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1) Thermosensitive and Nonthermosensitive R factors in *Enterobacteriaceae* and *Vibrio comma*

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Abstract

R factors isolated from the natural sources were classified into three groups. The first group of R factors, fi^+ R, was characterized by its inhibitory activity against the F-mediated fertility of host bacteria, and they were extraordinary unstable in the cells of *V. comma*. The second group, fi^- R factors, was specified according to their ability to restrict coexisting bacteriophages such as plkc, lambda and Tl. The R factors belonging to the second group were also very unstable in *V. comma*. The third group was thermosensitive R factors. Contrary to other nonthermosensitive R factors, this type R factor was rather stably maintained even in the cholera vibrios at 25C to 37C, although it was quickly diluted out from the host vibrios grown at 43C because of its temperature-sensitive replication, as in *E. coli*. Since the third group R factor is capable of producing new thermosensitive recombinant R harbourng increased number of resistance markers with fi^+, nonthermosensitive, multiple resistance factors, a possibility of increasing multiple drug resistant cholera vibrios by such R factors was suggested.

Introduction

Since Akiba et al. (2) and Ochiai et al. (13) first reported in 1959, an extrachromosomal, transferable drug resistance factor, R in *Enterobacteriaceae*, various studies have been performed. It is now understood that R factors responsible for bacterial drug resistance concern following chloramphenicol, tetracycline, streptomycin, sulfonamide, aminobenzyl penicillin, spectinomycin, gentamicin and mercury. (2, 13, 6, 3, 18)

It is also established that R factors can be transmitted from one strain to another by conjugation among all species of *Enterobacteriaceae*, (5, 12) *Pseudomonas aeruginosa*, (9) some species of *Pasteurella* (4) and various vibrios including cholera pathogens (1).

Transfer of R factors to *V. comma* from *E. coli* or *Shigella* has brought about a little discussion in Japan because of difficulty of its technique. Kuwahara et al. (8) reported in 1963 that an R factor controlling multiple drug resistance could be transferred from *Shigella flexneri* to *V. comma*, i. e. cholera vibrios and ElTor vibrios. Abe, Goto and Kuwahara (1) also presented results of successful R-transfer to *V. comma*, nonagglutinable vibrios and *Aeromonas hydrophila* from *E. coli* by conjugation, in 1966. Whereas, Yamada et al. (22) claimed that transfer of the R factor to *V. comma* was not confirmed so far as a synthetic agar medium was employed as the selective plate.

Since bacterial drug resistance due to R factors has been considered to play an important role in infections of the gastro-intestinal tract as well as of the urinary tract (2, 13, 16), possibility of transfer of R factors to *V. comma* is an interesting problem not only in the microbial genetics but also in clinical view point.

Another recent topic on R factors is the discovery of a thermosensitive R factor. Terawaki, Takayasu and Akiba (17) found in 1967 a kanamycin resistance factor of which replication is strongly suppressed by

higher temperature especially at 42C or more. This R factor was designated formerly as R(KM)^t and have currently changed to the Rtsl factor.

This paper deals with genetic behaviors of the Rtsl and various nonthermosensitive R factors in *V. comma* and *E. coli*.

Materials and Methods

Media. Penassay broth (Difco) was employed as liquid medium. MacConkey agar (Eiken, Japan), Lederberg's EMS agar (10) containing either 1% lactose or 1% maltose as the sole carbon source and supplemented with apropriate vitamins or amino acids, and TCBS agar (7, 11 : Eiken, Japan) were used as plating media.

Microorganisms. *E. coli* JE948 carrying R100, *E. coli* YA5 having the Rtsl factor and *E. coli* YA31 inheriting the NR73 factor were employed as initial donors of an fi$^+$ nonthermosensitive multiple resistance factor, a thermosensitive kanamycin resistance factor, and fi$^-$ nonthermosensitive tetracycline-streptomycin-sulfonamide resistance factor, respectively. The first and second strains have already listed, (23) and the third strain was given by M. Yoshikawa and described in Table 1. *E. coli* YA13 mentioned in the Table 1 was also used as the donor of thermosensitive recombinant between the Rtsl and R100 factors. *E. coli* CSH2 R$^-$ strain, *V. comma* E1, *V. comma* E2, *V. comma* E3, and *V. comma* E4 were employed as the recipients of R factors.

Drugs. Kanamycin sulfate (Takeda Chem, Ind. Co. Ltd., Osaka, Japan), dihydrostreptomycin sulfate (Takeda), tetracycline hydrochloride (Japan Lederly, Tokyo, Japan), chloramphenicol (Yamanouchi Pharm. Co. Lts., Tokyo, Japan) and sulfisoxazole (Yamanouchi) were employed and abbreviated as KM, SM, TC, CM and SA, respectively.

Method for mutual transfer between a strain carrving Rtsl and one bearing fi$^-$ NR73. *E. coli* CSH2 carrying the Rtsl factor was used as the donor of Rtsl and recipient of NR73, and *E. col* YA31 was employed as the opposite donor and recipient. *E. coli* CSH2 R$^-$ and *E. coli* W3630 R$^-$ were also employed as the recipients of NR73 and Rtsl, respectively. The strains were separately cultured in drug-free Penassay broth at 25C, 37C and 43C for 18 hours. Equal volumes of the donor and recipient cultures and

TABLE 1 *List of strains and their properties*

Strain name		Taxon	Phenotypic properties								Episomes or Plasmids	Reference
Gurrent	Former		Lac	Mal	Met	CM	TC	SM	SA	KM		
JE948	W3630	*E. coli*	+	−	+	R	R	R	R	S	R100	23
YA5	W3630	*E. coli*	+	−	+	S	S	S	S	R	Rts1	23
YA11	Hfr-C	*E. coli*	+	+	−	S	S	S	S	R	Rts1	23
YA13	W3630	*E. coli*	+	−	+	S	S	R	R	R	Rts2	23
YA31	W3630	*E. coli*	+	−	+	S	R	R	R	S	NR73	
CSH2		*E. coli*	+	+	−	S	S	S	S	S	None	
E1		*V. comma*	+	+	+	S	S	S	S	S	None	1 E1Tor
E2		*V. comma*	+	+	+	S	S	S	S	S	None	1 〃
E3		*V. comma*	+	+	+	S	S	S	S	S	None	1 〃
E4		*V. comma*	+	+	+	S	S	S	S	S	None	1 〃

Following abbreviations were used : Lac, lactose ; Mal, maltose ; Met, methionine ; CM, chloramphenicol ; TC, tetracycline ; SM, streptomycin ; SA, sulfonamide ; KM, kanamycin ; +, fermentable for sugars and non-requiring for amino acids ; −, Non-fermentable for sugars and requiring for amino acids ; S, sensitive ; R, resistance.

fresh Penassay broth were mixed in all combinations, and aliquots were incubated at 25C, 37C and 43C in accordance with the subculture temperatures for 6 hours without shaking. One tenth ml of each mating mixture was spread on EMS agar plates containing 1% maltose, 50 μg/ml of methionine and 25 μg/ml of SM, or on those containing 1% lactose and 50 μg/ml of KM. By the former plates, only the conjugal progenies of *E. coli* CSH2 inheriting the NR73 factor were selected, and the conjugal progenies of *E. coli* W3630 derivatives (W3630 and YA31) inheriting Rtsl were selected by the latter plates, because of inability to ferment of maltose in *E. coli* W3630 derivatives and of methionine-requiring character of *E. coli* CSH2 derivatives.

Method for transfer of various R factors from E. coli R⁺ substrains of W3630 to E. coli CSH2 R⁻ strain. Transfer of an fi⁺, nonthermosensitive R100 factor from *E. coli* JE948 to *E. coli* CSH2 R⁻ strain was performed in the same manner described before, except using the EMS-maltose plates containing methionine and 25 μg/ml of CM.

Method for traosfer of R factors from E. coli W3630 R⁺ substrains to V. comma. In this experiment also, *E. coli* JE948, YA31 and YA5 were employed as the donor of R100, NR73 and Rtsl factors, respectively. And *V. comma* ElTor type strains of E1, E2, E3 and E4 were used as the recipients. All strains of cholera vibrios were isolated in Philippine in 1961, and they are R⁻ and drug-sensitive. These strains were bestowed from Dr. S. Kuwahara. *E. coli* and *V. comma* strains were separately cultured in drug-free Penassay broth at 25C, 37C and 43C for 18 hours. One volume of *V. comma* culture, one forth volumes of *E. coli* culture and two volumes of fresh Penassay broth were mixed, and incubated at 25C, 37C, 43C for 24 hours without shaking. One tenth ml of any mixed culture was spread on TCBS selective plates (pH : 8.0) containing 5 μg/ml of CM, 50 μg/ml of TC or 100 μg/ml of KM. On the TCBS plates containing the antibiotics, the donor strain, *E. coli*, could not grow because of alkaline pH of the medium and of bacteriostatic action of the ingredients, the R⁻ recipient vibrios were also unable to form their colonies because of bacteriostatic action of the antibiotics. Only *V. comma* (recipient) inheriting any R factors was selected by that plate.

Method for testing the resistance level or resistance pattern of V. comma inheriting R factors. Colonies of *V. comma* appearing on the drug-containing TCBS plates were purified on the same selective medium containing the same amount of the antibiotic. The purified colonies were picked up with sterile tooth sticks, suspended in a minute amount of fresh Penassay broth poured in the holes of Microtiter tray, and then tested for their drug resistance levels and patterns by streaking on the TCBS plate series containing increased amounts of CM, TC, SM, SA or KM.

Method for examination of the stabilities of R factors in E. coli and in V. comma. Purified one colony confirmed to inherit any R factor, of *E. coli* or *V. comma* was transferred into drug-free Penassay broth, divided into 2 tubes, and incubated at 25C and 43C for 24 hours. Then, the culture was streaked on drug-free MacConkey plates or TCBS plates, followed by incubation at 37C for 24 hours. Drug resistance levels and patterns of appearing colonies of *E. coli* and *V. comma* were tested by the direct replica method on McConkey plates and TCBS plates containing various amounts of CM, TC, SM, SA, or KM, respectively. If any R factor was so unstable that colonies tested by the replica method were found to be all sensitized, comparison of colony numbers obtained with drug-free plates and drug-containing plates was performed to calculate the proportion of sensitized (R⁻) cells to resistant (R⁺) cells.

Results

Slight one-way immune of E. coli carrving fi⁻. noothermosensitive R factor against the Rtsl factor. As shown in Table 2, conjugal transfer frequency of the Rtsl factor to a recipient cells already carrying another fi⁻ nonthermosensitive NR73 factor was somewhat lower than to an R⁻ cells of the same

TABLE 2 *Mutual transfer of the thermosensitive Rts1 (kan) and fi⁻, nonthermosensitive NR73 (tes. str. sul) factors*

Cultured microbes	Culture temperatures			Transfer frequency/Donor	
	Subcult.	Mixed	Select.	Rts1	NR73
E. coli CSH2 : Rts1	25	25	37	7.3×10^{-4}	—
+	37	37	37	3.3×10^{-5}	—
E. coli W3630 : R⁻	43	43	37	3.3×10^{-6}	—
CSH:Rts1	25	25	37	8.1×10^{-5}	1.1×10^{-5}
+	37	37	37	3.7×10^{-7}	1.5×10^{-1}
W3630 : NR73	43	43	37	$<10^{-8}$	7.3×10^{-3}
CSH2 : R⁻	25	25	37	—	4.2×10^{-6}
+	37	37	37	—	3.3×10^{-2}
W3630 : NR73	43	43	37	—	3.2×10^{-3}

Mixing culture of the donor and recipient was performed at 25C, 37C and 43C, for 6 hors without shaking

strain. Transfer frequency of the NR73 factor, however, was not influenced by the existence of Rtsl in the recipient strain. This is a slight one-way immune against the Rtsl factor by an fi⁻, nonthermosensitive NR73 factor. This table also indicates the optimum temperatures of nonthermosensitive R factors, either fi⁺ or fi⁻, are at 37C to 43C, while that of the Rtsl factor is at 25C. (23)

Comparison of the transfer frequencies of R factors at respective optimum temperature to E. coli and to V. comma. Table 3 presents the transfer frequencies of fi⁺ R100, fi⁻ NR73 and thermosensitive Rtsl factors by 6 hour mixed culture from *E. coli* W3630 substrains to R⁻ *E. coli* CSH2 strain, at respective optimum temperatures, i. e. 37C for R_{100} and NR73, and 25C for Rtsl. Whereas, transfer frequencies of these R factors from *E. coli* to *V. comma* were shown in Table 4 and 5. It is noteworthy that the conjugal transfer frequency of any R factor to *V. comma* from *E. coli* was much lower than that to *E. coli* another strain from the same *E. coli*, eventhough the duration of mixed culture for R-transfer to *V. comma* was 3 times longer than that to *E. coli*.

Change of drug resistance patterns and resistance levels in V. comma by inheriting R factors. When *V. comma* strains received the NR73 factor, the microbes became resistant not only to TC but also to SM and SA in conformity to the character of this R factor, even only 50 μg/ml of TC was used as the selective agent, as shown in Table 6. As inheriting the Rtsl, El Tor vibrios increased their KM-resistance

TABLE 3 *Transfer frequencies of the R100 (chl. tet. str. sul) : fi⁺, NR73 (tet. str. sul) : fi⁻ and Rts1 (kan) factors from E. coli W3630 : R⁺ strains to E. coli CSH2 : R⁻ strain at various temperatures*

Culture temperatures			Transfer frequency/Donor of		
Subcult.	Mixed	Select.	R100	NR73	Rts1
25	25	37	7.5×10^{-7}	4.2×10^{-6}	1.4×10^{-3}
37	37	37	1.3×10^{-5}	3.3×10^{-2}	2.9×10^{-4}
43	43	37	8.5×10^{-5}	3.2×10^{-3}	$<10^{-8}$

Mixed culture of the donor and recipient was performed at respective temperature for 6 hours without shaking

TABLE 4 *Transfer frequencies of the R100 (chl. tet. str. sul) : fi⁺ ; NR73 (tet. str. sul) : fi⁻ and Rts1 (kan) factors from E. coli W3630 : R⁺ strains to V. comma ; El Tor E2 : R⁻ strain at various temperatures*

Culture temperatures			Transfer frequency/Donor of		
Subcult.	Mixed	Select.	R100	NR73	Rts1
25	25	37	$<10^{-9}$	$<10^{-9}$	5.5×10^{-7}
37	37	37	1.6×10^{-8}	1.8×10^{-8}	$<10^{-9}$
43	43	37	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$

Mixed culture of the donor and recipient was performed at respective temperature for 24 hours without shaking

TABLE 5 *Transfer frequencies of the R100 (chl. tet. str. sul) : fi⁺, NR73 (tet. str. sul) : fi⁻ and Rts1 (kan) factors from E. coli W3630 : R⁺ strains to 4 R⁻ strains of V. comma at respective optimum temperatures*

Recipient strain	Transfer frequency / Donor of		
	Rts1	R100	NR73
V. comma E1	4.2×10^{-8}	1.7×10^{-7}	$<1.1\times10^{-9}$
V. comma E2	5.5×10^{-7}	1.6×10^{-8}	1.8×10^{-8}
V. comma E3	1.5×10^{-7}	2.9×10^{-7}	4.9×10^{-8}
V. comma E4	1.1×10^{-6}	1.5×10^{-7}	$<1.1\times10^{-9}$

Mixed culture of the donor and recipient was performed at 25C for Rts1, and at 37C for R100 and NR73, for 24 hours without shaking.

TABLE 6 *Change of drug resistance patterns of V. comma strains by inheriting the NR73 (tet. str. sul) : fi⁻ factor*

Strain	R factor	Maximum growth permitting concentration on TCBS plates (pH : 8.0) of (μg/ml)				
		CM	TC	SM	SA	KM
V. comma E1	None	0.25	25	25	50	50
V. comma E2	None	0.25	25	25	50	50
V. comma E3	None	0.25	25	25	50	50
V. comma E4	None	0.25	25	25	50	50
V. comma E1	NR73	could not be purified				
V. comma E2	NR73	0.25	100	800	1,000	50
V. comma E3	NR73	0.25	100	800	1,000	50
V. comma E4	NR73	could not be purified				

as high as more than 800 μg/ml. Whereas their R⁻, spontaneous KM-resistant mutants hardly grew on the TCBS plates containing only 100 μg/ml of KM, as indicated in Table 7. Though only 5 μg/ml of CM was employed as the selective agent, as mentioned in Table 7, *V. comma* inheriting the R100 factor was found to be resistant not only to CM but also to TC, SM and SA.

Stabilities of the R factors in the cells of E. coli and V. comma. Table 8 shows stabilities of R100,

TABLE 7 *Change of drug resistance levels or resistance patterns in four strains of V. comma by inheriting the Rts1, R100 and their recombinant R factors*

Strain	R factor	Maximum growth permitting concentration on TCBS plates (pH : 8.0) of (μg/ml)				
		CM	TC	SM	SA	KM
V. comma E1	Rts1*	0.25	25	25	50	800
V. comma E2	Rts1	0.25	25	25	50	800
V. comma E3	Rts1	0.25	25	25	50	800
V. comma E4	Rts1	0.25	25	25	50	800
V. comma E1	R100**	25	800	200	1,000	50
V. comma E2	R100	10	800	200	1,000	50
V. comma E3	R100	10	800	200	2,000	50
V. comma E4	R100	10	800	200	2,000	50
V. comma E1	Rts2***	0.25	25	400	2,000	800
V. comma E2	Rts2	0.25	25	400	2,000	800
V. comma E3	Rts2	0.25	25	400	2,000	800
V. comma E4	Rts2	not tested				

* Rts1 confers KM-resistance on its host and undergoes thermosensitive replication
** R100 confers the resistance to CM, TC, SM, and SA on its host and is nonthermosensitive
*** Rts2 confers the resistance to KM, SM and SA on its host, and is a thermosensitive recombinant between Rts1 and R100 (23)

TABLE 8 *Stabilities of the R100 (chl. tet. str. sul) : fi^+, NR73 (tet. str. sul) : fi^- and thermosensitive Rts1 (kan) factors in E. coli grown at 25C or 43C*

Host bacteria	R factor	Culture temperature (C)	Elimination frequency (%)
E. coli W3630	R100	25	0.**
		43	0.
	NR73	25	0.
		43	0.
	Rts1	25	0.
		43	91.9
	Rts2*	25	0.
		43	99.5
E. coli CSH2	Rts1	25	0.
		43	88.5
E. coli YA11 (Hfr)	Rts1	25	0
		43	90.3

* Rts2 is a recombinant between Rts1 and R100, confers the resistance to SM, SA and KM, and undergoes thermosensitive replication (23)
** Elimination frequencies of an R factor was examined by replica method (23)

NR73 and Rts1 factors in *E. coli* starins, and Table 9 indicates stabilities of the R factors in El Tor vibrios. As shown in the former Table, the R100 and NR73 factors were stably maintained in the cells of *E. coli* grown either at 25C or 43C, while the Rts1 factor was quickly eliminated from the host grown at

TABLE 9 *Stabilities of the R100 (chl. tet. str. sul) : fi⁺, NR73 (tet. str. sul) : fi⁻, and thermosensitive Rts1 (kan) and Rts2 (kan. str. sul) in V. comma grown at 25C or 43C*

Host vibrio	R factor	Culture temperature (C)	Elimination frequency (%)
V. comma E1	R100	25	99.99999
		43	99.97
	NR73	25	99.99999
		43	99.991
	Rts1	25	0.
		43	99.9999
	Rts2	25	8.3*
		43	99.99999
V. comma E3	Rts1	25	7.1
		43	99.99995

* Elimination of an R factor from cholera vibrios was examined by the replica method when the elimination frequency was low, and it was tested by comparison of colony numbers on drug-free TCBS plates and those on drug-containing plates when the frequency was high.

43C because of its thermosensitive replication. Contrary to the above results, stabilities of the R factors in El Tor vibrios were quite different from in *E. coli*. Nonthermosensitive fi⁺ R100 and fi⁻ NR73 factors were so unstable in any strain of *V. comma*, that even purification and conservation of R⁺ vibrios required to employ drug-containing media. The thermosensitive Rtsl factor, however, was rather stably maintained in *V. comma* at lower temperatures, such as 25C, and it was eliminated from the host grown at 43C as in *E. coli* or more quickly.

The author has already reported that the thermosensitive KM-resistant factor, Rtsl, can produce new thermosensitive recombinants with the nonthermosensitive fi⁺ R100 factor, harbouring resistance markers for KM, SM and SA, or for KM and SA, and rarely for SM and SA. (23) It is of interest that one investigates the stability of such thermosensitive recombinants between Rtsl and R100 in the cells of *V. comma*, since one parent, Rtsl, is rather stable at lower temperatures and another parent, R100, is extraordinary unstable in this microbe. As mentioned in Table 8 and Table 9, the recombinant R, Rts2 (23), demonstrated genetic behaviors similar to those of Rtsl but not of R100, either in *V. comma* or in *E. coli*. This fact may indicate that recombinants between Rtsl and a multiple drug resistance factor can induce certain multiple drug-resistant cholera vibrios.

Discussion

It has been confirmed that various R factors, nonthermosensitive fi⁺, nonthermosensitive fi⁻ and thermosensitive R factors, were transferred from *E. coli* to *V. comma*, eventhough the technique to select cholera vibrios just inheriting R factors was rather difficult. For instance, *V. comma* just inheriting nonthermosensitive fi⁺ R100 factor was precisely selected only with the TCBS selective plate containing 5 μg/ml of CM. At lower concentrations of CM, spontaneous CM-resistant mutant, which is R⁻, was selected, and at higher concentrations of the drug, even R100⁺ vibrios were uncapable of colony formation. For selection of *V. comma* just inheriting the Rtsl factor, only 100 μg/ml of KM was found to be critical. The R⁺ cells of *V. comma* repurified on the drug containing TCBS agar plates, however, demonstrated their CM- and KM-resistance as high as 10-25 μg/ml and 800 μg/ml, respectively. It is actually unknown the reason why *V. comma* just inheriting an R factor can be selected only with a selective plate containing

TABLE 10 *Tentative classification of naturally isolated R factors*

Group	Popular resistance patterns	Relation to other episomes or plasmids			
		F factor	Col factor	Phages	R factor
I	(chl. tet. str. sul) (tet) (chl. str. sul)	ffi$^+$ (inhibition of F-fertility)	cli$^-$ (stable coexisting)	spp$^-$ (no restriction)	Immune to ffi$^+$ R factors
II	(str. sul) (str) or (sul) (tet. str. sul)	ffi$^-$	cli$^+$ (mutual exclusion)	spp$_1{}^+$ (restriction of plkc, lumbda and T1)	Immune to ffi$^-$ R factors
III	(kan)^{ts} (kan, str. sul)^{ts} (str. sul)^{ts}	ffi$^-$	?	spp$_2{}^+$ (restriction of T2h, T4b, T4d and T6)	No distinct immune to I and II

very low concentrations of an antibitic. However, one may postulate that the phenotypic expression of R factors in *V. comma* is much slower than in *E. coli*. It is also noteworthy that ordinary nonthermosensitive R factors, either fi$^+$ or fi$^-$, are extraordinary unstable in *V. comma* at any temperature while thermosensitive R factors can be stably maintaintained in the host bacteria at least at low temperatures, below 37C. This fact may indicate that the thermosensitive Rtsl factor is quite different in its genetic natures from ordinary nonthermosensitive R factors. According to already known genetic characteristics of various R factors, and the obtained results, the author has tentatively classified naturally isolated R factors into three groups as indicated in Table 10. The first group occupies a major part of R factors and characterized by the ability to inherit the F-mediated fertility of host bacteria. (19, 24) So that, this group has been called as fi$^+$ R factors or F-type R factors. (19) R factors belonging to this group have been known to inherit resistance markers mainly for CM, TC, SM, and SA, some times for TC only, and rarely for CM, SM and SA. Other features of this group are as follows : no restriction of any coexisting phages as far as examined ; nonthermosensitive replication ; and low frequency of R-mediated fertility. (15, 19, 20, 21, 24, 25) The second group of R factors has been called as fi$^-$ R factors or colicinogenic factor-type, because they do not interfer the F-mediated fertility (20, 24), and they are easily excluded from the host bacteria carrying both this type R factor and certain colicinogenic factor, such as Ia and Ib. (14) Their own replication undergo nonthermosensitively, and usually harbour resistance markers for both or any of SM and SA, but rarely for TC, SM and SA, or TC and SM. A big feature of this group is a restrive activity against certain coexisting phages such as plkc, lumbda and Tl. The plating efficiencies of those phages are markedly suppressed in bacterial cells bearing this type R factors. And this fact has been named by Yoshikawa and Akiba (25) by spp (suppression of plaque formation of phages) or by restriction of phages by R factors(21). Another characteristic of this group is higher frequency of R-mediated fertility. (15) The third group is the thermosensitive Rtsl factor and its offsprings. In the Table, Rts2 and Rts3 are thermosensitive recombinants between the Rtsl and R100 factors. (23) Though this group R factors do not inhibit the F-mediated fertility, they also do not restrict coexisting the plkc, lumbda and Tl phages. (23) They, however, suppress the plating efficiencies of all T-even phages, T2h, T4b, T4d and T6, at lower temperatures, i. e. below 30C. It has been shown that the Rtsl factor and its offsprings are distinguished from other R factors not only by their temperature sensitive replication but also by many other genetic natures. (23)

It is of interest that R factors of the group I and II are extraordinally unstable in *V. comma* at any temperature while group III R factors are stably maintained in the microbe at least below 30C. From the clinical view point, it is obvious that the group I and II R factors have an important responsibility

for anti-chemotherapy infections of the gastrointestinal tract and of the urinary tract by any species of *Enterobacteriaceae*, although it is quite doubtful to consider that these R factors would induce multiple drug resistant cholera vibrios, because of their unstability in the latter microbes. On the other hand R factors involved to the third group, may induce drug resistant cholera vibrios as well as drug resistant *E. coli*, in stable form. Furthermore, the Rtsl factor of group III can produce new thermosensitive R factors harbouring increased numbers of resistance markers, i. e. Rts2 and Rts3 (23), by recombination with the R100 factor. This fact may suggest that multiple drug resistant cholera vibrios might increase in number if this type R factor would be widely spread in the natural field.

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