Note

Restriction Endonuclease Mapping of pUH1 in *Bacillus subtilis* (natto)

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In our previous papers,^{1~4)} we reported that γ -glutamyl-transpeptidase activity mainly participates in the biosynthesis of polyglutamate (PGA) in *Bacillus subtilis* (natto) Asahikawa, isolated from the commercial product, "natto," and that the function of the 5.7-kilobase plasmid isolated from the bacteria is in PGA production. In order to understand the role of the plasmid in PGA production, we further characterized the plasmid. This paper presents the restriction endonuclease cleavage site map of the plasmid, pUH1.

The plasmid was prepared from late-logarithmic-phase cells, lysed with lysozyme and sodium dodecyl sulfate (*i.e.*, cleared lysate) according to the method of Guerry *et al.*⁵⁾ Purification of covalently closed circular (CCC) plasmid DNA was performed by cesium chloride-ethidium bromide dye buoyant-density centrifugation, according to Humphreys *et al.*⁶⁾ The plasmid was cleaved with restriction enzymes such as *Bam*HI, *Bgl*II, *Eco*RI, *Hap*II, *Hinc*II, *Hind*III, *Kpn*I, *Pvu*II, *Sal*I, *Sma*I and *Xho*I, which were purchased from Takara Brewery Co. Ltd., Kyoto. The conditions for the reactions for the cleavage

of plasmid DNA by the various enzymes were the same as those recommended by the Bethesda Research Laboratory. The digested DNA was analyzed by horizontal electrophoresis on 1.0% agarose (Nakarai Chemicals, Ltd.) gel. The molecular sizes of the DNA fragments were estimated after calibration of the electrophoretic mobility of phage λ DNA fragments digested with

FIG. 1. Agarose Gel Electrophoresis of Fragments of pUH1 Digested with Two Restriction Endonucleases.

The plasmid DNA was cleaved with *Bam*HI and *Pvu*II (lane B), *Hinc*II and *Pvu*II (lane C), *Bam*HI and *Hinc*II (lane D), *Eco*RI and *Hinc*II (lane E), *Eco*RI and *Pvu*II (lane F) and *Bam*HI and *Eco*RI (lane G). As molecular size markers, *Hind*III fragments of phage λ DNA were used. Electrophoresis was performed in 1.0% agarose gel.

TABLE I. FRAGMENTS PRODUCED ON RESTRICTION
ENDONUCLEASE DIGESTION OF pUH1

Enzyme	Fragment size ^a (kb)			
	A	B	C	D
<i>Hinc</i> II	5.7			
<i>Pvu</i> II	5.7			
<i>Bam</i> HI	4.2	1.5		
<i>Eco</i> RI	4.5	1.2		
<i>Hind</i> III	3.9	0.8	0.6	0.4

^a Molecular weights were estimated by agarose gel electrophoresis, using *Hind*III fragments of phage λ DNA as the standards.

FIG. 2. Restriction Endonuclease Cleavage Map of pUH1.

The relative distances shown are from average fragment sizes determined on agarose gels.

*Hind*III. The restriction patterns are shown in Table I. *Hinc*II and *Pvu*II gave a single fragment, *Bam*HI and *Eco*RI two fragments, and *Hind*III four fragments. The average size of pUH1 estimated from these results was about 5.7 kilobases. Double digestions with single-size enzymes allowed unambiguous mapping of these sites relative to one another. Electrophoretic patterns of some double-digested samples are shown in Fig. 1. Based on these analyses, the map positions of the various restriction sites were determined and given as kilobase coordinates relative to the *Hinc*II restriction site at 0.0/5.7 kilobases (Fig. 2).

In attempts to ascertain the distribution of extrachromosomal DNA elements in strains of *B. subtilis*, Uozumi *et al.*⁷⁾ reported that plasmids in *B. subtilis* could be classified into six classes according to their molecular weights and restriction patterns. Our results showed that plasmid pUH1 had a different molecular weight and different restriction patterns from those six plasmid groups.

As bacilli are important in the fermentation industry and in the commercial production of antibiotics, enzymes and insecticides, the development of a molecular cloning system in *B. subtilis* is desirable. Many *B. subtilis* strains have been shown to contain a number of endogenous plasmids⁷⁻¹⁴⁾ and some of them were used as cloning vectors in *B. subtilis*. We believe pUH1 is also a possible candidate for a cloning vector in *B. subtilis*. Construction of a cloning vector using pUH1 is now in progress.

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