Volume 331, number 1,2, 81–85 FEBS 13009 September 1993

Physical map of the extremely thermophilic bacterium *Thermus thermophilus* HB27 chromosome

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Received 23 July 1993

The physical map of the chromosome of *Thermus thermophilus* HB27 was constructed using three restriction enzymes; *Eco*RI, *Ssp*I and *Mun*I by applying pulsed-field gel electrophoresis techniques. Although the genome size of 1.82 Mb was almost the same as that (1.74 Mb) reported for *T thermophilus* HB8 [Borges, K.M., and P.L. Bergquist. (1993) J. Bacteriol. 175, 103–110], the *Mun*I cleavage maps were different. A 240 kb plasmid was detected in HB27, and its physical map was also constructed. In addition, several genes were located on the chromosomal physical map.

Physical map; Extreme thermophile; *Thermus thermophilus*

1. INTRODUCTION

Extremely thermophilic bacteria can be used in various fields including fermentation industries due to their heat resistance. Among them, *Thermus thermophilus* and other *Thermus* strains are representative species, and various biochemical studies have been carried out these species. *T. thermophilus* is a Gram-negative, aerobic microorganism that can grow at temperatures ranging between 50 and 82°C. *Thermus* species are capable of incorporating foreign DNA through their natural transformation system [1]. Among several strains, *T. thermophilus* HB27 showed the highest transformation ability. We are currently carrying out genetic and biochemical studies on *T. thermophilus* using HB27 and its various mutants. We started to construct a physical map of the HB27 chromosome, and during our investigation other authors described the physical map of the *T. thermophilus* HB8 chromosome [2]. Comparison of the map of the HB27 chromosome with that of HB8 revealed that the two maps were different. Here, we report on the physical map of the HB27 chromosome together with the map of a newly found large plasmid pTT27.

2. MATERIALS AND METHODS

2.1. *Bacterial strains, plasmids and DNA manipulation*

Thermus thermophilus HB27 [3], its auxotrophic mutants, *Escherichia coli* JM109 [4], and pUC18/19 [4] were used. Restriction enzymes and T4 DNA ligase were supplied by either Toyobo or Takara. DNA manipulation was performed according to the standard methods [5].

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2.2. *Preparation of intact DNA in agarose plugs*

T. thermophilus HB27 was grown overnight in a TM medium [1] to the stationary phase. DNA samples were prepared in agarose blocks as described by Itaya et al. [6].

2.3. *Digestion of DNA in agarose blocks and electrophoresis*

Agarose blocks were soaked in an appropriate enzyme buffer for 30 min, and then immersed in the same buffer to which a 5 to 10 unit sample of *Eco*RI or *Ssp*I, or 1 unit of *Mun*I was added. The digestion usually required 12 h at 37°C.

Pulsed-field gel electrophoresis (PFGE) was performed on a 1% agarose gel (SeaKem GTG Agarose, FMC BioProducts) in 0.5 × Tris-borate buffer (45 mM Tris, 45 mM borate, 1 mM EDTA pH8.0) at 7 to 8 V/cm by using a CHEF-DR2 apparatus (Bio-Rad Laboratories) or a Biocraft apparatus at 13–15°C for 36 to 48 h. Various pulse time ramps were used depending on the molecular weights of the fragments for resolution. Phage lambda ladder DNA concatemers (Clontech), *Saccharomyces cerevisiae* chromosomal DNA (Bio-Rad Laboratories), lambda-*Hin*dIII digest (Takara), or Marker 7 (Nippongene) were used as size markers. Fragments smaller than 10kb were analyzed by using standard agarose gel electrophoresis (AGE) with lambda-*Bst*PI digest (Takara), or lambda-*Hin*dIII digest as size markers.

2.4. *Isolation of* Eco*RI linking clones*

To determine the order of the restriction fragments, linking clones for *Eco*RI fragments were used. An *Eco*RI linking clone is defined as a clone which contains a chromosomal fragment with an *Eco*RI site. In order to construct linking clones, the following two methods were used. (i) *T. thermophilus* HB27 chromosomal DNA was partially digested with *Msp*I and ligated with *Acc*I digested and dephosphorylated pUC18′ (a derivative of pUC18 in which the *Eco*RI site is lost). After transformation of *E. coli* JM109, all the colonies were collected by scraping the plates. Plasmid DNAs from the library were subsequently prepared, cleaved with *Eco*RI, and subjected to PFGE to recover the linearized DNA fragments [7]. They were re-ligated and used to transform *E. coli* JM109. Plasmid DNAs were prepared from the transformants thus obtained and restriction analysis was performed. (ii) The second method was similar to that of Ladefoged et al. [8]. Chromosomal DNA was completely cleaved with the following restriction enzymes; *Acc*I, *Bam*HI, *Kpn*I and *Sac*I, respectively. The fragments were self-ligated into circles, then treated

with *Eco*RI. The linearized fragments associated with the presence of an *Eco*RI site were ligated to pUC19 restricted by *Eco*RI and dephosphorylated. The ligation mixture was used to transform *E coli* JM109 and the plasmids were prepared from the transformants

2.5. *DNA transfer and hybridization*

DNA in the PFGE gels was transferred onto nitrocellulose (Schleicher and Schuell) or nylon membranes (Amersham) by capillary transfer using 20 × SSC (3 M NaCl, 0.3 M tri-sodium citrate at pH 7.0).

A non-radioactive labelling nucleotide, digoxygenin-11-UTP, was used for preparing the DNA probes. The random primer labelling technique, pre-hybridization and hybridization procedures and color development followed the protocol for the DNA labelling and detection kit (Boehringer Mannheim).

For the hybridization of the linking clones and gene probes, probe plasmids linearized with *Eco*RI were labelled. For the hybridization of isolated restriction fragments to PFGE-separated fragments digested with restriction enzymes, referred to as band hybridization, individual bands were cut out from the gels Each gel slice was heated to 95°C for 10 min to melt the agarose and denature the DNA, then labelling reagents were added, and the mixture was incubated at 37°C for 16 h.

3. RESULTS

3.1. *Digestion of T. thermophilus HB27 DNA by restriction endonucleases*

To obtain a relatively small number of fragments from the chromosome cleaved with restriction endonucleases, about 10 restriction enzymes were tested due to the relatively high GC content (70%) of the organism. Three restriction enzymes (*Eco*RI, *Mun*I, and *Ssp*I) which cleaved the chromosome into a small number of DNA fragments were found to be useful for the construction of the genome maps (Fig. 1). The estimated sizes of the fragments are listed in Table I.

3.2. *Physical map of the T. thermophilus HB27 chromosome*

To align the restriction fragments, we used two methods; (i) Southern hybridization with *Eco*RI linking clones, and (ii) band hybridization.

As there are 15 *Eco*RI sites on the total DNA of HB27 (Table I), it is assumed that 15 *Eco*RI linking clones should be present. Although experiments to recover all the linking clones were performed repeatedly, only 8 linking clones were obtained. These clones were used to probe nitrocellulose or nylon membranes onto which chromosomal DNA digested by each restriction enzyme had been transferred. The results of hybridization are listed in Table II, and Fig. 2 shows representative results. Based on the results, the alignment of the *Eco*RI fragments A-E-D-H1, B-K, and C-J was determined along with the relative positions of some of the *Mun*I and *Ssp*I fragments (Table II). The existence of a large plasmid consisting of three *Eco*RI fragments F, G, and I was suggested based on the results of hybridization of the linking clones VI, VII, and VIII and the sum of the F, G, and I fragments was about 240 kb.

Band hybridization experiments were performed to complete the physical map. Appropriate restriction fragments from one digestion were isolated from the gel after PFGE separation and then labelled and hybridized

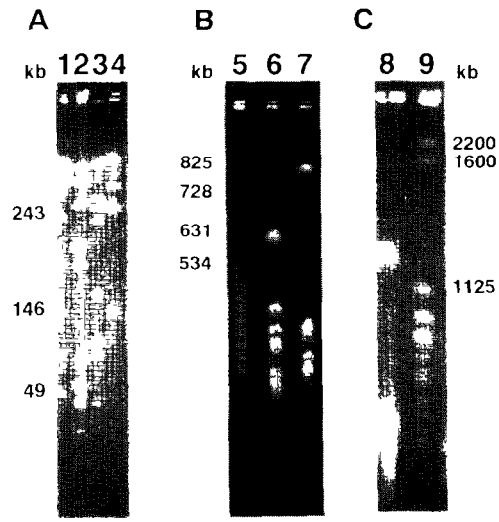

Fig. 1. *Eco*RI, *Ssp*I and *Mun*I digestion of the *T thermophilus* HB27 genome. The chromosome of HB27 was analyzed through digestion with *Eco*RI (lanes 2 and 6), *Ssp*I (lanes 3 and 7), or *Mun*I (lanes 4 and 8) followed by PFGE. Short (A, 20 s) and long (B and C, 96 s and 192 s, respectively) pulse times were used to resolve the restriction fragments generated by each restriction enzyme. Concatemeric lambda DNA molecular size markers are shown in lanes 1 and 5, and lane 9 contains the *S cerevisiae* chromosomes. Numbers indicate the length (in kilobases) of the molecular size markers.

Table I

Sizes of restriction fragments generated by cleavage of the *T thermophilus* HB27 total DNA

	*Eco*RI		*Ssp*L		*Mun*I	
	Band	Size (kb)	Band	Size (kb)	Band	Size (kb)
	A	595	A	815	A	1273
	B	343	B	267	B	288
	C	261	C	246	C1*	243
	D	208	D*	232	C2	243
	E	191	E	158	D	132
	F*	95	F1	104		
	G*	85	F2	104		
	H1	61	G	91		
	H2	61	H	38		
	I*	54	I*	10		
	J	41	J	1.5		
	K	37				
	L	18				
	M	6.0				
	N	1.3				
Total						
Chromosome		1823		1825		1936
Plasmid*		234		242		243

*Based on the results of hybridization experiments, it is suggested that these fragments was derived from a large plasmid.

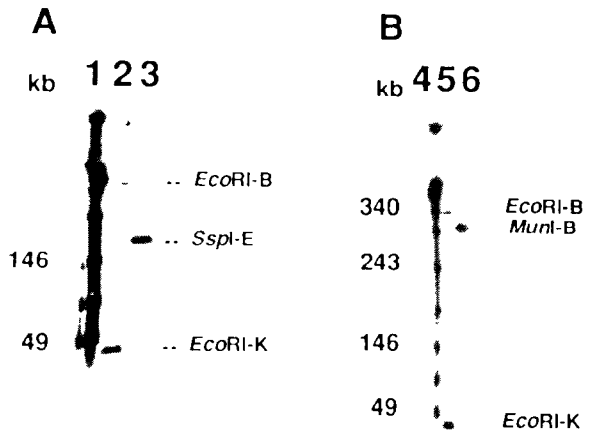

Fig. 2. Hybridization of linking clones to PFGE-separated fragments of *T. thermophilus* HB27 DNA digested with restriction enzymes. Linking clone II and lambda DNA were labelled with digoxygenin-11-UTP (DIG) and used to probe Southern transfers of lambda DNA concatemers (lanes 1 and 4), and *T thermophilus* HB27 DNA digested with *Eco*RI (lanes 2 and 5), *Ssp*I (lane 3), or *Mun*I (lane 6). Panels A and B correspond to two different PFGE separations.

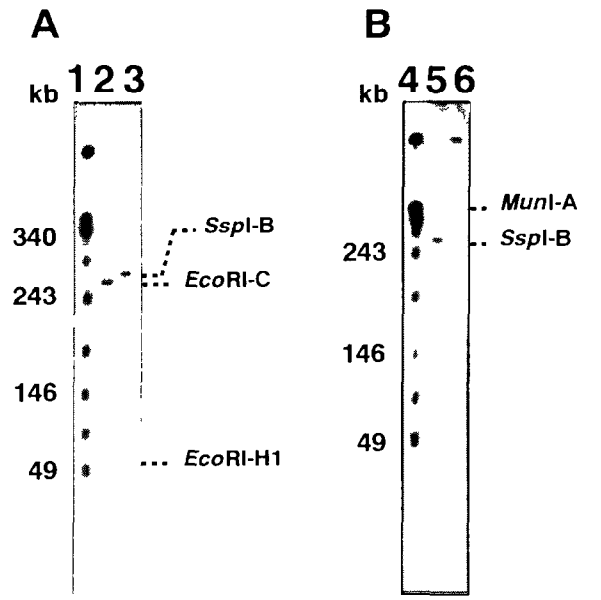

Fig. 3. Hybridization of the isolated *Ssp*I restriction fragment to PFGE-separated fragments of *T thermophilus* HB27 DNA digested with restriction enzymes. Isolated *Ssp*I restriction fragment B and lambda DNA were DIG-labelled and used to probe Southern transfers of lambda DNA concatemers (lanes 1 and 4), and *T. thermophilus* HB27 DNA digested with *Eco*RI (lane 2, *Ssp*I (lanes 3 and 5), or *Mun*I (lane 6). Panel A and B correspond to two different PFGE separations.

individually to PFGE separation of other restriction fragments. The results are listed in Table III, and Fig. 3 shows representative results.

All the restriction fragments were aligned by using these methods except for small fragments detected by conventional AGE (*Eco*RI – M, N; and *Ssp*I – J). These fragments were much smaller than other large fragments, and their absence from the physical map did not exert an appreciable effect on the alignment.

The presence of a 240 kb plasmid designated pTT27 was also confirmed by band hybridization (Table III). Screening of pTT27 was performed using the method of Casse et al. [9] and a plasmid band with a size of more than 200 kb was detected (data not shown).

The chromosomal physical map and the physical map of pTT27 are shown in Fig. 4. The genome size of HB27 was estimated at 1.82 Mb.

The cloned *T. thermophilus* genes were located on the chromosomal physical map using Southern hybridization. The gene probes used are listed in Table IV, and the map positions are shown in Table IV and above the map in Fig. 4. When two or more genes were found to be present in the same region, they are marked on the top of each other. The genes, *trpBA* [10], *trpEG* [11], and *proC* encoded tryptophan synthase B and A protein, anthranilate synthase I and II, and pyrroline-5-carboxylate reductase, respeectively. Although other fragments complemented each mutation locus on the chromosome, the corresponding gene product of each has

Table II

Hybridization of *Eco*RI linking clones

Probe (linking clone)	Fragment hybridized		
	*Eco*RI	*Ssp*I	*Mun*I
I	A, E	A	A
II	B, K	E	B
III	C, J	G	A
IV	D, E	A	A
V	D, H1	F1	A
VI	F, G	D	C1
VII	F, I	D	C1
VIII	G, I	I	C1

Table III

Hybridization of isolated restriction fragments

Probe (restriction enzyme and fragment)	Fragment hybridized		
	*Eco*RI	*Ssp*I	*Mun*I
*Eco*RI – A	A	A, E	A, B, D
*Eco*RI – B	B	C, E, F2	B, C2
*Eco*RI – C	C	B, G	A
*Eco*RI – L	L	C	A, C2
*Ssp*I – B	C, H1	B	A
*Ssp*I – C	B, H2, L	C	C2
*Ssp*I – D	F, G, I	D	C1
*Ssp*I – H	D	H	A
*Mun*I – B	A, B, K	E, F2	B
*Hum*I – D	A	A	D

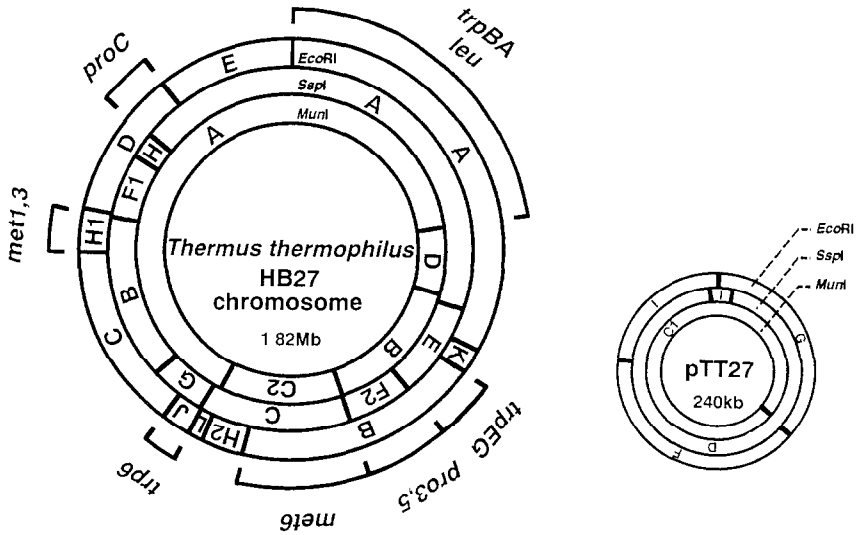

Fig 4. Physical maps of the chromosome of *T thermophilus* HB27 and plasmid pTT27, based on digestion with *Eco*RI, *Ssp*I and *Mun*I. Regions to which genes were mapped are indicated by bars.

not been identified yet. The size of the mapped areas ranged from 40 kb for the *trp6* gene up to 385 kb for the *leu* and *trpBA* genes.

4. DISCUSSION

The sum of the sizes of all the *Mun*I restriction fragments derived from the chromosome was 1.94 Mb (Table I). Since it was difficult to determine precisely the size of large fragments, such as the *Mun*I fragment A, the value was considered to be somewhat inaccurate. Thus we assumed that the genome size was 1.82 Mb based on the results of *Eco*RI and *Ssp*I digestions, in which the fragments were not very large.

Although the genome size of HB27 was similar to that of *T. thermophilus* HB8 [2], the *Mun*I cleavage map of *T. thermophilus* HB27 was markedly different from that of HB8. Since the physical map of the HB8 chromosome was constructed by using *Hpa*I, *Nde*I, and *Mun*I, we also used *Hpa*I and *Nde*I to cleave the HB27 chromosome. Cleavage patterns were different from those reported for HB8 (data not shown). Thus, it was concluded that the physical maps of the chromosomes of HB8 and HB27 were different from each other. Since the genes used for hybridization probes differed between our experiment and that of the other authors (only *trpBA* and *trpEG* genes were the same), it is difficult to analyze the difference in the genome organization between HB8 and HB27.

As the size of the chromosome of *T. thermophilus* HB27 was similar to that of *Streptococcus thermophilus* (1.7 Mb) [12], and the thermophilic archaebacterium *Thermococcus celer* (1.89 Mb) [13], it is suggested that a small genome size is a common feature to many types of bacteria occurring at high temperatures [2].

Cloning of other genes is now in progress. It is assumed that the cloning of a larger number of genes, and their use as hybridization probes, may contribute to refine the physical map of the HB27 genome.

Table IV

Location of cloned genes on the *T thermophilus* chromosome

Probe (gene)	Reference or source	Fragment hybridized		
		*Eco*RI	*Ssp*I	*Mun*I
leu	Y. Koyama	A	A	A
met1,3	This laboratory*	H1	B	A
met6	This laboratory*	B	C	C2
pro3,5	This laboratory*	B	F2	B
proC	This laboratory*	D	A	A
trpBA	[10]	A	A	A
trpEG	[11]	B	E	B
trp6	This laboratory*	J	G	A

*A chromosomal fragment which complemented each mutation was screened from the genomic library of *T thermophilus* HB27 constructed in our laboratory.

Acknowledgements. We are grateful to Dr. M. Itaya for the helpful discussions. We thank the investigators who supplied the cloned genes. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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