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INTERGENUS CELL FUSION BETWEEN L-FORM CELLS OF *PSEUDOMONAS AERUGINOSA* AND *ESCHERICHIA COLI*

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SUMMARY Intergenus cell fusion of prokaryotic bacteria was demonstrated for the first time; namely, fusion products doubly resistant to streptomycin and tetracycline were produced by polyethylene glycol treatment of a mixture of the streptomycin-resistant L-form of *Pseudomonas aeruginosa* and tetracycline-resistant L-form of *Escherichia coli*.

INTRODUCTION

In 1963, Okada and Tadokoro discovered that hemagglutinating virus of Japan (HVJ) induced cell fusion between Ehrlich ascites tumor cells from different mouse strains. Three years later, this work was followed by demonstration of HVJ-induced interspecies cell fusion between KB cells of human origin and Ehrlich ascites tumor cells or L cells both from mice or PS cells from pigs (Okada and Murayama, 1965). The formation of hybrids between mouse and man cells by HVJ treatment was confirmed by Harris and Watkins (1965). These pioneering studies on interspecies cell fusion have been confirmed and extended by a number of investigations, and have led to a new field of genetics, namely somatic cell genetics, which provides a novel and very useful method for genetic mapping of chromosomal genes in humans (Tischfield and Ruddle, 1974).

Interspecies or intergenus cell fusion of plant cells seems potentialy important in plant breeding, as illustrated by the finding that polyethylene glycol (PEG)-induced fusion between tomato and potato protoplasts resulted in protoplast heterokaryons, and after cell wall regeneration produced hybrid tomato-potato hybrid plants (Melchers, Sacrystan and Holder, 1978).

With respect to microbes, there are a few reports on cell fusion between different species of fungi, which are eukaryotes like animals and plants: e.g. hybridization by PEG-induced protoplast fusion between *Penicillium chrysogenum* and *Penicillinum notatum* (Anné and Peberdy, 1976) and between *Saccharo-*

mycopsis fibuligera and *Candida tropicalis* (Provost et al., 1978).

With respect to prokaryotic bacteria, hybridizations of several species of streptomyces by PEG-induced protoplast fusion have been reported (Godfrey, Ford and Huber, 1978). This achievement let us to expect that the cell fusion technique could be used for either better production of known antibiotics or development of novel antibiotics by streptomyces. Interspecies protoplast fusion between coagulase-positive *Staphylococcus aureus* and some coagulase-negative staphylococci induced by PEG was also demonstrated in a recent study of Götz, Ahrné and Lindberg (1981).

However, these have been no reports on hybridization between prokaryotic bacterial cells of different genera. In this paper, we report the induction by PEG of intergenus cell fusion products between the L-forms of *Pseduomonas aeruginosa* and *Escherichia coli.*

MATERIALS AND METHODS

1. *Test organisms and growth media*

The bacterial strains used in this study are shown in Table 1. L-form strains of *P. aeruginosa* (IFO 3455) and *E. coli* (Ohtani), abbreviated as Ps-L and Ec-L, respectively, in this report, were isolated by the conventional lysozyme-EDTA method. Ps-L was a generous gift from Dr. Yuzuru Homma, Kitazato Institute, and Dr. Akihiro Yamamoto, Chugai Pharmaceutical Co., Ltd. (Yamamoto and Homma, 1978a; 1978b). Ec-L was isolated in the Department of Bacteriology, Yokohama City University School of Medicine. Both L-form strains grew well in liquid or solid basal medium: Difco Brain-Heart-Infusion (BHI) broth supplemented with 4.5% NaCl, without or with 1.0% agar (Grade I, Wako Pure Chemical Ind., Osaka). For Ps-L, the minimum inhibitory concentration (MIC) of streptomycin sulfate (SM, Meiji Seika Co., Tokyo) was more than 2,000 μg/ml, and that of tetracycline (TC, Sigma Chemical Co.) was less than 1 μg/ml. Thus the Ps-L strain was resistant to SM, but sensitive to TC. The original Ec-L strain was susceptible to both SM and kanamycin (MIC <20 μg/ml and <5 μg/ml, respectively) but was resistant to TC (MIC >5 μg/ml and <50 μg/ml). TC-resistance of Ec-L was increased by serial subculture in liquid and solid basal medium supplemented with increasing concentrations of the drug to obtain a mutant, Ec-L(Tc^r), showing higher TC resistance (MIC >50 μg/ml). Other characteristics of Ps-L(Sm^r) were resistance to kanamycin (MIC >160 μg/ml of kanamycin sulfate, KM, Meiji Seika) and utilization of sucrose, whereas Ec-L(Tc^r) was susceptible to KM (MIC <5 μg/ml) and could not utilize sucrose, as described later. Ps-L(Sm^r) and Ec-L (Tc^r) were subcultured in liquid medium or on solid medium supplemented with SM (2,000 μg/ml) and TC (50 μg/ml), respectively and were repeatedly confirmed to maintain their original properties.

2. *PEG treatment to induce cell fusion*

The Ps-L(Sm^r) and Ec-L(Tc^r) strains were grown at 37 C in liquid basal medium supplemented with penicillin G (PC-G, Meiji, 500 U/ml) together with SM (2,000 μg/ml) and TC (50 μg/ml). Overnight cultures (10 ml each) of test strains were gently homogenized by agitation in a Thermo-Mixer (Thermonics Co., Tokyo). Samples of 2 ml of culture of each strain or mixtures of the two (one ml of each) were treated with PEG under the conditions described previously (Hirachi, Kurono and Kotani, 1979; 1980; Hirachi et al., 1982).

3. *Assay of formation of cell fusion products*

PEG-treated mixtures of the indicated combinations of Ps-L(Sm^r) and Ec-L(Tc^r) were incubated for enrichment culture in liquid basal medium containing PC-G (500 U/ml) unless otherwise stated, and then serially diluted 10-fold with liquid basal medium. Portions of 1 ml of appropriate dilutions were cultured in triplicate by the pour-plate method in solid assay medium at an agar concentration of 0.8% (See Table 1). The number of colonies (colony forming units, CFU) developed in each assay medium was determined after incubation for one week at 37 C.

4. *Cell lysates and culture filtrates of Ps-L(Sm^r) and Ec-L(Tc^r)*

Lysates and filtrates were prepared as described previously (Hirachi et al., 1980) to exclude the possibility that bacteriophages induced or deoxyribonucleic acid released by PEG treatment are involved in the emergence of doubly drug-resistant products on transduction and transformation, respectively.

TABLE 1. *Bacterial strains and assay media*

Abbreviations of L-forms	
Ps-L (Sm^r):	MIC SM >2,000 μg/ml
L-form of *P. aeruginosa* (IFO 3455)	KM >160 μg/ml
	TC <1 μg/ml
	Sucrose utilization (+)
Ec-L (Tc^r):	MIC TC >50 μg/ml
L-form of *E. coli* (Ohtani)	SM <20 μg/ml
	KM < 5 μg/ml
	Sucrose utilization (−)
Abbreviations of assay medium	Antibiotics were added to a solid basal medium whose agar content was reduced to 0.8% at the following final concentrations.
SM+TC	SM 1,000 μg/ml and TC 25 μg/ml
SM	SM 1,000 μg/ml
TC	TC 25 μg/ml
None	None

SM, Streptomycin; TC, Tetracycline; KM, Kanamycin.
Sm^r, Resistant to SM; Tc^r, Resistant to TC.

5. *Test for sucrose fermentation and KM resistance*

Mycoplasma broth base (BBL) supplemented with 4.5% NaCl, 1% sucrose and 0.002% phenol red, and liquid basal medium supplemented with KM (80 μg/ml), 0.8% glucose and 0.002% phenol red (KM-medium) were used. Test organisms were cultivated in these two media for 2 days at 37 C to examine acid production due to growth and fermentation of test sugars.

RESULT

1. *Emergence of products resistant to both SM and TC by cell fusion of Ps-L(Sm^r) and Ec-L (Tc^r)*

Table 2 shows that colonies doubly resistant to SM and TC developed at a fairly high frequency on PEG-treatment of a mixture of Ps-L(Sm^r) and Ec-L(Tc^r) and subsequent enrichment culture: colonies resistant to both drugs were recovered at a very low rate of $1/7.5\times10^8$ when a mixture of Ps-L(Sm^r) and Ec-L(Tc^r) was not treated with PEG (Experiment D). Doubly drug-resistant fusion products were obtained at a rate of $1/2.5\times10^6$ by enrichment culture of PEG-treated mixture of the two strains (Experiment A). This finding strongly suggests that the high frequency of emergence of doubly drug-resistant L-form cells was due to intergenus cell fusion induced with PEG. Control experiments (Experiments B and C) showed that the frequencies of development of colonies doubly resistant to SM and TC by Ps-L(Sm^r) and Ec-L(Tc^r) which were treated with PEG without the corresponding partner were less than $1/6.9\times10^9$ and only $1/3.3\times10^9$, respectively. These values were essentially the same as those for Ps-L(Sm^r) and Ec-L(Tc^r) treated under the conditions for Experiment E or F, indicating that the frequency of emergence of doubly drug-resistant L-form cells by spontaneous mutation with or without PEG-treatment was much lower than that obtained by simultaneous PEG treatment of the two strains.

2. *Check for possible involvement of genetic transfer mechanisms other than cell fusion*

Transformation: Addition of deoxyribonuclease (DNase-I, from bovine pancreas, DN-CL, Sigma) at a concentration of 10 μg/ml to

TABLE 2. *PEG-induced cell fusion between Ps-L(Sm^r) and Tc-L(Tc^r)*

Exp. No.	Composition of reaction mixture				No. of colonies[a] (ratio[b]) in medium supplemented with			
	Ps-L(Sm^r) ml	Ec-L(Tc^r) ml	PEG	DNase-I[c]	SM+TC	SM	TC	None
A	1	1	+	−	2.6×10^3 ($1/2.5\times10^6$)	2.8×10^9	4.3×10^9	6.7×10^9
B	2	0	+	−	0 ($<1/6.9\times10^9$)	6.6×10^9	0	6.9×10^9
C	0	2	+	−	3.0 ($1/3.3\times10^9$)	9.3	9.9×10^9	1.0×10^{10}
D	1	1	−	−	1.1×10 ($1/7.5\times10^8$)	7.3×10^9	7.5×10^9	8.2×10^9
E	2	0	−	−	0 ($<1/4.5\times10^9$)	4.4×10^9	0	4.5×10^9
F	0	2	−	−	4.7 ($1/1.2\times10^9$)	9.7	5.1×10^9	5.5×10^9
G	1	1	+	+	3.4×10^3 ($1/2.0\times10^6$)	4.3×10^9	4.8×10^9	6.9×10^9

[a] Mean CFU in triplicated cultures (per ml of enrichment culture).
[b] Ratio of CFU in the respective assay medium to that in control medium.
[c] DNase-I (10 μg/ml) was added to the reaction mixture for PEG treatment and the medium for enrichment culture.

TABLE 3. *No induction of doubly drug-resistant products by PEG treatment of mixtures of intact L-form cells and osmotic cell shockate*

Exp. No.	Compositon of reaction mixture			No. of colonies[a] (ratio[b]) in medium supplemented with			
	Ps-L(Sm^r)	Ec-L(Tc^r)	PEG	SM+TC	SM	TC	None
A	Intact cells	Intact cells	+	2.3×10^3 ($1/2.6\times10^6$)	4.7×10^9	4.1×10^9	7.3×10^9
D	Intact cells	Intact cells	−	7.6 ($1/6.6\times10^8$)	3.2×10^9	3.8×10^9	5.0×10^9
H	Intact cells	Osmotic shockate[c]	+	0 ($1/3.4\times10^9$)	3.0×10^9	0	3.4×10^9
I	Osmotic shockate[c]	Intact cells	+	1.7 ($1/3.4\times10^9$)	3.7	4.9×10^9	5.7×10^9

[a], [b] See Table 2.
[c] Cells grown in 10 ml of the liquid basal medium were harvested by centrifugation and suspended in distilled water (0.8 ml). The cell suspension was vigorously shaken in a Thermo-mixer to disrupt the cells by osmotic shock. The disrupted cell suspension was supplemented with 0.2 ml of NaCl solution (22.5%) at a final concentration of 4.5%. Portions of 0.1 ml of an osmotic shockate from the 1.0 ml L-form culture were incubated with intact cells from one ml of culture of the L-form.

reaction mixtures at the steps of PEG treatment and enrichment culture did not affect the rate of emergence of doubly drug-resistant products (Table 2, Experiment G). Other evidence that transformation was not involved is shown in Table 3. Doubly drug-resistant colonies were recovered at a frequency of $1/2.6\times10^6$ when a mixture of intact L-forms of

TABLE 4. *No induction of doubly drug-resistant products by treatment of intact L-form cells with a filtrate of PEG-treated cells*

Exp. No.	Composition of reaction mixture			No. of colonies[a] (ratio[b]) in medium supplemented with			
	Ps-L(Sm^r)	Ec-L(Tc^r)	PEG	SM+TC	SM	TC	None
A	Intact cells	Intact cells	+	2.5×10^3 ($1/1.6\times10^6$)	2.1×10^9	2.3×10^9	4.1×10^9
D	Intact cells	Intact cells	−	8.3 ($1/5.9\times10^8$)	4.3×10^9	4.0×10^9	4.9×10^9
J	Intact cells	Filtrate[c]	+	0 ($<1/7.3\times10^9$)	6.1×10^9	0	7.3×10^9
K	Filtrate[c]	Intact cells	+	0.7 ($1/3.0\times10^9$)	2.3	1.9×10^9	2.1×10^9

[a, b] See legend to Table 2.

[c] Cells harvested by centrifugation (5,000 *g* for 20 min) of 4 ml of the respective L-form strains in the liquid basal medium were suspended in 4 ml of PEG, and the suspension was stirred vigorously for 10 min at 37C, and centrifuged (12,000 *g* for 20 min). The supernatant fluid was passed through a Millipore filter (0.45 μm in pore size). One ml of filtrate from 2 ml of culture was incubated with intact cells from one ml of culture of the partner L-form.

TABLE 5. *No induction of doubly drug-resistant products by enrichment culture of Ps-L(Sm^r) and Ec-L(Tc^r) separately treated with PEG*

Exp. No.	Composition of reaction mixture			No. of colonies[a] (ratio[b]) in medium supplemented with			
	Ps-L(Sm^r)	Ec-L(Tc^r)	PEG	SM+TC	SM	TC	None
A	Intact cells	Intact cells	+	3.0×10^3 ($1/3.1\times10^6$)	6.0×10^9	7.8×10^9	9.2×10^9
D	Intact cells	Intact cells	−	7.3 ($1/9.2\times10^8$)	4.0×10^9	4.7×10^9	6.7×10^9
L	PEG-treated cells[c]	PEG-treated cells[c]	(+)[c]	0.3 ($1/2.8\times10^{10}$)	5.1×10^9	3.4×10^9	8.3×10^9

[a, b] See legend to Table 2.

[c] Ps-L(Sm^r) and Ec-L(Tc^r) was each treated with PEG. The PEG drained off as completely as possible, and the preparation were mixed for enrichment culture.

Ps-L(Sm^r) and Ec-L(Tc^r) were exposed to PEG (Experiment A), but negligibly small numbers of doubly drug-resistant colonies were recovered from mixtures of the intact L-form of Ps-L(Sm^r) and osmotic shocked Ec-L(Tc^r) cells (Experiment H), and *vice versa* (Experiment I).

Involvement of phage transduction: The possibility that bacteriophages induced by PEG transfer the drug-resistant genes in the development of doubly drug-resistant products was excluded by the experiments presented Tables 4 and 5. As shown in Table 4, with a combination of a filtrate of Ec-L(Tc^r) and intact Ps-L(Sm^r) cells (Experiment J) or a filtrate of Ps-L(Sm^r) and intact Ec-L (Tc^r) cells (Experiment K), the frequency of doubly drug-resistant colony development was as low as on spontaneous mutation, and was far less than that in Experiment A, where a mixture of intact cells of Ps-L(Sm^r) and Ec-L(Tc^r) was submitted to PEG treatment. Another experiment was done with an enrichment culture of a mixture of Ps-L(Sm^r)

TABLE 6. *Effect of temperature of PEG treatment of a mixture of Ps-L(Sm^r) and Ec-L(Tc^r) on the emergence of fusion products doubly resistant to SM and TC*

Exp. No.	PEG	Temperature (C)	No. of colonies[a] (ratio[b]) in medium supplemented with	
			SM+TC	None
I	+	37	2.7×10^3 ($1/3.2\times10^6$)	8.7×10^9
II	−	37	8.7 ($1/1.1\times10^9$)	9.3×10^9
III 1	+	0	2.0×10^3 ($1/4.3\times10^6$)	8.6×10^9
2	+	0	2.1×10^3 ($1/3.6\times10^6$)	7.6×10^9
IV 1	−	0	7.0 ($1/1.1\times10^9$)	7.8×10^9
2	−	0	5.7 ($1/5.4\times10^8$)	3.1×10^9

[a,b] See legend of Table 2 for details.

and Ec-L(Tc^r), which had been separately treated with PEG under the standard experimental conditions and then separated from PEG as completely as possible. As shown in Table 5 (Experiment L), essentially no products resistant to both SM and TC were obtained.

Table 6 shows that the frequency of emergence of fusion products doubly resistant to SM and TC on PEG treatment of Ps-L(Sm^r) and Ec-L(Tc^r) was temperature-independent. This finding seems consistent with a report of Gabor and Hotchkiss (1979) that the frequency of emergence of protoplast fusion products between different strains of *Bacillus subtilis* is independent of the temperature of PEG treatment. The finding also suggests that the conjugation mechanism was not involved appreciably in the emergence of doubly drug-resistant products in the present experiment, since the conjugation process of bacteria is in general temperature-dependent.

3. *No requirement of enrichment culture for isolation of fusion products*

In our previous study (Hirachi et al., 1980) on PEG-induced cell fusion of SM1000R-L (Sm^r) and EM10R-L(Em^r, resistant to erythromycin) substrains of *S. aureus* L-forms, strain EMT-1, no doubly drug-resistant fusion products (recombinants) were obtained unless enrichment culture was done after PEG treatment. In contrast, the combination between Ps-L(Sm^r) and Ec-L(Tc^r) produced fusion products doubly resistant to SM and TC at as high a rate as $1/8.4\times10^3$ without enrichment culture after PEG treatment (Table 7, Experiment Ao). This rate was much higher than that ($1/2.6\times10^6$) obtained when post PEG-treatment enrichment culture was done for 18 h (Table 7, Experiment A).

4. *Other characteristics of doubly drug-resistant fusion products*

Finally we analyzed the characteristics of the fusion products of Ps-L(Sm^r) and Ec-L (Tc^r) other than resistance to SM and TC. In all 273 colonies of fusion products on assay medium containing SM and TC were picked up at random, and inoculated into liquid basal medium supplemented with SM (1,000 μg/ml) and TC (25 μg/ml) (41 colonies did not grow at this step and were lost). Portions of 0.5 ml of the growing cultures were assayed for resistance to kanamycin and for sucrose utilization (Table 8). The percentage of fusion products with characteristics of the Ps-L type (sucrose utilization and KM resistance) of total doubly drug-resistant fusion products was 6.5%, while that of the fusion products of Ec-L type (no sucrose utilization and no resistance to KM) was 14.2%. The percentage of fusion products that did not utilize sucrose and was resistant to KM, i.e. an intermediate type, was 79.3%. The opposite intermediate type that utilized sucrose and was sensitive to KM was not detected.

DISCUSSION

Transformation, transduction and conjugation are known to be mechanisms of genetic transfer in bacteria, including *E. coli* and *P.*

TABLE 7. *Effect of enrichment culture after PEG treatment on the frequency of emergence of doubly drug-resistant fusion products of Ps-L(Sm^r) and Ec-L(Tc^r)*

Exp. No.	Duration of enrichment h	Composition of reaction mixture			No. of colonies[a] (ratio[b]) in medium supplemented with			
		Ps-L(Sm^r) ml	Ec-L(Tc^r) ml	PEG	SM+TC	SM	TC	None
Ao	0	1	1	+	8.7×10^2 ($1/8.3\times10^3$)	4.9×10^6	5.0×10^6	7.3×10^6
Bo		2	0	+	0 ($<1/7.6\times10^6$)	6.5×10^6	0	7.6×10^6
Co		0	2	+	0 ($<1/6.1\times10^6$)	0	5.6×10^6	6.1×10^6
Do		1	1	−	2.3 ($1/2.9\times10^6$)	6.9×10^6	7.1×10^6	9.0×10^6
A	18	1	1	+	2.5×10^3 ($1/2.6\times10^6$)	5.5×10^9	5.7×10^9	6.6×10^9
B		2	0	+	0 ($<1/3.9\times10^9$)	3.1×10^9	0	3.9×10^9
C		0	2	+	0.7 ($1/7.4\times10^9$)	1.3	4.9×10^9	5.2×10^9
D		1	1	−	9.3 ($1/4.6\times10^8$)	2.0×10^9	2.3×10^9	4.3×10^9

[a,b] See legend of Table 2 for details.

TABLE 8. *Characteristics of fusion products of Ps-L(Sm^r) and Ec-L(Tc^r) other than resistant SM and TC*

		Resistance[b] to KM		Total
		+	−	
Sucrose utilization[a]	+	15 (6.5)	0 (0.0)	15 (6.5)
	−	184 (79.3)	33 (14.2)	217 (93.5)
Total		199 (85.8)	33 (14.2)	232 (100)

[a] My coplasma broth base supplemented with 4.5% NaCl, 1% sucrose and phenol red used.
+ Growth and acid production were examined after 2 days' culture.
− Growth, but acid production was detected during 5 days' incubation.

[b] Liquid basal medium supplemented with 4.5% NaCl, KM (80 μg/ml), 0.8% glucose and phenol red was used
+ Growth and acid production after 2 days' culture.
− Neither growth nor acid production was detected during 5 days' incubation.

Values are numbers of colonies showing KM resistance and sucrose utilization. Values in parenthese are percentages of total colonies.

aeruginosa. However, the present study demonstrated that the emergence of fusion products doubly resistant to SM and TC from a mixture of Ps-L(Sm^r) and Ec-L(Tc^r) was not due to any of the above mechanisms. This conclusion is based on the following findings: 1. The requirement of PEG treatment for formation of fusion products (Table 2). 2. Exclusion of the possibility that the mutation rate was increased by PEG treatment of Ps-L(Sm^r) or Ec-L(Tc^r) (Table 2). 3. Exclusion of the participation of DNA capable of transformation (Table 2, 3). 4. The improbability of involvement of either induction or maturation of transducing phage on PEG treatment (Table 4, 5). 5. The independence of induction of the fusion products on the temperature of PEG treatment (Table 6). 6. The absence of requirement for the enrichment culture (Table 7).

The findings that enrichment culture after PEG treatment was not needed to obtain doubly drug-resistant fusion products by PEG-induced cell fusion of Ps-L(Sm^r) and Ec-L(Tc^r) in contrast to our previous experi-

ence with a combination of SM1000R-L (Sm^r) and EM10R-L(Em^r) substrains of *S. aureus* L-forms (strain EMT-1), indicates that the development of double drug-resistance observed in this study does not need a period of phenotypic expression, and consequently is not due to other genetic transfer mechanisms. It is well known that recombinants produced by transformation, transduction and conjugation need a period of phenotypic expression. In this connection, it shoud be maintained that the drug-resistances of Ps-L(Sm^r) and Ec-L(Tc^r) used in the present study were inherent properties of the respective strains, while the drug-resistance of the substrains of *S. aureus* described above was induced by selection in our laboratory. Therefore, it seems likely that the drug resistance of the substrains of *S. aureus* L-form is due to alterations of ribosomes, whereas the resistances of Ps-L(Sm^r) and Ec-L(Tc^r) are caused by drug modifying enzymes. The difference in requirment of enrichment culture in the present and previous studies might reflect the above differences in mechanisms by which the L-form strains tested showed drug resistance. The decrease in frequency of doubly drug-resistant fusion product development after enrichment culture for 18 h compared with that obtained immediately after PEG treatment might be due to either a longer generation time of resulting fusion products than that of parental L-form, or lower stability of the genetic traits, due to, for example formation of complementary and non-complementary diploids (Hochkiss and Gabor, 1980). It was shown (Table 8) that though Ps-L (Sm^r) exhibited resistance to KM as well as SM, 14.2% (33/232) of the fusion products resistant to SM (and TC) were sensitive to KM. This finding indicates that KM resistance of Ps-L(Sm^r) is dissociable from SM resistance, and suggests that with Ps-L(Sm^r), the mechanism of KM resistance may be different from that of SM resistance. It may be added here in this connection that there are two mechanisms of KM and SM resistance: 1. Mutational alterations of either 50s or 30s ribosomal subunits with KM, and those of 30s subunit with SM result in reduced affinity for the drugs, and consequently insensitivity of polypeptide synthesizing ability to the drugs (Choi, Nishimura and Tanaka, 1980) and 2. formation of drug modifying enzymes (Young et al., 1980).

It is unknown why the rate of development of one intermediate cell type that did not utilize sucrose was resistant to KM was extremely high (79.3%), but that of the opposite intermediate type that utilized sucrose and was sensitive to KM was not detected.

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