TRANSDUCTION AND TRANSFORMATION BETWEEN *BACILLUS SUBTILIS* AND *BACILLUS NATTO*

HATSUO AOKI¹, HIUGA SAITO, and YONOSUKE IKEDA

Institute of Applied Microbiology, University of Tokyo, Tokyo

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A group of aerobic spore-bearing bacilli which have long been used in this country as fermenters in manufacturing "natto" was named "*Bacillus natto*" by SAWAMURA (1) in 1879, and it comprises a variety of strains whose taxonomic features are not sharply distinguishable from those of other aerobic spore bearers. Current studies on the biochemical as well as genetic relationship between *B. subtilis* and *B. natto* have revealed that those two bacilli should be considered as one species. For instances, MARMUR *et al.* (2,3) reported the success of DNA hybrid formation between the two species and also described the transfer of genetic traits from *B. natto* to *B. subtilis* by DNA.

To make this point clearer, the authors attempted to employ the phage mediated transduction technique established by THORNE (4) and TAKAHASHI (5) in *B. subtilis* as well as the transformation technique established by SPIZIZEN (6). The experiments were done between *B. subtilis* and several strains of *B. natto*. The DNA composition of these bacilli and the transducing phage will be the subject of subsequent publications.

MATERIALS AND METHODS

Bacterial strains. Seven strains of *B. natto* listed in Table 1 were used. They were stock cultures at the Institute of Applied Microbiology, University of Tokyo. Strains 1212-52 (*try*⁻) and 1259-87 (*arg*⁻) were derived from strains 1212 and 1259, respectively, by X ray irradiation. Strain 23 is a wild type strain of *B. subtilis* Marburg. Strain 171-15 (*try-arg*⁻) was derived from *B. subtilis* Marburg 160 (*try*⁻).

Bacteriophage. S-1 is a temperate phage, isolated by TAKAGI (7), which is capable of transducing genetic traits from *B. subtilis* 23 to auxotrophic mutants. The phage titer was assayed by the agar layer method using strain 23 as an indicator.

Media. NB broth: containing per liter of distilled water, Bacto nutrient broth, 14 g; Bacto peptone, 7 g; Bacto yeast extract, 4 g; and glucose, 1 g. Minimal medium: containing per liter of water, K₂HPO₄, 7 g; KH₂PO₄, 3 g; Na₃-citrate·2H₂O, 0.5 g; (NH₄)₂SO₄, 1 g; MgSO₄·7H₂O, 0.2 g; and glucose, 5 g. Glucose and MgSO₄·7H₂O were autoclaved separately. Supplemented medium: for the detection of transductants and transformants, the minimal medium

¹ Present address: Fujisawa Pharmaceutical Industries, Ltd., Koganei City, Tokyo.

was supplemented with one of the following amino acids, L-tryptophan 50 $\mu\text{g}/\text{ml}$ and L-arginine 100 $\mu\text{g}/\text{ml}$.

Transduction experiment. For the preparation of phage suspension, a strain of *B. natto* or *B. subtilis* was lysogenized with phage S-1 and cultivated overnight in NB broth at 37°. The cells were centrifuged at 12,000 rpm for 15 min. The suspension thus prepared contained about 10^9 per ml of infective phages in the case of *B. subtilis*. The titer was generally low when the phage multiplied in strains of *B. natto*. Recipient cells were cultivated in NB broth overnight at 37°, and after dilution to one twentieth with fresh NB broth incubated further for 3 hr to gain $5-6 \times 10^8/\text{ml}$ of cells.

One ml of the recipient cell suspension and 0.1 ml of the phage suspension were mixed together and kept at 37° for 15 min with shaking. The mixture was diluted with 10 ml of chilled saline (0.85%) and centrifuged. The cells were suspended in one ml saline and 0.1 ml was plated on an appropriate agar medium prepared for the detection of transductants. Colonies were counted after 2 days' incubation at 37°.

Transformation experiment. Transforming DNA was prepared from the cells of *B. natto* by the method described by SAITO and MIURA (8). The conditions employed for the performance of transformation were the same as those described previously (6, 9).

RESULTS

Plating efficiencies of phage S-1 on B. natto

To know the plating efficiencies of phage S-1 on different strains of *B. natto*, aliquots containing equal numbers of phage particles were plated with preincubated cells. Table 1 shows the relative numbers of plaques appeared on each strain. Generally speaking, the efficiencies on strains of *B. natto* were lower than that on the standard strain, *B. subtilis* 23.

Table 1. Plating efficiencies of phage S-1 on strains of *B. natto*.

Strain		E.O.P. ^a of Phage S-1	Requirement
<i>B. natto</i>	1028	6.6×10^{-1}	Not determined
	1212	2.8×10^{-1}	None
	1151	2.7×10^{-1}	Biotin
	1259	4.7×10^{-1}	None
	1143	1.9×10^{-5}	Biotin
	1109	1.6×10^{-1}	Not determined
	1163	1.7×10^{-2}	"
	1212-52	—	Tryptophan
	1259-87	—	Arginine
<i>B. subtilis</i>	171-15 (<i>try</i> ₁₆₀ <i>arg</i> ₁₇₁)	0	Tryptophan & arginine
"	23	1.0	None

^a Efficiency of plating.

Transduction from B. natto to B. subtilis

As seen in Table 2, 5 out of the 7 strains tested were found to be able to transfer their genetic traits to *B. subtilis* by the mediation of phage S-1. Because the infective titer of the phage suspension prepared from *B. natto* was generally low, it was difficult to set the m.o.i. at the desired value.

Table 2. Transduction of *try* and *arg* markers in *B. natto* to *B. subtilis* 171-15 by phage S-1.

Donor		m.o.i. ^a	Transductant No./plate	
			<i>arg</i> ⁺	<i>try</i> ⁺
<i>B. natto</i>	1028	0.4	5,500	3,030
	1212	10 ⁻³	2,736	2,616
	1191	10 ⁻³	14,200	14,000
	1259	0.5	444	48
	1143	10 ⁻⁵	1,284	1,384
	1109	1.0	0	0
	1163	10 ⁻²	0	0
<i>B. subtilis</i>	23	0.3	1,200	2,500

^a m.o.i.; multiplicity of infection. 5×10^7 recipient cells were used.

In some strains, especially in 1191 and 1143, the frequency of transduction was considerably higher than that in *B. subtilis* 23. This might have been due to the presence of larger numbers of defective phages. The reason why the defective phages are present so abundantly in *B. natto* lysates is not clear.

When *B. subtilis* 23 was used as a donor, the frequency in the transduction of *try* marker was about twice as high as that in the *arg* marker. On the other hand, the *arg* marker was transduced more frequently than the *try* marker when some strains of *B. natto* were used as donors.

Table 3. Transformation of *B. subtilis* 171-15 by DNA from *B. natto*.

Donor		DNA conc. μg/ml	Transformant No./plate ^a	
			<i>arg</i> ⁺	<i>try</i> ⁺
<i>B. natto</i>	1028	0.1	13,000	15,000
	1212	"	12,500	10,400
	1191	"	13,200	9,950
	1259	"	600	500
	1143	"	28,000	2,000
	1109	"	4,340	3,070
	1163	"	15,000	20,250
<i>B. subtilis</i>	23	0.01	4,970	4,300

^a $2 \sim 5 \times 10^7$ cells were used as recipients.

Transformation from B. natto to B. subtilis

DNAs prepared from strains of *B. natto* were given to *B. subtilis* 171-15 (*try*⁻*arg*⁻). The frequencies are shown in Table 3. It is noteworthy that strains 1109 and 1163, which gave no transductant, were able to transform the recipient strains.

Transduction and transformation from B. subtilis to B. natto

Phages and DNA prepared from *B. subtilis* 23 were given to *B. natto* 1212-52 (*try*⁻) and 1259-87 (*arg*⁻). As shown in Table 4, in which the data

Table 4. Transduction and transformation from *B. subtilis* to *B. natto* and from *B. natto* to *B. natto*.

Recipient	Vector	Donor		
		W23	1212	1259
1212 - 52 <i>try</i> ⁻	DNA ^a	1400 ^c	300	400
	S-1 ^b	40	104	130
1259 - 87 <i>arg</i> ⁻	DNA ^a	0	0	0
	S-1 ^b	0	100	1150

^a Concentration was about 0.1 µg/ml.

^b m.o.i. was 0.1~0.3.

^c Using 2~5×10⁷ cells as recipients, transductants and transformants are expressed as No./plate.

for transduction and transformation from *B. natto* to *B. natto* are also shown, the *try* marker in strain 1212-52 was transduced as well as transformed by the phage and DNA from 23, whereas the *arg* marker in strain 1259-87 was not. The absence of transduction and transformation in the latter may be ascribed to their low frequencies as seen in Tables 2 and 3.

DISCUSSION

As described above, the genetic similarity between *B. subtilis* and *B. natto* was evidenced by the results obtained by the transduction as well as transformation technique. It is still early to conclude, however, which of the results obtained by the two techniques may be regarded as more significant in discussing the taxonomic correlation between the two species in question. As far as the present experiments are concerned, the frequencies of transformation were comparatively constant regardless of the difference in donor strains and genetic markers, while the frequencies of transduction fluctuated significantly depending on the strains and markers used.

One significant point is the *arg*: *try* ratio as given in Table 2. As has been reported by TAKAGI (7), the ratio is invariably 1 : 2 when strain 23 is used as the donor. Meanwhile, the ratio obtained with strains of *B. natto* as

donors was approximately 1 : 1, with an exception of the value 10 : 1 obtained with strain 1259. This fact together with the observation that the phage from strain 1190 or 1163 could not transduce genetic traits to strain 171-15 although the DNA could cause transformation suggest that these two genetic events involves different mechanisms. Further discussion on the problem will be made elsewhere in connection with the results obtained with other kinds of aerobic bacilli.

SUMMARY

Seven strains of *B. natto* investigated were found to be all vulnerable to phage S-1, while five among them were able to transfer their genetic traits to *B. subtilis* by the phage. All strains, including the two which could not transduce, were able to transform the genetic markers in *B. subtilis* by the DNA. The transduction as well as the transformation from *B. subtilis* to *B. natto* was also successful. Based on these results, the genetic similarity between *B. subtilis* and *B. natto* was discussed.

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