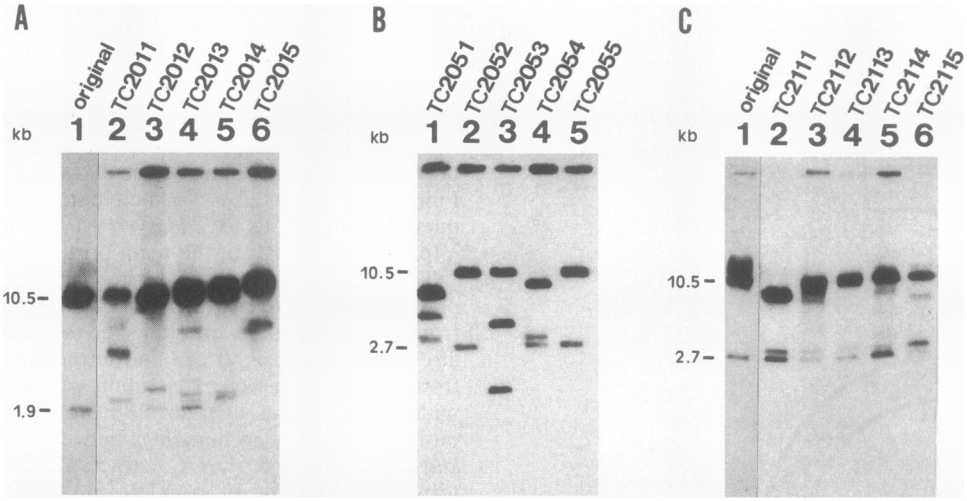

FIG. 2. Southern hybridization analysis of DNA from *S. kluyveri* transconjugants. Total DNA extracted from *S. kluyveri* transconjugants was separated by agarose gel electrophoresis, and then Southern hybridization was carried out by using a ^{32}P-labeled YIp5 probe. (A) Lane 1, original plasmid pAY201 digested with *Hin*dIII; lanes 2 through 6, genomic DNA from transconjugants induced by pAY201 (designated TC2011 to TC2015) digested with *Hin*dIII. (B) Lanes 1 through 5, genomic DNA from transconjugants induced by pAY205 (designated TC2051 to TC2055) digested with *Hin*dIII. (C) Lane 1, original plasmid pAY211 digested with *Hin*dIII; lanes 2 through 6, genomic DNA from transconjugants induced by pAY211 (designated TC2111 to TC2115) digested with *Hin*dIII.

2A, lane 2 and Fig. 3A, lane 3). This indicates the appearance of structurally altered plasmids with which *E. coli* transformants cannot be obtained. These results suggest that *oriV* or kanamycin and tetracycline resistance genes of the pAY plasmid may have been lost during conjugative transmission. On the other hand, both pAY201 and pAY211 introduced by the conventional transformation technique (4) maintained their original structure in *S. kluyveri* transformants, as shown in Fig. 4. These results suggest that the structural alteration is due to the conjugative transfer mechanism of plasmids rather than the characteristics of plasmids themselves. This raises another question, i.e., whether the plasmid alteration is due to adaptation for survival in host transconjugants or whether it is merely due to the conjugation mechanism. To investigate this, we retransmitted the pAY plasmids of *E. coli* transformants to *S. kluyveri* by conjugation. The result was that almost none of the altered pAY plasmids showed an increase in conjugation frequency (data not shown). Furthermore, Southern analysis of the yeast transconjugants indicates that the plasmid was altered again during the second conjugation (data not shown). Consequently, these results indicate that the structural alteration of the pAY plasmids is due to the conjugation mechanism and not to survival adaptation in *S. kluyveri*. Although pAY plasmids introduced by transformation did not change their structure at high frequency as described above, we cannot exclude the possibility that the pAY plasmid alteration is due

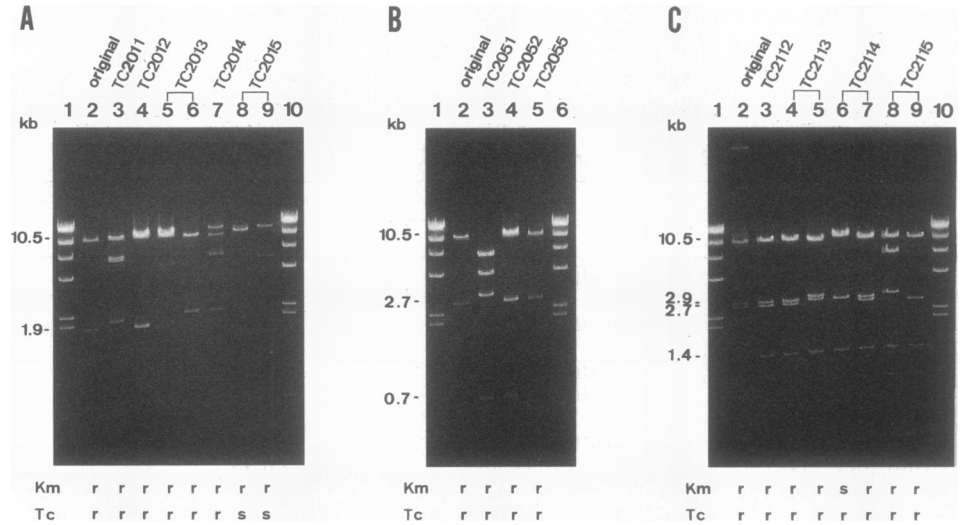

FIG. 3. Agarose gel electrophoresis of pAY plasmids recovered from *S. kluyveri* transconjugants. Above each lane is indicated the transconjugant from which the plasmid was prepared, transformed into *E. coli*, and subsequently analyzed after reextraction. For example, TC2051 indicates pAY205-induced transconjugant no. 1, from which the plasmid was transformed into *E. coli*. r and s at the bottom in the figure indicate kanamycin (Km) or tetracycline (Tc) resistance and sensitivity, respectively. Lane 1 in each panel contains molecular size markers.

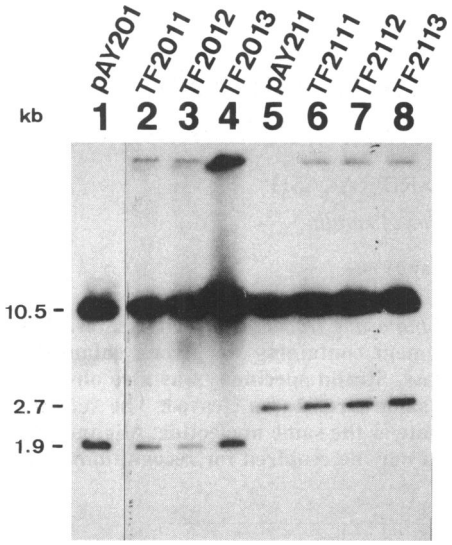

FIG. 4. Southern hybridization analysis of DNA from conventional *S. kluyveri* transformants. Transformation of *S. kluyveri* was carried out with pAY201 or pAY211. Southern hybridization of DNA from the transformants was carried out with ^{32}P-labeled YIp5 probe. Lane 1, original plasmid pAY201 digested with *Hin*dIII; lanes 2 through 4, genomic DNA from transformants (by pAY201 DNA) digested with *Hin*dIII; lane 5, original plasmid pAY211 digested with *Hin*dIII; lanes 6 through 8, genomic DNA from transformants (by pAY211 DNA) digested with *Hin*dIII.

to their reversible integration into the *S. kluyveri* genome. However, we could not obtain positive evidence on integration by Southern analysis of the genome of the *S. kluyveri* transconjugants by using the conjugatively altered plasmid as a probe (data not shown). The lack of integration may be attributed to the evolutionary differences between *S. cerevisiae* and *S. kluyveri* (11, 14) because the chromosomal integration in *S. cerevisiae* is caused by the homologous recombination between *URA3* on the plasmids and *ura3* on the host chromosome (9).

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REFERENCES

1. **Bagdasarian, M., R. Lurz, B. Rückert, F. C. H. Franklin, M. M. Bagdasarian, J. Frey, and K. N. Timmis.** 1981. Specific-purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors, and a host-vector system for gene cloning in *Pseudomonas*. Gene **16:**237–247.
2. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41:**459–472.
3. **Figurski, D. H., and D. R. Helinski.** 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in *trans*. Proc. Natl. Acad. Sci. USA **76:**1648–1652.
4. **Fujimura, H.** 1991. Transformation of the yeast *Saccharomyces kluyveri* by *Saccharomyces cerevisiae*-based plasmids. FEMS Microbiol. Lett. **82:**149–152.
5. **Heinemann, J. A.** 1991. Genetics of gene transfer between species. Trends Genet. **7:**181–185.
6. **Heinemann, J. A., and G. F. J. Sprague.** 1989. Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. Nature (London) **340:**205–209.
7. **Kojima, H.** Ph.D thesis. University of Tokyo, Japan.
8. **Nishikawa, M., K. Inomata, T. Sekito, K. Suzuki, and K. Yoshida.** 1993. Trans-kingdom conjugation as a symbiotic plasmid transfer between bacteria and yeasts, p. 537–544. *In* S. Sato, M. Ishida, and H. Ishikawa (ed.), Endocytobiology. Tübingen University Press, Tubingen, Germany.
9. **Nishikawa, M., K. Suzuki, and K. Yoshida.** 1990. Structural and functional stability of IncP plasmids during stepwise transmission by trans-kingdom mating: promiscuous conjugation of *Escherichia coli* and *Saccharomyces cerevisiae*. Jpn. J. Genet. **65:**323–334.
10. **Nishikawa, M., K. Suzuki, and K. Yoshida.** 1992. DNA integration into recipient yeast chromosomes by trans-kingdom conjugation between *Escherichia coli* and *Saccharomyces cerevisiae*. Curr. Genet. **21:**101–108.
11. **Okamoto, K., K. Suzuki, and K. Yoshida.** 1991. Physical mapping and RFLP analysis of mtDNAs from the ascosporogenous yeasts: *Saccharomyces exiguus*, *S. kluyveri* and *Hansenula wingei*. Jpn. J. Genet. **66:**709–718.
12. **Sikorski, R. S., W. Michaud, H. L. Levin, J. D. Boeke, and P. Hieter.** 1990. Trans-kingdom promiscuity. Nature (London) **345:** 581–582.
13. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76:**1035–1039.
14. **Yoshida, K., T. Hisatomi, and N. Yanagishima.** 1989. Sexual behavior and its pheromonal regulation in ascosporogenous yeasts. J. Basic Microbiol. **29:**99–128.