Note

Cloning and Identification of an Exogenous Thermostable α -Amylase Gene residing on the *Bacillus subtilis* ChromosomeShoji SHINOMIYA, Kunio YAMANE,*
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We have previously reported the transformation of *Bacillus subtilis* Marburg strain with non homologous DNA harbouring the thermostable α -amylase gene of a thermophilic bacterium, *Thermophile* V2 and the isolation of *B. subtilis* transformant (207-SV1) producing thermostable α -amylase.¹⁾ The enzymatic characterization of the extracellular thermostable α -amylase was conducted in comparison with the *B. subtilis* α -amylase. Two α -amylases were found to be equally insensitive to EDTA but were different from each other in thermostability, optimum temperature, electrophoretic mobility on polyacrylamide gel and immunological specificity.²⁾ The genetic analysis of two α -amylase genes revealed that the structural gene of

the thermostable α -amylase derived from the strain, *Thermophile* V2, was integrated into a different chromosomal locus from that of the *B. subtilis* α -amylase structural gene.²⁾ The structural gene of the thermostable α -amylase was named *amyV2*⁺.

In order to study the secretion mechanism of the thermostable α -amylase, we constructed the specialized transducing phage of *B. subtilis* temperate phage ρ 11 containing the *amyV2*⁺ gene by the method named "prophage transformation" that have been originally reported by Kawamura *et al.*³⁾ It was difficult, however, to isolate this *amyV2*⁺ fragment from recombinant DNA molecule of ρ 11 since the phage ρ 11 contains a large DNA molecule of 78 megadaltons and the DNA generates a number of fragments by digestion with restriction endonucleases such as *Eco*RI, *Bam*HI, *Sal*I, *Pst*I, *Pvu*II and *Xba*I.⁴⁾ We reconstructed the specialized transducing phage containing the *amyV2*⁺ gene using another *B. subtilis* temperate phage ϕ 105 which contains a rather small DNA molecule of 24 megadaltons. The DNA fragment carrying the *amyV2*⁺ gene was obtained by partial digestion of *Sau*3A of ρ 11 containing the *amyV2*⁺ gene and the DNA fragment was inserted between the *Bam*HI and *Bgl*II recognition sites of ϕ 105*hisA* DNA by the prophage transformation method. The ϕ 105 DNA had neither the *Bam*HI nor the *Bgl*II site while the *hisA* containing DNA portion had unique sites for these enzymes. Fortunately, the *amyV2*⁺ gene in the reconstituted ϕ 105*amyV2*⁺ could be recovered on a 1.5 megadaltons *Bam*HI-*Bgl*II DNA fragment.

Using this *Bam*HI-*Bgl*II fragment of the *amyV2*⁺ gene as a probe, a southern hybridization experiment was carried out to confirm that the *amyV2*⁺ gene was truly derived from the strain, *Thermophile* V2. Figure 1 shows the southern hybridization pattern after several *B. amylo-*

TABLE I. DETECTION OF Amy⁺ TRANSFORMANTS WITH VARIOUS BACTERIAL DNAs

Transformation was carried out with *B. subtilis* M07-SV10 (*amyV2-10*, *metB5*) as recipient. *amyV2-10* is a mutant allele of the thermostable α -amylase structure gene of M07-SV1. About 1 μ g of each bacterial DNA and 0.02 μ g of ϕ 105*metB*⁺ DNA to select the MetB⁺ transformant were added to the recipient cells, simultaneously, in the transformation experiment.

Donor DNAs	No. of transformants		
	Selected marker	Unselected marker	Amy ⁺ MetB ⁺ (%)
	MetB ⁺	Amy ⁺	
Thermophile V2	100,000	0	—
<i>B. amyloliquefaciens</i>			
strain Fukumoto (F)	10,000	80	0.8
IAM1521 (H)	10,000	76	0.76
IAM1522 (N)	10,000	80	0.8
IAM1523 (K)	10,000	72	0.72
<i>B. subtilis</i>			
M07-SV1 (<i>amyV2</i> ⁺)	325	10	3.0

FIG. 1. Southern Hybridization of the ^{32}P -Labelled DNA Fragment Containing the *amyV2⁺* Gene to Various Bacterial DNAs.

Each bacterial DNA was digested with *Eco*RI, electrophoresed in 0.8% agarose and electro-blotted onto a nitrocellulose filter as in ref. 5. The ^{32}P -labelled DNA fragment carrying the *amyV2⁺* gene was prepared by the rapid end-labelling method.⁶⁾ Hybridization was performed as in ref. 7. (1 ~ 6) are UV photographs of ethidium bromide stained gel, while (7 ~ 12) are autoradiographs: (1, 7) *Thermophile* V2 DNA; (2, 8) *B. subtilis* M07-SV1 DNA; (3, 9) *B. amyloliquefaciens* strain Fukumoto DNA (F); (4, 10) *B. amyloliquefaciens* IAM1522 DNA (N); (5, 11) *B. amyloliquefaciens* IAM1521 (H); (6, 12) *B. amyloliquefaciens* IAM1523 DNA (K).

liquefaciens DNAs including *Thermophile* V2 were digested with *Eco*RI. It should be noted that no band was detected in the lane of *Thermophile* V2 DNA, but two bands were seen in the lane of DNAs of *B. amyloliquefaciens* strain Fukumoto (F), *B. amyloliquefaciens* IAM1521 (H), *B. amyloliquefaciens* IAM 1522 (N), *B. amyloliquefaciens* IAM 1523 (K) and *B. subtilis* M07-SV1 (*amyV2⁺*), suggesting that the *amyV2⁺* gene did not originate from the strain, *Thermophile* V2. A transformation experiment was performed to confirm this result further. The strain, *B. subtilis* M07-SV1 (*amyV2⁻*), isolated from *B. subtilis* M07-SV1 (*amyV2⁺*) by treatment with NTG was used as the recipient cell. The *Amy⁺* transformants were obtained with DNAs from *B. amyloliquefaciens* strain Fukumoto

(F), *B. amyloliquefaciens* IAM1521 (H), *B. amyloliquefaciens* IAM1522 (N), *B. amyloliquefaciens* IAM1523 (K) and *B. subtilis* M07-SV1 (*amyV2⁺*) but not obtained with DNA from *Thermophile* V2 (Table I). The results indicate that the thermostable α -amylase gene integrated into the *B. subtilis* chromosome appears to be derived from *B. amyloliquefaciens* spp. It is plausible that the DNA of *B. amyloliquefaciens* was taken for that of *Thermophile* V2 in the first transformation experiment. However, this α -amylase is of no disadvantage in research on the secretion mechanism of α -amylase or for the application of the leader sequence of α -amylase and is even more advantageous than that of *B. subtilis* from the view point of thermostability. Based on this, we change the name of

the genotype of this α -amylase gene which has already been designated as *amyV2* to *amyF*.

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