Note

Specific Host Range of *Bacillus subtilis* (natto) Phages Associated with Polyglutamate ProductionToshio HARA, Atsushi SHIRAISHI,*
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"Natto," produced by growing *Bacillus subtilis* (natto) on steamed soybeans, is one of the most traditional fermented foods in Japan. It is a viscous material consisting of polysaccharide (a levan-form fructan) and polyglutamate (PGA).^{1,2)} Fujii³⁾ showed that the PGA consists of L- and D-glutamate, and the proportion of the two isomers in PGA varies from 40 to 93% depending cultivation conditions.⁴⁾

In a previous paper,⁵⁾ we reported that the 5.7-kb plasmid, encoding the γ -glutamyltranspeptidase (γ -GTP) gene responsible for PGA production, exists widely in *B. subtilis* (natto) strains showing biotin requirement for

growth. The host range of bacteriophages isolated from an abnormal fermented "natto" completely coincides with the 5.7-kb plasmid-harboring strains of *B. subtilis* (natto). These findings have led to the suggestion that the infective bacteriophages for *Bacillus* species which can produce PGA as a capsular or extracellular mucilaginous material might recognize PGA as a receptor on phage adsorption.

Fuji *et al.*⁶⁾ reported that PN phages isolated from an abnormal fermented "natto" could be classified from the host range into 4 groups and serologically into 3 groups. All *B. subtilis* (natto) strains were sensitive to the isolated phages, whereas *B. subtilis* strains including variants of *B. subtilis* sometimes referred to as *B. mesentericus* and *B. licheniformis* were resistant. The present investigation dealt with the host range of isolated phages of *B. subtilis* (natto) strains, representative transformants and the cured strains derived with acridine orange.

Bacteriophages PM, PN-1 and PN-19 described previously^{6,7)} were used in this work. Phage titrations were performed by the double layer method and other general techniques were performed according to Adams.⁸⁾ The strains which gave lytic zones after incubation at 37°C were taken as being sensitive. Curing experiments were carried out by the method described previously.⁹⁾ *B. subtilis* (natto) strains were rapidly cured of the ability to produce PGA concomitantly with the loss of γ -GTP activity with acridine orange (Table I). As shown in Table I, the strains cured of PGA productivity became resistant to all the "natto" phages used, PM, PN-1 and PN-19.

To clarify whether such susceptibility to "natto" phages was caused by a stringly substance (PGA), PGA-producing transformants were derived from *B. subtilis*

TABLE I. PHAGE HOST RANGE AGAINST THE PARENTAL AND CURED STRAINS, AND REPRESENTATIVE TRANSFORMANTS

Strain	PGA productivity	γ -GTP activity ^a (mU/ml)	Phage host range ^b		
			PM	PN-1	PN-19
<i>B. subtilis</i> (natto)					
Asahikawa	+	424	+	+	+
Miura	+	157	+	+	+
Takahashi No. 5	+	90	+	+	+
Cured strain (parental strain)					
HA-1 (Asahikawa)	—	2.7	—	—	—
HM-1 (Miura)	—	3.3	—	—	—
HT-1 (Takahashi No. 5)	—	14.4	—	—	—
Transformant ^c (donor strain)					
3F1 (Asahikawa)	+	147	+	+	+
M5B4 (Miura)	+	142	+	+	+
F1~9 (Takahashi No. 5)	+	233	+	+	+
<i>B. subtilis</i> Marburg 168	—	6	—	—	—

^a After cultivation, cells were removed by centrifugation and then enzyme activities in the culture fluid were determined.

^b +, sensitive; —, resistant.

^c *B. subtilis* Marburg 168 was used as recipient.

Marburg 168 by DNA-mediated transformation using DNA of *B. subtilis (natto)* strains. The susceptibility of the parental strains and representative transformants to "natto" phages is also shown in Table I. All strains of *B. subtilis (natto)* were sensitive to all phages isolated from an abnormal fermented "natto," whereas *B. subtilis* Marburg 168 which produced only a small amount of γ -GTP and cannot produce a stringy substance around colonies was resistant. All transformants of Marburg 168 examined, showing stringy substance productivity, showed high γ -GTP productivity and were sensitive to all used phages.

These results indicate that PGA might be associated with phage absorption. In fact, Hongo and Yoshimoto^{10,11)} reported that a depolymerase capable of decomposing PGA was formed when *B. subtilis (natto)* was infected with a bacteriophage, and they suggested that PGA produced by *B. subtilis (natto)* might be associated with phage adsorption.

Many studies on the genetic as well as the biochemical relationship between *B. subtilis* and *B. subtilis (natto)* have revealed that these two bacilli should be considered as one species. For instance, Marmur *et al.*^{12,13)} reported the successful DNA hybrid formation between the two species and also described the transfer of genetic traits from *B. subtilis (natto)* to *B. subtilis* by DNA. Furthermore, Aoki *et al.*¹⁴⁾ reported that transduction as well as transformation from *B. subtilis* to *B. subtilis (natto)* was successful. However, Amaha *et al.*¹⁵⁾ and Kida *et al.*¹⁶⁾ reported that *B. subtilis* and *B. subtilis (natto)* can be classified separately on the basis of whether biotin is essential for growth or not.

The usefulness of bacteriophages in determining the relatedness between two species of bacteria has been discussed by Sneath.¹⁷⁾ However, if plasmids are concerned in the host range of bacteriophages, it is impossible to formulate any general principle relating phage susceptibility with the taxonomic position of a *Bacillus* species. We define that *B. subtilis (natto)* harbors a 5.7-kb plasmid encoding the γ -GTP gene responsible for PGA production. Therefore, the results demonstrated here might be helpful for distinguishing *B. subtilis* and *B. subtilis (natto)*. An

approach of this kind may continue to be useful in the future.

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