TEMPERATURE SENSITIVE R PLASMID ORIGINATED FROM SALMONELLA TYPHIMURIUM

Yoko Yoshida, Yoshiro Terawaki* and Rintaro Nakaya*

Department of Public Health Microbiology, The Institute of Public Health, 4-6-1 Shiroganedai, Minato-ku, Tokyo 108, Japan.

*Department of Microbiology, Tokyo Medical and Dental University School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan.

Received May 17,1974

SUMMARY

Tetracycline(TC) resistance in a strain of Salmonella typhimurium was easily transfered to an Escherichia coli K12 substrain at 30 C, but rarely at 37 C. Whereas, transfer of the other concomitant resistances, sulfathiazole(SA) and streptomycin(SM) were not restricted at 37 C. In addition, these drug resistances easily segregated either to (SA SM) or (TC) exclusively on the conjugal transfer. These indicate that (SA SM) and (TC) resistances are carried separately in two independent R plasmids in this S. typhimurium strain. Our additional transfer experiments between E. coli strains suggest that these R plasmids exist as seperate replicon also in E. coli, but they tend to associate on conjugal transfer.

In addition to the temperature sensitive transfer, TC resistance was specifically eliminated either from S. typhimurium or E. coli host cell at 42 C.

INTRODUCTION

R plasmids originated from Salmonella are mostly of fi^- type and their drug resistances easily segregate to various patterns on conjugal transfer (1). Contrary to these, R plasmids originally carried in Shigella are of fi^+ type and are rare to segregate on conjugal transfer. In addition some R plasmids in Salmonella are composed of separate replicon, i.e. sex factor or $\triangle$ factor and r-determinant (2-4). This type of R plasmid is designated as class 2 R plasmid (5,6).

Recently, we have examined R plasmids in Salmonella which were isolated clinically in Tokyo area since 1966, and found an R plasmid of which transfer of tetracycline resistance was specifically restricted at 37 C. In this communication we will report some genetic characteristics of this R plasmid.

MATERIALS AND METHODS

Bacterial strains and R plasmids. S. typhimurium ST 197-69, isolated from a patient of enterocolitis was resistant to more than 100 μg/ml of sulfathiazole(SA), dihydrostreptomycin(SM) and tetracycline(TC), respectively. These drug resistances were transferable, that is, they were carried by (an) R plasmid, R197. Segregants of R197 were designated as R197-1 (SA SM) and R197-2 (TC). E. coli K12 CSH-2A (F^- lac^+ met, nalidixic $acid^r$, $rifampicin^r$) and E. coli K12 W677 (F^- lac^- thr leu thi) were used as recipient strains on conjugal transfer of the drug resistances.

Selection of transconjugants. Selective plate of transconjugants CSH-2A mated with ST 197-69 was B medium (7) agar plate containing 0.001% of 2, 3, 5-triphenyl tetrazolium chloride, 40 μg/ml of methionine, 1.0% of lactose and 20 μg/ml of nalidixic acid (NA). Selection of transconjugants W677 mated with CSH-2A/R^+ was made by using A medium (8) agar plate containing each 20 μg/ml of threonine and leucine, 1 μg/ml of thiamine and 0.2% of glucose. These plates also contained either one of SA(50 μg/ml), SM (25 μg/ml) or TC(12.5 μg/ml), or with appropriate combinations of these three drugs as selective agent. Methods of mating were described in the legend to Table 1.

fi type of R plasmids It was determined by examining whether transfer of chromosomal gene of E. coli K12 W1895 (HfrC lac^+ met) was inhibited by the presence of the R plasmids in its cytoplasm. E. coli K12 W4753 (F^- lac^-) was employed as a recipient strain.

RESULTS

Transfer of the drug resistance. The transfer frequencies of drug resistance were in order of 10^{-4} per donor cell when conjugation was made between ST 197-69 and CSH-2A at 30 C using either one of SA, SM or TC as selective drug (Table 1). At 37 C or 42 C, however, the transfer of TC resistance was specifically restricted, whereas SA and SM resistances were transfered as high as at 30 C. Transconjugants obtained by the mating at 30 C always

Table 1. Transfer frequency of R plasmid 197 from S. typhimurium to E. coli K12 CSH-2A

Temperature (C) of mating	Temperature (C) of plating	Selective drug	Transfer frequency	Transconjugant resistance pattern	Transconjugant %
30	30	SA	6.1×10^{-4}	SA SM	100
		SM	4.5×10^{-4}	SA SM	100
		TC	5.2×10^{-4}	TC	100
		SA SM TC	1.0×10^{-7}	SA SM TC	100
30	37	SA	3.6×10^{-4}	SA SM	100
		SM	4.3×10^{-4}	SA SM	100
		TC	3.7×10^{-4}	TC	100
37	37	SA	5.7×10^{-4}	SA SM	100
		SM	2.8×10^{-4}	SA SM	100
		TC	$< 10^{-8}$	-	
		SA SM TC	$< 10^{-8}$	-	
37	30	SA	2.6×10^{-4}	SA SM	100
		SM	1.7×10^{-4}	SA SM	100
		TC	$< 10^{-7}$	-	
42	42	SA	7.9×10^{-5}	SA SM	100
		SM	2.1×10^{-5}	SA SM	100
		TC	$< 10^{-8}$	-	
		SA SM TC	$< 10^{-8}$	-	

Overnight culture of donor strain ST 197 in penassay broth (Difco) at 30 C with shaking was mixed with recipient strain CSH-2A at a ratio of 1 : 10 and grown in fresh penassay broth for 3hr either at 30 C, 37 C or 42 C. The mixture was plated on the selective plates and cultured for 48hr. The other unselected drug resistances of these transconjugants were examined by replica plating methods.

harvored resistances of either (SA SM) or (TC) exclusively, provided that single drug was used as the selective agent. If these three drugs were used simultaneously to select transconjugants CSH-2A (SA SM TC), the transfer became very poor even if the mating was done at 30 C. These results indicate that ST 197-69 carries two different R plasmids, i.e. R197-1 (SA SM) and

Table 2. Transfer frequency of R plasmid 197 from E. coli K12 CSH-2A to E. coli K12 W677

Temperature (C) of mating	Temperature (C) of plating	Preculture of donor	Selective drug	Transfer frequency	Transconjugant resistance pattern	Transconjugant %
30	30	30 C	SA	1.6×10^{-3}	SA SM TC	99.2
					SA SM	0.8
			SM	1.8×10^{-4}	SA SM TC	99.5
					SA SM	0.5
			TC	3.2×10^{-5}	SA SM TC	100
		37 C	SA	2.9×10^{-3}	SA SM TC	94
					SA SM	6.0
			SM	1.5×10^{-3}	SA SM TC	99
					SA SM	1.0
			TC	2.9×10^{-5}	SA SM TC	100
37	37	30 C	SA	$\geq 6.0 \times 10^{-3}$	NT*	
			SM	5.0×10^{-4}	SA SM TC	98
					SA SM	2.0
			TC	$< 10^{-7}$	-	
		37 C	SA	1.4×10^{-2}	SA SM TC	90
					SA SM	10
			SM	5.3×10^{-3}	SA SM TC	97
					SA SM	3.0
			TC	$< 10^{-7}$	-	
37	30	30 C	SA	1.3×10^{-3}	SA SM TC	99
					SA SM	1.0
			SM	4.6×10^{-4}	SA SM TC	99
					SA SM	1.0
			TC	7.0×10^{-6}	SA SM TC	100
		37 C	NT*			
42	42	30 C	SA	3.5×10^{-4}	SA SM TC	100
			SM	4.8×10^{-5}	SA SM TC	99.5
					SA SM	0.5
			TC	$< 10^{-7}$	-	
		37 C	SA	1.6×10^{-3}	SA SM TC	86
					SA SM	14
			SM	6.5×10^{-4}	SA SM TC	96
					SA SM	4.0
			TC	$< 10^{-7}$	-	

CSH-2A/R^+ and W677 were mixed and transconjugants W677/R^+ were selected. Mating procedures were the same as the experiment in Table 1. *, none tested.

R197-2 (TC). As shown in Table 1, only the temperature of mating did affect the transfer of TC resistance but those of donor culture and plating culture did not.

Secondary transfer of the drug resistances was tested between CSH-2A/R197 (SA SM TC) and W677. As shown in Table 2, transfer of drug resistances was again temperature sensitive when transconjugants were selected by TC. Contrary to the mating between ST 197-69 and CSH-2A, however, almost all transconjugants received SA SM and TC simultaneously as long as the donor was grown at 30 C, even in which mating was done at 37 C or 42 C and single drug was used for selection. These suggest a possibility that R(SA SM) and R(TC) exist as separate replicon also in CSH-2A but they have a tendency to associate to each other at least on conjugal transfer. Here we must memtion that even in the mating between CSH-2A/R^+ and W677, some of transconjugants (- 14%) received only (SA SM) resistances if the donor was grown at 37 C (Table 2) indicating that association between R197-1 and R197-2 in CSH-2A might be inhibited at higher temperature.

Stability of the R plasmids. Curing of TC resistance from ST 197-69, CSH-2A/R197 or CSH-2A/R197-2 were examined resulting that nearly 10% of TC sensitive cells were observed in any population within 6 to 7 generation at 42 C (Table 3). On the other hand, TC resistance at 30 C or 37 C (data not shown) and SA or SM resistances at 42 C were quite stable. This elimination of TC resistance, especially from CSH-2A/R197-2 at 42 C, means instability of R197-2 itself, in another words, it was not caused by the coexistence of R197-1.

As reported previously, a temperature sensitive R plasmid Rtsl made its host cell phenotypically temperature-sensitive (9). We, therefore, examined growth of the host cell at 42 C turbidometrically. Neither ST 197-69, CSH-2A/R197 nor CSH-2A/R197-2 was temperature sensitive in cell growth (Fig. 1,2), indicating that presence of TC sensitive cells in each population at 42 C was not the result of overgrowth of R^- cells but might be caused by positive curing of R(TC) from its host.

fi type of the R plasmids. W1895/R197-1 and W1895/R197-2 were constructed by cross with W677/R197-1 and W677/R197-2. Transfer of lac of these R^+

Table 3. Stability of R plasmid at 42 C

Host	R plasmid	Temperature of shaking	Gegeration	% curing of	
				TC	SA SM
S. typhimurium	R197	42 C	6- 7	10	0
			20-21	Exp.1 19	0
				Exp.2 29	0
		30 C	20-21	0	0
CSH-2A	R197	42 C	6- 7	9	0
			20-21	23	0
		30 C	20-21	0	0.5
CSH-2A	R197-2	42 C	6- 7	9	NT
			20-21	14	NT
		30 C	20-21	0	NT

Overnight culture at 30 C of each R^+ strains was diluted to 10^{-2} and 10^{-6} by fresh Penassay broth and grown to full growth either at 30 C or 42 C. Then, the cultures were plated on Penassay broth agar plates and TC and other drug resistances of resulting colonies were examined by replica plating method.

W1895 were not inhibited by the presence of R197-1 or R197-2, showing that both of these R plasmids belong to fi^- type (Table 4).

DISCUSSION

An R plasmid R197 (SA SM TC) in ST 197-69 always segregated either to R197-1 (SA SM) or R197-2 (TC) by the cross with CSH-2A if either one of drugs SA, SM or TC was used for selection of transconjugants. In addition transfer of TC resistance alone was temperature sensitive. These findings suggest that R197-1 and R 197-2 would exist as two separate replicon in ST 197-69. This interpretation is supported by the fact that the cotransfer of (SA SM) and (TC) was very poor even the mating was done at 30 C. Though both R197-1 and R197-2 are of fi^- type, it is possible that such plasmids could coexist in the same cell if they belong to different incompatibility group (10,11) of plasmid to each other. Contrary to the mating between ST 197-69 and CSH-2A, SA SM and TC resistances in CSH-2A were

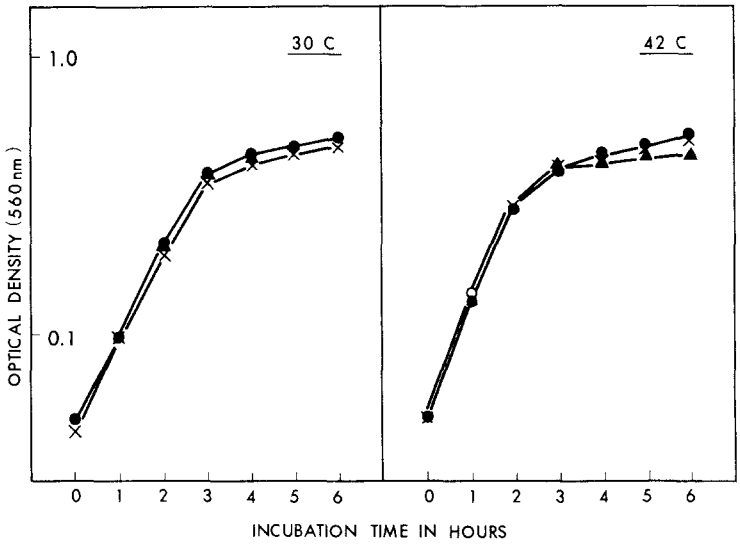

Fig. 1. Growth of S. typhimurium R^+ and R^- strains. ST 197-69(●–●) and its spontaneous segregants ST 197-69/R(SA SM)(▲–▲), ST 197-69/R(TC) (○–○) and ST 197-69/R^-(×–×) was grown at 30 C and diluted to approximately 5 X 10^7/ml in Penassay broth, respectively. Then, each of these was grown at 42 C or 30 C with shaking, and their growth were monitored turbidometrically by using Coleman junior II type spectrophotometer every one hour.

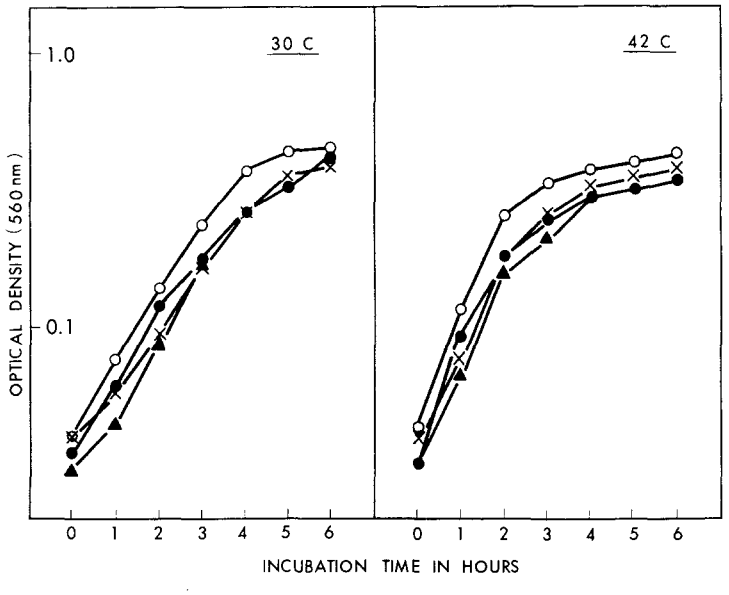

Fig. 2. Growth of E. coli K12 CSH-2A R^+ and R^- strains. Methods were the same as the experiment in Fig. 1. R197 (●–●), R197-1 (▲–▲), R197-2 (○–○), R^-(×–×).

Table 4. Inhibition of F-mating by the presence of R plasmid 197

Temperature of mating	R plasmid	Mating frequency mediated by F
30 C	197	1.7×10^{-4}
	197-1	1.0×10^{-4}
	197-2	1.1×10^{-4}
	R^-	9.3×10^{-4}
37 C	197	2.4×10^{-4}
	197-1	3.9×10^{-4}
	197-2	2.4×10^{-4}
	R^-	2.1×10^{-4}

Mating procedures were the same as the experiment in Table 1.

transfered simultaneously to W677 even if the mating was made at 37 C and selected either by SA or SM. It appears from this finding that R197-1 and R197-2 might combine forming single replicon in CSH-2A. But this is unlikely since transfer of TC resistance still remained temperature sensitive if TC was used for selection and in addition R(SA SM TC) in W677 obtained by the cross with CSH-2A easily segregated to R(SA SM) and R(TC) on conjugal transfer (data not shown). Therefore, we would consider that in CSH-2A R197-2 and R197-1 would interact to each other and as a result one is mobilized by the other on conjugal transfer although they exist as separate replicon. This state of association of the R plasmids was not observed in S. typhimurium. Whether the sex factor and r-determinant of each R197-1 and R197-2 exist as separate unit like class 2 R plasmid is presently unknown.

Specific curing of TC resistance at 42 C might derived from temperature sensitive replication of R197-2 since overgrowth of R^- cell against R^+ cell was not observed and the cured cell never recovered TC resistance at 30 C.

Molecular studies of these R plasmids are now in progress and its results will appear elsewhere.

ACKNOWLEDGMENTS

The authors wish to thank Drs. S. Sakai and H. Zenyogi for their kind supplying of Salmonella strains.

REFERENCES

1. Yoshikawa, M., Nagashima, S. and Matsushima, S. (1971) Japan. J. Microbiol. 15, 425-436.
2. Anderson, E. S. and Lewis, M. J. (1965) Nature, 27, 843-849.
3. Anderson, E. S. and Natkin, E. (1972) Mol. Gen. Genetics, 114, 261-265.
4. Milliken, C. E. and Clowes, R. C. (1973) J. Bacteriol, 113(2), 1026-1033.
5. Clowes, R. C. (1972) Bacteriol. Rev. 36(3), 361-405.
6. Embden, J. V. and Cohen, S. N. (1973) J. Bacteriol., 116(2), 699-709.
7. Nakaya, R. and Rownd, R. (1971) J. Bacteriol. 106, 773-783.
8. Nakaya, R., Nakamura, A. and Yamada, C. (1966) Igaku-no-Aumi, 56, 363-373.
9. Terawaki, Y., Kakizawa, Y. and Takayasu, H. (1968) Nature, 219, 284-285.
10. Chabbert, Y. A., Scavizzi, M. R., Witchitz, J. L., Gerband, G. R. and Bouanchaud, D. H., (1972) J. Bacteriol. 112, 666-675.
11. Grindley, N. D. F., Humphreys, G. O. and Anderson, E. S. (1973) J. Bacteriol. 115, 387-398.