STUDIES ON THE TRANSFORMATION OF STREPTOMYCIN—DEPENDENCY IN *STAPHYLOCOCCUS**

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Introduction

In 1928 Griffith described the first successful genetic transformation of capsular type in *Diplococcus pneumoniae.* In 1944 Avery *et al.* showed that the transforming agent is deoxyribonucleic acid (DNA). Up to now, transformation phenomena have been observed in various bacteria such as: *Diplococcus pneumoniae* (Avery, 1944), *Haemophilus influenzae* (Alexander and Leidy, 1951), *Shigella paradysenteria* (Well, 1947), *Bacillus subtilis* (Spizizen, 1958), *Rhizobium* (Balassa, 1963), *Neisseria meningitidis* and various *Neisseria* (Catlin and Cunninghan, 1960, 1961). The transformation reactions between *Pneumococcus* and *Streptococcus* were reported by Braco *et al.* in 1957. The transformation of *Streptococci* to streptomycin resisance wtih DNA from homologous and heterologous strains of streptomycin-resistant *Streptococci* and Streptomycin-resistant *Pneumococci* were reported by Perry and Slade in 1962. The effects of interspecific transformation on the linkage of various antibiotic-resistane marker of *Haemophilus influenzae* and *Haemophilus parainfluenzae* were reported by Nicket and Goodgal in 1964. But there is no report of transformation of *Staphylococcus.* Therefore in our laboratory, we used the streptomycin-dependent *Staphylococcus* as marker for transformation. However the transformation of *Staphylococcus* failed when recipient cells was not pretreated. On the other hand when they were properly treated with penicillin G, a successful trnsformation was obtained. The results of this are reported in this paper.

Materials and Methods

Bacterial strains. *Staphylococcus aureus* 209 p designated as wild type was used for the recipient cell. As shown in Table 1, the wild type does not grow in

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streptomycin concentrations higher than 5 μg/ml. Streptomycin-dependent *Staphylococcus aureus* 209 p designated as S-d type was used for the donor cell. It was obtained from the wild type by Lederberg's (1952) spontaneous mutation and selection method. As shown in Table 1, it can not be propagated on solid media containing a streptomycin concentration less than 5 μg/ml.

DNA preparation. DNA was extracted from S-d type. A 50 ml quantity of 12-hours cultures of S-d type was prepared in brain heart infusion medium (B. B. L.) containing 1,000 μg/ml of streptomycin. Then it was inoculated into 1,000 ml of the the same medium and incubated for 12 hours at 37°C. Sodium

Table 1. *The response of wild type and streptomycin-dependent type of* ***Staphylococcus aureus*** *209p to various streptomycin concentrations*

Streptomycin Conc. (μg/ml)	Growth	
	Wild type	S-d type
0	##	—
0.05	##	—
1	##	—
2.5	##	—
5	+	+
7.5	—	+
10	—	#
25	—	#
100	—	##
1,000	—	##
10,000	—	##
80,000	—	#
100,000	—	+

Wild type: *Staphylococcus aureus* 209p wild type
S-d type: Streptomycin-dependent type, isolated from the wild type by the spontaneous mutation and selection method.
Incubation period: 37°C for 24 to 48 hours.
—: No colonies
+: Few colonies
#: More colonies than +
##: Many colonies; same as the maximum growth in the control which had no streptomycin.

citrate was added (0.05 M) at the end of the growth period to inhibit deoxyribonuclease action. Cells were harvested by centrifugation and then washed four times with a solution containing 0.1 M sodium chloride and 0.015 M sodium citrate. Then, 32 grams of the washed cells were treated according to the method of Blabel (1961). The yield from this extraction method was 6.7 mg of DNA. The final DNA was sterilized by precipitation with ethanol overnight, after

the DAN was redissolved in 10 ml of normal saline solution. The sterility of the DNA was checked and in no case was contamination observed. Stock solutions of DNA were kept at 4°C.

Medium. Nutrient broth (Difco) was used for the experiments. The streptomycin-resistant type (designated as S-r type) and S-d type were grown on streptomycin supplemented nutrient agar. The preparation of streptomycin nutrient agar plates for S-r type, S-d type and for transformation reactions was as follows: a test tube containing 10 ml of nutrient agar was sterilized and after cooling to 50°C, sufficient dihydrostreptomycin sulfate was added to reach a final concentration of 1,000 μg/ml, then it was poured in to petri dishes (9 cm. in diameter).

Chemicals. The streptomycin used in this study was crystalline dihydrostreptomycin sulfate, purchased from the Takeda Company, Japan. The penicillin G used in this study was crystalline penicillin G sodium, purchased from the Banyu Company, Japan. The penicillin G was diluted in a pH 7.0 phosphate buffer solution.

Preparation of competent cells. Highly competent cells of *staphylococcus* were obtained following penicillin G treatment. The wild type was grown on a nutrient broth at the ratio of one loopful of bacteria to 100 ml of nutrient broth, then it was incubated at 37°C with shaking for 12 hours. Then 10 ml. of the bacterial suspension was poured into test tubes. Next 0.1 ml. of the various concentrations of penicillin G solutions was added to each test tube so as to adjust penicillin G concentration to 0, 1, 10, 50, and 100 units per ml. Then each tube was incubated in water both at 37°C for different intervals of time: 0, 10, 30, 60, and 120 minutes respectively. Then the bacterial suspension in each tube was washed twice with 10 ml nutrient broth containing 10% glucose by centrifugation at 3,000 rpm for 15 minutes.

Transformation procedure. The competent cells were suspended in 10 ml of transformation nutrient broth, then divided into two tubes each containing 5 ml. One tube was used for transformation reaction and the other was used for viable cell counts: 0.1 ml of various concentrations of DNA was added to each tube. The mixture was incubated at 37°C for different intervals of time: 0, 10, 30 minutes, etc. Then it was treated with deoxyribonuclease at the same concentrations as DNA was added at 37°C for 30 minutes to digest the free DNA. After washing twice with nutrient broth containing 10% glucose by centrifugation at 3,000 rpm for 15 minutes, the cells were inoculated on nutrient agar plates which contained 1,000 μg/ml streptomycin by over-lay-technique. After incubation of 24 to 48 hours at 37°C, colonies of S-r and S-d variant types grew in a randomly mixed fashion on the same plate. The purification of S-r and S-d types was performed according to the method of Chiu (1964). The S-d

colonies thus obtained by the purification were cultivated on nutrient agar plates containing a concentration of streptomycin 1,000 μg/ml at 37°C for 18 hours. The harvest was washed with normal saline solution ten times and then inoculated on the media without streptomycin and the media with streptomycin 1,000 μg/ml so as to make sure that they were strepromycin-dependent. The other tube to be used for viable cell count was washed with normal saline solution once and then inoculated on the nutrient agar plates without streptomycin.

Sensitivity tests. S-r types and S-d types were obtained by transformations. This experiment was designed to investigated how many properties the transformed cells received from the donor cells. Only 10 colonies of S-d type and S-r type were selected at random. They were cultured on an agar slant containing 1,000 μg/ml streptomycin for 18 hours. After the cells were harvested, they were washed ten times with normal saline solution in order to remove the streptomycin. The cells were then cultured on media containing various concentrations of strptomycin, and incubated for 24 to 48 hours. The shape and cell growth of the coloeies on media containing various concentrations were boserved with the naked eye.

Calculation of transformation frequency. The transformation frequency was calculated by dividing the number of colonies of S-r type or S-d type obtained from transformation with the number of total cells in viable cell counts. For example, if the number of total cells is 3.5×10^8 and the number of transformed S-r colonies is 6, then the frequency of transformation for S-r is $\frac{6}{3.5 \times 10^8} = 1.7 \times 10^{-8}$

Back mutation rate of S-d type. A slant culture of S-d type on the medium containing streptomycin 1,000 μg/ml was harvested and washed in 10 ml normal saline solution. The suspension was inoculated in streptomycin medium at the rate of 0.1 ml on each plate. The colonies on these plates were subjected to purification procedure and sensitivity test. From the number of colonies of back mutant and the number of total cells the back mutation rate of S-d type was calculated.

Result

The optimal conditions for obtaining competent cells were obtained by treating the cells with various penieillin G concentrations for various durations. As shown in Table 2, when penicillin G was at concentrations of 1 unit/ml to 10 units/ml. It was observed that the longer reaction time used the higher frequence of transformation was obtained. However it was noticed that penicillin G was at concentrations of 50 units/ml to 100 units/ml showing that the

Table 2. *The relationship between frequency of transfomation and various concentrations and durations of treatment of penicillin G in* ***Staphylococcus aureus*** *209p wild type.*

Pen. Conc. (unit/ml)	Time (min.)	Frequency of transformation		
		Total cells	S-r	S-d
0	0	3.5×10^{8}	1.7×10^{-8}(6)	0
	10	4.1×10^{8}	1.5×10^{-8}(6)	0
	30	4.3×10^{8}	2.9×10^{-8}(8)	0
	60	4.9×10^{8}	1.4×10^{-8}(7)	0.2×10^{-8}(1)
	120	5.2×10^{8}	1.7×10^{-8}(9)	0.19×10^{-8}(1)
1	0	32.2×10^{7}	0.2×10^{-7}(8)	0.031×10^{-7}(1)
	10	1.3×10^{7}	26.10×10^{-7}(38)	0.15×10^{-7}(2)
	30	1.2×10^{7}	70.00×10^{-7}(84)	6.67×10^{-7}(8)
	60	1.0×10^{7}	119.00×10^{-7}(119)	2.00×10^{-7}(2)
	120	1.0×10^{7}	128.00×10^{-7}(128)	4.00×10^{-7}(4)
10	0	31.8×10^{6}	1.16×10^{-6}(37)	0.09×10^{-6}(3)
	10	5.2×10^{6}	22.70×10^{-6}(118)	1.10×10^{-6}(6)
	30	1.7×10^{6}	99.40×10^{-6}(163)	10.58×10^{-6}(18)
	60	1.2×10^{6}	45.60×10^{-6}(56)	10.00×10^{-6}(12)
	120	0.4×10^{6}	200.00×10^{-6}(82)	31.70×10^{-6}(13)
50	0	20.6×10^{5}	2.23×10^{-5}(46)	0.19×10^{-5}(4)
	10	8.6×10^{5}	14.10×10^{-5}(122)	1.70×10^{-5}(15)
	30	1.2×10^{5}	17.50×10^{-5}(21)	1.60×10^{-5}(2)
	60	3.2×10^{5}	4.06×10^{-5}(13)	0.30×10^{-5}(1)
	120	3.1×10^{5}	4.50×10^{-5}(14)	0.30×10^{-5}(1)
100	0	18.4×10^{5}	4.20×10^{-5}(78)	0.70×10^{-5}(13)
	10	3.3×10^{5}	7.00×10^{-5}(23)	0.30×10^{-5}(1)
	30	2.8×10^{5}	6.07×10^{-5}(17)	0.35×10^{-5}(1)
	60	1.2×10^{5}	6.60×10^{-5}(8)	0 (0)
	120	1.0×10^{5}	6.00×10^{-5}(6)	0 (0)

The numbers in brackets show the unmber of transformed colonies.
Inoubation period: 37°C for 24 to 48 hours.
Penicillin G was diluted in a pH 7.0 phosphate buffer solution.
Media were nutrient ager plates with 1000 μg/ml of streptomycin.
DNA concentration: 10 μg/ml
DNA reaction time: 30 minutes
Frequency of transformation; number of traneformed colonies÷number of total cells

longer the reauion time used the lower frequencyly transformation was the result. The relationship between penicillin G concentrations and the reaction

duration was found to be inversely proportional to one another. For example, at a penicillin G concentration of 1 unit/ml, the highest frequency of transformation was observed to be 128×17^{-7} for S-r type in 120 minutes and the lowest frequency was observed to be 0.031×10^{-7} for S-d type in 0 minutes. At a penicillin G concentration of 10 units/ml, the highest frequency of transformation was observed to be 31.7×10^{-6} for S-d type in 120 minutes, the lowest freguency was observed to be 0.09×10^{-6} for S-d type in 0 minutes and 1.16×10^{-6} for S-r type in 0 minutes. At a penicillin G concentration of 100 units/ml the highest frequency of transformation was observed to be 0.7×10^{-5} for S-d type in 0 minutes and 7×10^{-5} for S-r type in 10 minutes and the lowest frequency was observed to be 0×10^{-5} for S-d type in 120 minutes and 6×10^{-5} for S-r type in 120 minutes. Why did the highest frequency of transformation take place with the penicillin G concentration of 100 units/ml at 0 minutes for S-d type. It may be that penicillin G has some effect on the cells during centrifugation. In this experiment no S-d type cells were observed in the control tubes which were not treated with penicillin G, i. e. tubes of penicillin concentarion 0. From Table 2, it can be noted that the most successful transformation took place when the cells were pretreated with 10 units/ml penicillin G for 30 minutes. Henceforth, this optical condition was used in the following experiments.

As shown in Table 3, the different concentrations of DNA were added to the recipient cells which were pre-treated with 10 units/ml penicillin G for 30 minutes. When the cells were not exposed to DAN, no colony of S-d type was obtained. When the cells were exposed to 1 μg/ml of DNA for 30 minutes, the frequency of transformation was obtained at 45×10^{-6} for S-r type and 1.4×10^{-6} for S-d type. Whereas those exposed to 100 μg/ml of DNA for 30 minutes showed a frequency of transformation of 33.6×10^{-6} for S-r type

Table 3. *The relationship between the incidence of transformation and DNA concentration.*

DNA concentraion (μg/ml)	Frequency of transformation		
	Total cells $\times 10^{6}$	S-r $\times 10^{-6}$	S-d $\times 10^{-6}$
0	3.5	1.1 (4)	0 (0)
1	3.5	45 (157)	1.4 (5)
10	3.7	31 (126)	2.4 (9)
50	3.4	41.7 (142)	3.4 (12)
100	3.9	33.6 (131)	3.5 (14)

The numbers in brackets represent number for transformed colonies.
Incubation period: 37°C for 24 to 48 hours.
The recipient cells were pre-treated with 10 units/ml penicillin G for 30 minutes.
DNA reaction time: 30 minutes

and 3.5×10^{-6} for S-d type. It is evident that the number of transformants obtained is directly dependent upon the concentrations of DNA, the transformation can occur at DNA concentration of 1 μg/ml.

The data in Table 4 demonstrate that within 30 minutes of DNA exposure time, the transformation can reach its maximum with 10 μg/ml of DNA, when the recipient cell is pretreated by 10 units/ml penicillin G for 30 minutes.

Table 4. *The relationship between incidence of transformations and DNA durations.*

Time (min.)	Frequency of transformation		
	Total cells $\times 10^6$	S-r 10^{-6}	S-d 10^{-6}
0	1.28	2.3 (3)	0 (0)
10	1.23	33.3 (41)	3.3 (4)
30	1.37	86.9 (119)	11.7 (16)
60	2.26	63.7 (144)	7.96 (18)
120	1.52	76.3 (116)	8.6 (13)

The numbers in brackets represent number of transformed colonies.
Incubation period: 37°C for 24 to 48 hours.
The recipient cells were pre-treated with 10 units/ml penicillin G for 30 minutes.

Table 5 shows the sensitivity test of S-d type obtained by transformation.

Table 5. *The responses of streptomycin-dependent type obtained by transformation to various streptomycin concentrations*

Sm. Conc. (μg/ml)	Strain No.									
	1	2	3	4	5	6	7	8	9	10
0	−	−	−	−	−	−	−	−	±	−
0.01 to 0.1	−	−	−	−	−	−	−	−	±	±
0.5	−	−	−	−	−	−	−	−	++	+
1	−	−	−	±	±	±	±	±	++	++
5	−	±	+	+	+	+	+	+	++	+++
10	+	+	++	++	++	+++	+++	+++	+++	+++
50	++	++	++	+++	+++	+++	+++	+++	+++	+++
100 to 10,000	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
50,000	++	++	+	++	++	++	+++	++	+++	++
200,000	+	+	±	+	+	±	+	+	+	+

Incubation period: 37°C for 24 to 48 hours.
−: No colonies
+: Few colonies
++: More colonies than +
+++: Many colonies, same as the maximum growth in the control which contained no streptomycin.
±: Few, very small colonies

The test strains of S-d type from No. 1 to No. 8 have similar action on stropomycin as a donor strain. However, No. 9 and No. 10 show some difference in that they can continue to grow at streptomycin concentrations of 0.01 μg/ml.

Table 6 shows the sensitivity test of S-r type obtained by transformation. The test strains of S-r type from No. 1 to No. 6 have more or less the same reaction on streptomycin concentrations of 0 μg/ml to 100,000 μg/ml. However, No. 1 to No. 6 received higher resistance to streptomycin through the transformation of DNA from the donor cells. The strains No. 7, 8 and 10 all have the same response to streptomycin but the response is lower than No. 1 to No. 6 as they cannot grow in 5,000–100,000 μg/ml of streptomycin media. The strain No. 9 is essentially an intermediate between S-d type and S-r type.

Table 6. *The responses of streptomycin-resistant type obtained by transformation to various streptomycin concentrations.*

Sm. Conc. (μg/ml)	Strain No.									
	1	2	3	4	5	6	7	8	9	10
0	###	###	###	###	###	###	###	###	+	###
0.01	###	###	###	###	###	###	###	###	+	###
0.1	###	###	###	###	###	###	###	###	+	###
0.5	###	###	###	###	###	###	###	###	+	###
1	###	###	###	###	###	###	###	###	+	###
5	###	###	###	###	###	###	###	###	+	##
10	###	###	###	###	###	###	###	###	##	##
50	###	###	###	###	###	###	###	###	###	##
100	###	###	###	###	###	###	+	##	###	##
1,000	###	###	###	###	###	###	+	+	###	+
5,000	###	###	###	###	###	###	±	+	###	±
10,000	##	##	###	##	##	###	±	±	###	±
50,000	±	##	##	+	+	##	±	±	##	—
100,000	±	+	##	±	+	+	±	±	±	—

Incubation period: 24 to 48 hours at 37°C.

—: No colonies

+: Few colonies

##: More colonies than +

###: Many colonies, same as the maximum growth in the control which had no streptomycin.

±: Few, very small colonies

To summarize, both the streptomycin dependent types and the streptomycin-resistant types can be obtained if DNA of the streptomycin-dependent type is employed in the transformation reactions.

The spontaneous mutation and selection method was employed to isolate

the strepptomycin-resistant type and the streptomycin-dependent type from the wild type. The results and mutation frequency obtained are recorded in Table 8. A comparison of Table 8 with Table 2, 3 and 4 shows that the spontaneous mutation frequency of S-r type was 1×10^{-6} but that the frequency of transformation of S-r type was 1×10^{-4} to 1×10^{-5}. The frequency of spontaneous mutation of S-d type was 7×10^{-9} but the frequency of transformation of S-d type was 1×10^{-6} to 1×10^{-5}. The results showed that the frequency of transformation of S-d type was 1×10^{3} time more than the frequency of spontaneous

Table 7. *The responses of back mutation strains from the streptomycin-dependent type to various streptomycin concentrations.*

Sm. Conc. (μg/ml)	Strain No.									
	1	2	3	4	5	6	7	8	9	10
0	##	##	##	##	##	##	##	##	##	##
0.1	##	##	##	##	##	##	##	##	##	##
1	##	##	##	##	##	##	##	##	##	##
5	##	##	##	##	##	##	##	#	#	+
10	##	##	##	##	+	#	#	+	+	+
50	##	##	#	##	±	+	+	±	±	±
100	#	#	+	#	±	±	±	±	−	−
500	+	+	±	±	±	±	±	−	−	−
1,000	+	±	±	±	±	±	±	−	−	−
10,000	±	±	±	±	−	−	−	−	−	−

Incubation period: 37°C for 24 to 48 hours.
−: No colonies
+: Few colonies
#: More colonies than +
##: Many colonies, same as the maximum growth in the control which had no streptomycin.
±: Few, very small colonies

Table 8. *The mutation rate of resistant type and dependent type by the spontaneous mutation and selection method*

Exp. No.	Mutation rate		
	Resistant type $\times 10^{-6}$	Dependent type $\times 10^{-9}$	Total No. of cells $\times 10^{8}$
1	1.1 (138)	0	1.24
2	1.16 (163)	7 (1)	1.41
3	1.04 (133)	0	1.29
4	1.04 (124)	8.3 (1)	1.20
5	1.00 (122)	0	1.13
6	0.98 (134)	0	1.38

The number in brackets show the number of colonies.

mutation and the frequency of transformation of S-r type was 1×10^1 to 1×10^2 times more than the spontaneous mutation rate.

The back mutation rate of S-d type was calculated to be 1.3×10^{-10}, which is lower than the frequency of spontaneous mutation of S-d type 7×10^{-9}. Ten strains were selected from the back mutants, and their responses to streptomycin are shown in Table 7.

Discussion

The mechanism of transformation has been studied in great detail in *Pneumococcus, Haemophilus, Bacillus subtilis, Streptococcus* and *Rhizobium*, and in these organisms the following stages have been distinguished: 1) development of competent bacteria, 2) adsorption of DNA on the bacteria surface, 3) penetration of DNA, 4) genetic integration of the transforming marker, and 5) expression of the new phenotypes. In these processes, the most important stage is the development of competence.

Avery (1944) showed that the competence in *Pneumococcus* occurs in the latter half of the logarithmic phase of growth. Spizizen *et al.* (1950) also observed that this is the same period in which competence is acquired in *Bacillus subtilis*. Similarly, *Haemophilus influenzae* reaches its highest peak of competence during the early stationary phase (Alexander and Leidy, 1951). Perry and Slade (1962) showed that optimal competence in *Streptococcus* was attained in the early logarithmic phase. Balassa showed that the competence in *Rhizobium* occurs in the beginning of the exponential phase of growth. The most propitious moment is in the first two divisions after the latent phase. But there is no transformation in *Staphylococcus aureus* 209 p, when exposed to DNA at various stages of growth. However, *Staphylococcus aureus* 209 p can be made competent by treatment either with a low concentration of penicillin G for a long period of time, or with high concentration for a short period of time in the logarithmic phase growth. The maximum transformation was obtained after incubation of the cell at 37°C for 12 hours followed by treatment of the cells with 10 units/ml to 50 units/ml of penicillin G for 30 mintes. According to the reports of Donald and Straminger (1965) and wise and Park (1965), penicillin G has a selective action to gram-positive microorganisms, because it inhibits the enzyme acting on the glycose sugar unit of the high-energy uridine pyrophosphate compound of *Staphylococcus*, and it inhibits the action of glycosidase, thus prevents the cell wall formation. The data in Table 2 indicate that the lower penicillin G concentrations with a longer reaction time can cause incomplete cell wall formation. Therefore, this enables the DNA to penetrate the cells, leading to successful transformation. The possibility exists that enzymes and physical barriers play a prominent role in the transformation of

some bacteria. The use of additional markers, the screening on a large number of strains, and the study of optimal conditions may result in transformation of other kind of organisms.

According to the report of Alexander and Leidy (1951), the minimal requirement for DNA was 0.3 μg/ml to 0.03 μg/ml for the transformation of *Haemophilus influenzae* in three minutes. According to the report of Avery (1944), the concentration of DNA required for trangformation of Pneumococcus is 0.06 μg/ml with a reaction time of 30 minutes. In this experiment, the transformation took place at DNA concentration of 1 μg/ml, and the transformation frequency increased with the increase of DNA concentration. The optimum conditions for transformation were found to be a concentration of 10 μg/ml of DNA with a reaction time of 30 minutes.

The streptomycin-dependent type was isolated from the wild type. It does not grew in the absence of streptomycin, but it grows very slowly in a medium containing 10 μg/ml of streptomycin, and does not attain a maximal rate of growth until a concentration of 1,000 μg/ml is reached.

The genetic analysis of streptomycin-dependent mutants in *E. coli*, *Salmonella typhimurium* and *Rhizobium* have revealed the existence of complex locus. According to the reports of Balassa (1963) in *Rhizobium* dependence is controlled by two regions of the streptomycin-resistance locus, one of which confers resistance upon the bacterium, the other confers dependence. This situation was similar in Staphylococcus, as shown in Tables 2, 3, 4, 5, 6, 7. Transformation of the sensitive strain with DNA from the dependent mutant produced several types of transformed colonies, resistants and dependents. The transformation was analyzed according to the method of Balassa (1963). The result is the same as being obtained by Balassa (1963).

DNA of the streptomycin-dependent strain can, therefore, transfer either resistance to a low concentration of streptomycin, or the entire resistance of the donor strain, or more rarely, dependence itself.

Summary

1. *Staphylococcus aureus* 209 p could become competent by treatment either with low concentrations of penicillin G for a long period of time, or with high concentrations for a short period of time in the logarithmic phase of growth. The maximum transformation was obtained by treating cells with 10 units/ml of Penicillin G for 30 minutes.
2. The transformation could take place at DNA concentration 1 μg/ml, and the transformation frequency increase with the increase of DNA concentration.
3. The optimum condition for transformation was found to be a concentration of 10 μg/ml of DNA with a reaction time of 30 minutes.

4. Both streptomycin-dependent type and streptomycin-resistant type occurred in the transformation by DNA of streptomycin-dependnt *Staphylococcus aureus* 209 p to the wild type.
5. The frequency of transformation of streptomycin-dependent type was 1×10^3 times more than the spontaneous mutation rate. The frequency of transformation of streptomycin-resistant type was 1×10^1 to 1×10^2 times more than the spontaneous mutation rate.
6. In the transformations, the amount of streptomyin-resistant type was 10 times more than streptomycin-dependent type.
7. It was found that 80% of the streptomycin-dependent colonies transformed by DNA had a similar response to streptomycin as the donor cells, and 20% of these showed a different response.

鏈黴素依賴性葡萄狀球菌形質轉換之研究

吳 榮 洋

1. 金黃色葡萄狀球菌 209p (*Staphylococcus aureus* 209p) 在其生長對數期 (logarithmic phase) 用低濃度盤尼西林G (penicillin G) 長時間處理，或以高濃度短時間處理，均可得形質轉換 (transformation) 的 competent cell。最適宜的條件爲用 10 units/ml 的盤尼西林G處理30分鐘。

2. 當 DNA 之濃度爲 1 μg/ml 時有形質轉換發生，其頻度隨 DNA 濃度之增加而增大。

3. 形質轉換最適合的條件爲以 DNA 10 μg/ml 處理30分鐘。

4. 鏈黴素依賴性金黃色葡萄狀球菌 (streptomycin-dependent *Staphylococcus aureus* 209p)的DNA轉換予其野生型(wild type)時能得鏈黴素依賴性型 (streptomycin-dependent type) 及抵抗性型 (streptomycin-resistant type)的二種金黃色葡萄狀球菌。

5. 依賴性型形質轉換發生頻度爲其自然突變頻度 (spontaneous mutation rate) 的1,000倍，低抗性型形質轉換發生頻度則爲自然突變頻度之10至100倍。

6. 抵抗型形質轉換量爲依賴性型之10倍。

7. 由形質轉換所得依賴性型，其中 80%對鏈黴素之反應與 donor cell 相同，其餘 20%對鏈黴素則顯示不同的反應。

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