Mapping of 61 genes on the refined physical map of the chromosome of *Thermus thermophilus* HB27 and comparison of genome organization with that of *T. thermophilus* HB8

Kazuyuki Tabata and Takayuki Hoshino

Author for correspondence: Takayuki Hoshino. Tel: +81 298 53 6782. Fax: +81 298 53 4605.
e-mail: takachan@sakura.cc.tsukuba.ac.jp

Institute of Applied
Biochemistry, University of
Tsukuba, Ibaraki 305, Japan

We have constructed refined physical maps of the chromosome (1.82 Mb) and the large plasmid pTT27 (250 kb) of *Thermus thermophilus* HB27. A total of 49 cleavage sites with five restriction enzymes, *EcoRI*, *SspI*, *MunI*, *EcoRV* and *ClaI*, were determined on the maps. The location of 61 genes was determined by using as probes 64 genes cloned from *T. thermophilus* or other *Thermus* strains. Comparison of the genomic organization of the chromosomes of *T. thermophilus* HB27 and HB8 revealed that they were basically identical, but some genes were located in different regions. Among 32 genes whose locations were determined on both the HB27 and the HB8 chromosomes, the copy number of *rpsL-rpsG-fus-tufA*, the locations of *glyS*, *pol*, and one copy of *nusG-rplK-rplA* were different. The *IS1000* sequence was located only in one region on the HB27 chromosome. In contrast, *IS1000* sequences were scattered over four regions on the chromosome of HB8. As each region in which *glyS*, *pol*, or one copy of *nusG-rplK-rplA* are present also contained *IS1000* in HB8, it is suggested that *IS1000* may play an important role in genomic rearrangements in *Thermus* strains.

Keywords: *Thermus thermophilus*, physical map, insertion sequence

INTRODUCTION

Thermus thermophilus is a Gram-negative, aerobic micro-organism which can grow at temperatures above 75 °C, and is one of the most widely studied of the thermophilic bacteria. *Thermus* strains are known to produce unique substances such as carotenoids as well as many thermostable enzymes and proteins. We have isolated various auxotrophic mutants, carotenoid under- and over-producing mutants from *T. thermophilus* HB27 and carried out genetic studies of the strain (Hoshino *et al.*, 1993a, 1994a, c, d; Kosuge *et al.*, 1994; Tabata *et al.*, 1994). We showed that carotenogenic genes occur in a cluster and are located on the plasmid pTT27 whose size is about 250 kb in *T. thermophilus* HB27 (Tabata *et al.*, 1994).

One of the characteristics of the *Thermus* strains is that they are capable of incorporating foreign DNA through a natural transformation system (Koyama *et al.*, 1986). Among the *Thermus* strains examined, *T. thermophilus*

HB27 showed the highest transformation ability (Koyama *et al.*, 1986). Therefore, studies aimed at using this transformation system as a tool for genetic analysis and molecular breeding, including improvement of the host vector system, have been conducted using *T. thermophilus* HB27 (Hidaka *et al.*, 1994; Hoshino *et al.*, 1993b, 1994b) in our laboratory.

In recent years, we have constructed physical maps of the *T. thermophilus* HB27 chromosome and the large plasmid pTT27 using three restriction enzymes, *EcoRI*, *SspI* and *MunI* together with the determination of the location of 11 genes (Tabata *et al.*, 1993). A comparison of the chromosomal maps among the strains belonging to the same genus has provided information on differences in genomic organization and possible genomic rearrangements that occur within the genus (Ladefoged & Christiansen, 1992). The physical map of the *T. thermophilus* HB8 chromosome was constructed independently using *HpaI*, *NdeI* and *MunI* (Borges & Bergquist, 1993; P. L. Bergquist, personal communication). Although the genome size of HB27 (1.82 Mb) is almost the same as that reported for HB8 (1.74 Mb), the cleavage patterns of

Abbreviation: DIG, digoxigenin-11-UTP.

several restriction endonucleases and the *MunI* cleavage maps were different (Tabata *et al.*, 1993). Thirty five genes were mapped on the HB8 chromosome. Since the numbers of genes or gene clusters used for probes to determine genomic locations were not large for either the HB8 or the HB27 chromosomes, and only two gene clusters were commonly used, it is difficult to determine the difference in the genome organization between HB8 and HB27 (Tabata *et al.*, 1993).

In this paper, we have used *EcoRV* and *ClaI* to refine the physical maps of the HB27 chromosome and pTT27, and have identified a total of 49 restriction enzyme cleavage sites on the HB27 chromosome. Moreover, the location of a total of 61 genes was determined on the maps. Comparisons between the refined HB27 maps and the HB8 chromosomal map revealed a significant similarity in the genome organization of the two strains. However, some genes were located in different regions of the genomes in both strains. The possible role of insertion sequence IS1000 (Ashby & Bergquist, 1990) in genomic rearrangements is also discussed.

METHODS

Bacterial strains and plasmids. *T. thermophilus* HB27 (Sakaki & Oshima, 1975), *Escherichia coli* JM109 (Yanisch-Perron *et al.*, 1985), pUC19 (Yanisch-Perron *et al.*, 1985) were used. *T. thermophilus* HB27 was grown in TM medium (Koyama *et al.*, 1986) at 70 °C.

Preparation of DNA, DNA manipulations and PFGE. DNA manipulation was performed according to standard methods (Sambrook *et al.*, 1989). Restriction endonucleases were supplied by Toyobo or Takara. Preparation procedures of intact DNA in agarose plugs and their digestion were described previously (Tabata *et al.*, 1993). PFGE was performed on a 1% (w/v) agarose gel (SeaKem GTG agarose; FMC BioProducts) in 0.5 × Tris/borate buffer (45 mM Tris, 45 mM borate, 1 mM EDTA, pH 8.0) at 7.5 V cm⁻¹ by using a CHEF-DR2 apparatus (Bio-Rad) at 13–15 °C for 36 h. Various pulse time ramps were used depending on the molecular masses of the fragments for resolution. Phage lambda ladder DNA concatemers (Clontech or FMC BioProducts), *Saccharomyces cerevisiae* chromosomal DNA (Bio-Rad), or lambda/*HindIII* digest (Takara) were used as size markers. Fragments smaller than 10 kb were analysed by using standard agarose gel electrophoresis with lambda/*HindIII* or lambda/*BstPI* digests (Takara) as size markers.

Isolation of two *EcoRV* linking clones. To complete the *EcoRV* cleavage map, i.e. to determine the order of *EcoRV* fragments C, J, and O (Fig. 3), two linking clones for *EcoRV* fragments were used. To construct these, *T. thermophilus* HB27 total DNA was partially digested with *MspI* and ligated with *AccI*-digested and dephosphorylated pUC19. After transformation of *E. coli* JM109, all the colonies were collected by scraping the plates and then resuspended in 0.85% saline. Plasmid DNA from the library was subsequently prepared, cleaved with *EcoRV*, and subjected to PFGE to recover linearized DNA fragments (Ito & Sakaki, 1988). They were re-ligated and used to transform *E. coli* JM109. Plasmid DNAs which were prepared from the transformants were transferred onto nitrocellulose membranes, and hybridized with a probe of the largest restriction fragment of the chromosome digested with *EcoRI* and *MunI* (shaded region in Fig. 3). Plasmid clones that hybridized with the probe, namely pEV016 and pEV034, were used as hybridization probes as described below.

Cloning of small fragments produced by digestion by rare-cutting endonucleases. *T. thermophilus* HB27 total DNA was digested with *EcoRI*, *SspI*, *EcoRV*, or *ClaI*, run on an agarose gel and the following six restriction fragments were recovered: *EcoRI* fragments M and N, *SspI* fragment J, *EcoRV* fragment R, and *ClaI* fragments E and F. Each fragment was ligated to pUC19 and introduced into *E. coli* JM109 by transformation. Clones containing each of these fragments were prepared and used as hybridization probes. Since no clone containing *ClaI* fragment E was obtained, it was directly labelled with digoxigenin-11-UTP (DIG) and used as a hybridization probe.

DNA transfer and hybridization. DNA fragments in the agarose gel were transferred onto a nitrocellulose filter (Schleicher & Schuell) by capillary transfer using 20 × SSC (3 M NaCl, 0.3 M tri-sodium citrate at pH 7.0). A non-radioactive labelling nucleotide, DIG, was used for preparing the DNA probes. The random primer labelling technique, pre-hybridization, hybridization procedures and colour development were performed according to the protocol of the DNA labelling and detection kit (Boehringer Mannheim). Gene probes of *Thermus* strains were generously supplied by the researchers indicated in Table 5. For the hybridization of isolated restriction fragments with PFGE-separated fragments digested with other restriction enzymes (band hybridization), individual bands were cut from the gels. Each gel slice was heated to 95 °C for 10 min to melt the agarose and denature the DNA, labelling reagents were added, and the mixture was incubated at 37 °C for 16 h.

RESULTS

Digestion of HB27 DNA with *ClaI* or *EcoRV*

In our previous experiments on the physical map of the chromosome of HB27 based on digestion with *EcoRI*, *SspI*, and *MunI* (Tabata *et al.*, 1993), it was confirmed that two restriction endonucleases, *ClaI* and *EcoRV*, cleaved

Fig. 1. *EcoRI*, *SspI*, *MunI*, *ClaI* and *EcoRV* digestion of the *T. thermophilus* HB27 total DNA. The total DNA of HB27 was analysed through digestion with *EcoRI* (lanes 2 and 7), *SspI* (lane 3), *MunI* (lane 4), *ClaI* (lane 5) or *EcoRV* (lane 8), followed by PFGE. Pulse time was 25 s (a) and 10 s (b). Concatemeric lambda DNA molecular mass markers are shown in lanes 1 and 6, and lane 9 contains the lambda/*HindIII* digest.

Table 1. Sizes of restriction fragments generated by cleavage of *T. thermophilus* HB27 total DNA

<i>EcoRI</i>		<i>SspI</i>		<i>MunI</i>		<i>ClaI</i>		<i>EcoRV</i>	
Band	Size (kb)	Band	Size (kb)	Band	Size (kb)	Band	Size (kb)	Band	Size (kb)
A	595	A	815	A	1273	A	1300	A	523
B	343	B	267	B	288	B	232	B1	174
C	261	C	246	C1*	243	C	167	B2	174
D	208	D*	232	C2	243	D	103	C	156
E	191	E	158	D	132	E	4.1	D*	126
F*	99	F1	104			F	1.1	E	122
G*	88	F2	104					F	116
H1	61	G	91					G	113
H2	61	H	38					H	86
I*	57	I*	10					I1	80
J	41	J	1.5					I2	80
K	37							I3*	80
L	18							J	57
M	6.0							K	41
N	1.3							L1	31
								L2	31
								M*	28
								N	20
								O	17
								P	12
								Q*	9.2
								R	3.9
Total DNA									
Chromosome:	1823		1825		1936		1807		1837
Plasmid:*	246		242		243		—†		243

* Fragment was derived from the large plasmid pTT27.

† There was no *ClaI* site on pTT27.

the chromosome (and a large plasmid pTT27 of about 250 kb) into 6 and 22 fragments, respectively. These were useful as rare-cutting endonucleases for the HB27 genome. Fig. 1 shows the typical PFGE banding pattern produced by these restriction endonucleases. The estimated sizes of the fragments are listed in Table 1. Band sizes were determined by comparison to size markers. The mean size of each band was determined by several independent measurements.

Construction of *EcoRV* cleavage maps of the HB27 chromosome and pTT27

(i) **Southern hybridization with *EcoRI* linking clones.** Eight *EcoRI* linking clones, which were employed to align the *EcoRI* restriction fragments (Tabata *et al.*, 1993), were used to determine the positions of several *EcoRV* restriction fragments. These clones were used to probe nitrocellulose membranes onto which chromosomal DNA digested by *EcoRV* had been transferred. Generally, a single *EcoRI* linking clone used as a probe should produce a single band when hybridized against a membrane carrying fragments of an *EcoRV* digest.

The results of the hybridizations (Table 2) allowed the positions of *EcoRV* restriction fragments A, B1, B2, D, F,

G, M and Q on the *EcoRI* cleavage map to be determined. Among eight *EcoRI* linking clones, clone II, which contains the *EcoRI* site between *EcoRI* restriction fragments B and K, also contained the *EcoRV* site about 2 kb away from the *EcoRI* site. Since the '*EcoRI* and *EcoRV* linking clone' actually hybridized with two *EcoRV* restriction fragments, B1 and G (Fig. 2a), it was confirmed that these fragments were linked.

(ii) **Band hybridization experiments.** Band hybridization experiments were performed to determine the positions of the *EcoRV* restriction fragments in relation to the *EcoRI*, *SspI* and *MunI* fragments. Appropriate restriction fragments from one digestion were isolated from the gel after PFGE separation and then labelled and hybridized individually with other restriction fragments separated by PFGE. The results are listed in Table 3.

The positions of *EcoRV* restriction fragments E, H, I1, I3, K, L2, N and P were determined. Although it was indicated that fragments I2 and L1 were located between fragments E and G, and that fragments C, J and O were located between fragments B2 and E, the order of these fragments could not be determined.

The result of hybridization of *MunI* fragment D with the fragments generated by the double digestion of total

Table 2. Hybridization of linking clones

Probe (linking clone)	Fragment hybridized:		
	<i>EcoRV</i>	<i>ClaI</i>	<i>EcoRI</i>
<i>EcoRI</i> linking clone			
I	B2	A	A, E
II	B1, G	A	B, K
III	A	D	C, J
IV	F	A	D, E
V	A	A	D, H1
VI	M	ND	F, G
VII	D	ND	F, I
VIII	Q	ND	G, I
<i>EcoRV</i> linking clone			
pEV016	C, E	NT	A
pEV034	B2, J	NT	A

ND, Not detected.

NT, Not tested.

Table 3. Hybridization of isolated restriction fragments

Probe (restriction enzyme and fragment)	Fragment hybridized:			
	<i>EcoRV</i>	<i>EcoRI</i>	<i>SspI</i>	<i>MunI</i>
<i>EcoRI</i>				
A	B2, C, E, G I2, J, L1, O	A	NT	NT
B	B1, H, I1, P	B	NT	NT
D	A, F	D	NT	NT
E	B2, F	E	NT	NT
G	I3	G	NT	NT
H (H1 and H2)	A, H, K	H	NT	NT
J	A, L2	J	NT	NT
L	N	L	NT	NT
<i>SspI</i>				
C	H, I1, K, N	B, H2, L	C	NT
<i>MunI</i>				
D	E, I2, L1	A	NT	D

NT, Not tested.

Fig. 2. Hybridization of linking clones with PFGE-separated fragments of *T. thermophilus* HB27 DNA digested with restriction enzymes. (a) *EcoRI* linking clone II and lambda DNA or (b) *EcoRV* linking clone pEV034 and lambda DNA were labelled with DIG and used to probe Southern transfers of lambda concatemers (lanes 1 and 4), and *T. thermophilus* HB27 DNA digested with *EcoRI* (lanes 2 and 5) or *EcoRV* (lanes 3 and 6).

DNA with *EcoRV* and *MunI* revealed that fragment I2 contained a *MunI* site. Hence, the order of I2 and L1 was determined as shown in Fig. 3 (–E–L1–I2–G–).

(iii) **Southern hybridization with two *EcoRV* linking clones.** The order of the fragments C, J and O could not be determined by band hybridization because there was no restriction site for rare-cutting endonucleases which were used to construct cleavage maps previously (Tabata *et al.*, 1993). Therefore, we isolated *EcoRV* linking clones for

the region and used them to align these fragments. Due to the recovery of *EcoRV* linking clones by the method described in Methods, two linking clones named pEV016 and pEV034 were isolated. The probe of pEV016 hybridized with *EcoRV* restriction fragments C and E, and that of pEV034 hybridized with fragments B2 and J (Fig. 2b). The order of fragments C, J and O was –B2–J–O–C–E–.

Based on the combination of the three different methods described above, all *EcoRV* restriction fragments separated by PFGE were aligned. The deduced *EcoRV* cleavage map of the HB27 chromosome and that of the large plasmid pTT27 are depicted in Figs 3 and 4.

Construction of *ClaI* cleavage maps of the HB27 chromosome and pTT27

A *ClaI* cleavage map of the HB27 chromosome was constructed by utilizing *EcoRI* linking clones and band hybridization experiments by applying similar methods to those used for the construction of the *EcoRV* cleavage maps (Tables 2 and 4). The chromosomal map is shown in Fig. 3. There was no *ClaI* site on pTT27.

Location of small fragments produced by digestion by rare-cutting endonucleases

Digestion of HB27 intact DNA with the rare-cutting endonucleases used in the previous study (Tabata *et al.*, 1993) and this work gave small fragments of less than 6 kb (see Table 1). Hybridization with these fragments as probes enabled us to determine the location of the fragments on the physical map. The *EcoRI* fragments M and N, *SspI* fragment J, and *ClaI* fragments E and F were determined to fall between *EcoRI* fragment J and L, D

Table 5. Location of cloned genes on the *T. thermophilus* chromosome

Gene(s)	Gene product(s)	Strain*	Fragment hybridized:†					Reference/source
			<i>EcoRV</i>	<i>EcoRI</i>	<i>SspI</i>	<i>MunI</i>	<i>ClaI</i>	
<i>argCB</i>	N-Acetylglutamate 5-semialdehyde dehydrogenase N-Acetylglutamate 5-phosphotransferase	HB27	B1	B	F2	B	C	This laboratory
<i>crtB</i>	Phytoene synthase	HB27	D	I	D	C1	ND	Hoshino <i>et al.</i> (1993a)
<i>fabB</i>	3-Oxoacyl synthase	HB27	A	C	B	A	A	This laboratory
<i>leu</i>	Leucine biosynthesis‡	HB27	O	A§	A§	A§	A	Y. Koyama
<i>met-1, -3</i>	Methionine biosynthesis‡	HB27	A	H1§	B§	A§	A	This laboratory
<i>met-6</i>	Methionine biosynthesis‡	HB27	I1	B§	C§	C2§	B	This laboratory
<i>proBA</i>	γ -Glutamyl kinase γ -glutamyl phosphate reductase	HB27	B1	B§	F2§	B§	C	Kosuge <i>et al.</i> (1994)
<i>proC</i>	Δ^5 -Pyrroline-5-carboxylate reductase	HB27	F	D§	A§	A§	A	Hoshino <i>et al.</i> (1994c)
<i>trp6</i>	Tryptophan biosynthesis‡	HB27	A	J§	G§	A§	D	This laboratory
<i>trpBA</i>	Tryptophan synthetase	HB27	B2	A§	A§	A§	A	Koyama & Furukawa (1990)
<i>aspS</i>	Aspartyl tRNA synthetase	HB8	A	D	F1	A	A	Poterszman <i>et al.</i> (1993)
<i>atpAB</i>	ATPase (V-type)	HB8	C	A	A	A	A	Tsutsumi <i>et al.</i> (1991)
<i>ba3</i>	Cytochrome oxidase <i>ba₃</i>	HB8	B2	A	A	A	A	J. A. Fee
<i>caa3</i>	Cytochrome oxidase <i>caa₃</i>	HB8	I1	B	C	C2	B	Mather <i>et al.</i> (1991, 1993)
<i>ffs</i>	4-5S RNA	HB8	B1	B	F2	B	C	Struck <i>et al.</i> (1988)
<i>gltX</i>	Glutamyl tRNA synthetase	HB8	A	C	B	A	A	Nureki <i>et al.</i> (1992)
<i>glyS</i>	Glycyl tRNA synthetase	HB8	A	C	B	A	A	R. Kreutzer
<i>groES-groEL</i>	Chaperonin	HB8	H	B	C	C2	B	M. Yoshida
<i>icd</i>	Isocitrate dehydrogenase	HB8	L1	A	A	D	A	Miyazaki <i>et al.</i> (1992)
<i>ileS</i>	Isoleucyl tRNA synthetase	HB8	B2	A	A	A	A	O. Nureki
IS1000	Insertion sequence	HB8	D, I3, L1, M	A, F, G	A, D	C1, D	A	Ashby & Bergquist (1990)
<i>ldh</i>	Lactate dehydrogenase	HB8	B2	A	A	A	A	Park & Kreutzer (1994)
<i>lig</i>	DNA ligase	HB8	B2	A	A	A	A	Barany & Gelfand (1991); Lauer <i>et al.</i> (1991)
<i>metS</i>	Methionyl tRNA synthetase	HB8	C	A	A	A	A	Nureki <i>et al.</i> (1991)
<i>nox</i>	NADH oxidase	HB8	A	C	B	A	A	Park <i>et al.</i> (1992)
<i>nusA-infB</i>	NusA, IF-2	HB8	A	D	F1	A	A	R. Kreutzer
<i>nusG-rplK-rplA</i>	Ribosomal proteins	HB8	G, H	A, B	C, E	B, C2	A, B	Heinrich <i>et al.</i> (1992)
<i>pfk</i>	Phosphofructokinase I	HB8	B1	B	F2	B	C	Xu <i>et al.</i> (1991)
<i>pheST</i>	Phenylalanyl tRNA synthetase	HB8	B1	B	F2	B	C	Kreutzer <i>et al.</i> (1992)
<i>pol</i>	DNA polymerase	HB8	B2	E	A	A	A	Asakura <i>et al.</i> (1993)
<i>recA</i>	RecA	HB8	B1	B	E	B	C	Kato & Kuramitsu (1993)
<i>rnhA</i>	RNaseH	HB8	I2	A	A	D	A	Itaya & Kondo (1991)
<i>rnp</i>	RNA subunit of RNaseP	HB8	B2	A	A	A	A	Hartmann & Erdmann (1991)
<i>rpsL-rpsG-fus-tufA</i>	Ribosomal proteins	HB8	G, H	A, B	C, E	B, C2	A, B	Seidler <i>et al.</i> (1987)
<i>rrn16S</i>	16S rRNA	HB8	G, N	A, H2, K, L	C, E	B, C2	A, B	Hartmann & Erdmann (1989)
<i>rrn23S-5S</i>	23S-5S rRNA	HB8	A, P	B, C	B, C, F2, G	A, C2	A, B, D	Hartmann <i>et al.</i> (1987)
<i>trpEG</i>	Anthranilate synthase I and II	HB8	B1	B§	E§	B§	C	Sato <i>et al.</i> (1988)
<i>tsf</i>	Elongation factor Ts	HB8	F	E	A	A	A	R. Kreutzer
<i>xylA</i>	Xylose isomerase	HB8	ND	ND	ND	ND	ND	Dekker <i>et al.</i> (1991)
<i>apt</i>	Aminopeptidase T	YT1	J	A	A	A	A	Motoshima <i>et al.</i> (1990)
<i>aql</i>	Aqualysin I	YT1	ND	ND	ND	ND	ND	Terada <i>et al.</i> (1990); Lee <i>et al.</i> (1991)
<i>cpt</i>	Carboxypeptidase	YT1	H	B	C	C2	B	H. Matsuzawa
<i>gap</i>	Glyceraldehyde-3-phosphate dehydrogenase	YT1	F	E	A	A	A	Hecht <i>et al.</i> (1989)
<i>sod</i>	Superoxide dismutase	YT1	A	C	B	A	A	H. Motoshima
<i>scsBA</i>	Succinyl-CoA synthetase	AT62	A	C	B	A	A	Nishiyama <i>et al.</i> (1991)
<i>mdh</i>	Malate dehydrogenase	EP00276	A	C	B	A	A	Park & Kreutzer (1994)
<i>spc</i>	Ribosomal proteins	EP00276	G	A	E	B	A	Jahn <i>et al.</i> (1991)
<i>lac</i>	α , β -Galactosidase	T2	ND	ND	ND	ND	ND	Koyama <i>et al.</i> (1990)

ND, Not detected.

* HB8, *T. thermophilus* HB8; YT1, *T. aquaticus* YT1; AT62, *T. flavus* AT62; EP00276, *T. aquaticus* EP00276; T2, *Thermus* strain T2.

† Fragment letters refer to those illustrated in Fig. 3.

‡ A chromosomal fragment which complemented each mutation was screened from the genomic library of *T. thermophilus* HB27.§ Data are from Tabata *et al.* (1993).

|| Personal communication.

Fig. 5. Hybridization of cloned gene probe. Insertion sequence IS1000 and lambda DNA were DIG-labelled and used to probe Southern transfers of *T. thermophilus* HB27 DNA digested with *EcoRI* (lane 1) or *EcoRV* (lane 2), lambda/*HindIII* digest (lane 3) and lambda DNA concatemers (lane 4).

rRNA genes) up to about 200 kb (fragment containing succinyl CoA synthetase genes). Two or more copies of the 16S rRNA genes, 23S–5S rRNA genes, *nusG-rplK-rplA*, *rpsL-rpsG-fus-tufA*, and insertion sequence IS1000 were located on the genome. No signal was detected when probes for either the gene encoding aqualysin I (*aql*) from *T. aquaticus* YT1, α , β -galactosidase (*lac*) from *Thermus* strain T2, or xylose isomerase (*xylA*) from *T. thermophilus* HB8 were used (Table 5).

The probe of IS1000 hybridized with *EcoRV* restriction fragments I (I1, I2 and/or I3) whose sizes were 80 kb (Fig. 5). From the hybridization experiment with other rare-cutting restriction endonucleases, it was obvious that the IS1000 probe did not hybridize with fragment I1 (Table 5). Because the IS1000 probe also hybridized with the *EcoRV* + *MunI* restriction fragment whose size was 80 kb (data not shown), it was also obvious that the IS1000 probe did not hybridize with fragment I2. Thus, it was confirmed that IS1000 was present on *EcoRV* restriction fragments D, I3, L1 and M (Table 5).

DISCUSSION

In this paper we describe the construction of more detailed maps of the *T. thermophilus* HB27 chromosome and large plasmid pTT27 than in a previous study (Tabata *et al.*, 1993). A total of 49 cleavage sites for the restriction endonucleases *EcoRI*, *SspI*, *MunI*, *EcoRV* and *ClaI* on the HB27 chromosome and 15 sites for *EcoRI*, *SspI*, *MunI*, *NotI* and *EcoRV* on the pTT27 were determined, and the location of 61 cloned *Thermus* genes was also determined. As a result, restriction sites are indicated every 35–40 kb on the HB27 chromosome and every 15 kb on the large plasmid on average. However, the cleavage sites of the restriction endonucleases used were unevenly distributed

on the map and several regions contained more sites for restriction endonucleases than the remaining parts of the chromosome. This apparent lack of homogeneity may be due to differences in the G + C contents among chromosomal regions.

The sums of the sizes of all the *EcoRV* or *ClaI* restriction fragments derived from the chromosome were 1837 and 1807 kb, respectively, which was in good agreement with previous determinations of the genome size, 1.82 Mb (Tabata *et al.*, 1993). The same applied to the sizes of pTT27.

Three *Thermus* hybridization probes failed to hybridize with the *T. thermophilus* HB27 chromosome. Since it was shown that *T. thermophilus* HB27 lacked protease activity, the absence of hybridization for the aqualysin I gene (*aql*) probe is not surprising. As in the case of *T. thermophilus* HB8 (Borges & Bergquist, 1993; P. L. Bergquist, personal communication), no signal was detected when probes for xylose isomerase gene were used. However, the α , β -galactosidase gene (*lac*) also failed to hybridize with the HB27 chromosome although *T. thermophilus* HB27 was reported to have weak and constitutive α , β -galactosidase activities (Koyama *et al.*, 1990). The reason for these results is unknown.

Construction of the physical map and assignment of cloned genes were also carried out for another *Thermus* strain, *T. thermophilus* HB8 (Borges & Bergquist, 1993; P. L. Bergquist, personal communication). In a previous report, we stated that the cleavage patterns of HB27 chromosome and that of HB8 were different, and moreover the *MunI* physical maps of these two strains were quite dissimilar (Tabata *et al.*, 1993). When the location of genes on the HB27 chromosome was compared with that of the HB8 chromosome, a considerable similarity was evident in many genes in spite of differences in the physical maps (Fig. 6). These results suggest that, although the nucleotide sequence of the HB27 chromosome may be different from that of the HB8 chromosome, the genome structure in terms of locations of genes may be fundamentally similar.

Differences between these two strains included the copy number of *rpsL-rpsG-fus-tufA* (one for HB8 and two for HB27), lack of *lac* in HB27, location of *glyS*, *pol* and of one copy of *nusG-rplK-rplA*, and both copy numbers and location of the insertion sequence IS1000 (Fig. 6). Although IS1000 sequences were scattered all over the HB8 chromosome, only the region within *EcoRV* restriction fragment L1 contained the IS1000 sequence in the HB27 chromosome. Interestingly, each region on which *glyS*, *pol* or one copy of *nusG-rplK-rplA* existed also contained IS1000 in HB8 (Fig. 6). In *E. coli*, it was suggested that a gene flanked by two insertion sequences was moved as a composite transposon (Umeda & Ohtsubo, 1989). It is interesting to speculate that these regions on the chromosome may be derived by IS-mediated transposition.

It was observed that deletions of the large plasmid pTT27 occurred in derivatives of *T. thermophilus* HB27 (Tabata *et al.*

Fig. 6. Physical map of *T. thermophilus* strains HB27 and HB8. Restriction sites for *MunI* and the positions of the functional loci are indicated. Thick lines indicate regions in which *IS1000* exist. For presentation, each of the circular genomes is shown linearized from the *MunI* site between *MunI* fragment A and D for HB27, and 4 and 6 for HB8. The HB8 map is a personal communication from P. L. Bergquist.

al., 1994). In other bacteria, pairs of identical insertion sequences as well as gene duplication induce such chromosomal instabilities (Anderson & Roth, 1981; Windom *et al.*, 1988). In HB27, *IS1000* sequences were present not only on the chromosome but also on pTT27. Hybridization data indicated that several *IS1000* sequences were present in pTT27 as described in Fig. 4. The probe of *IS1000* hybridized more strongly with *EcoRI* restriction fragment F and *EcoRV* fragment D than with *EcoRI* fragment G, *EcoRV* fragments I3 and M. This suggests either that the number of *IS1000*s is different in these two regions, or that the insertion sequence(s) present in the latter region show a relatively lower homology with *IS1000*. Although it remains to be determined whether duplicated sequences such as *IS1000* also cause instability in HB27, these naturally occurring deletions on pTT27 may conceivably have arisen by homologous recombination within the closely spaced *IS1000* sequences.

Our results suggest that there is a close relationship between *T. thermophilus* HB27 and HB8. These cleavage maps also provide relevant information concerning the

structural relationship among the chromosomes of other *Thermus* strains. Moreover, the detailed cleavage map of HB27 should improve the mapping of every *Thermus* gene cloned in the future.

ACKNOWLEDGEMENTS

We thank all the scientists who supplied cloned genes. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

- Anderson, P. & Roth, J. (1981). Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (*rrn*) cistrons. *Proc Natl Acad Sci USA* **78**, 3113–3117.
- Asakura, K., Komatsubara, H., Soga, S., Yomo, T., Oka, M., Emi, S. & Urabe, I. (1993). Cloning, nucleotide sequence, and expression in *Escherichia coli* of DNA polymerase gene (*polA*) from *Thermus thermophilus* HB8. *J Ferment Bioeng* **76**, 265–269.

- Ashby, M. K. & Bergquist, P. L. (1990). Cloning and sequence of IS1000, a putative insertion sequence from *Thermus thermophilus* HB8. *Plasmid* **24**, 1–11.
- Barany, F. & Gelfand, D. H. (1991). Cloning, overexpression and nucleotide sequence of a thermostable DNA ligase-encoding gene. *Gene* **109**, 1–11.
- Borges, K. M. & Bergquist, P. L. (1993). Genomic restriction map of the extremely thermophilic bacterium *Thermus thermophilus* HB8. *J Bacteriol* **175**, 103–110.
- Dekker, K., Yamagata, H., Sakaguchi, K. & Udaka, S. (1991). Xylose (glucose) isomerase gene from the thermophile *Thermus thermophilus*: cloning, sequencing, and comparison with other thermostable xylose isomerases. *J Bacteriol* **173**, 3078–3083.
- Hartmann, R. K. & Erdmann, V. A. (1989). *Thermus thermophilus* 16S rRNA is transcribed from an isolated transcription unit. *J Bacteriol* **171**, 2933–2941.
- Hartmann, R. K. & Erdmann, V. A. (1991). Analysis of the gene encoding the RNA subunit of ribonuclease P from *T. thermophilus* HB8. *Nucleic Acids Res* **19**, 5957–5964.
- Hartmann, R. K., Kröger, B., Vogel, D. W., Ulbrich, N. & Erdmann, V. A. (1987). Characterization of cloned rDNA with *Thermus thermophilus* HB8. *Biochem Int* **14**, 267–275.
- Hecht, R. M., Garza, A., Lee, Y.-H., Miller, M. D. & Pisegna, M. A. (1989). Nucleotide sequence of the glyceraldehyde-3-phosphate dehydrogenase gene from *Thermus aquaticus* YT-1. *Nucleic Acids Res* **17**, 10123.
- Heinrich, T., Schröder, W., Erdmann, V. A. & Hartmann, R. K. (1992). Identification of the gene encoding transcription factor NusG of *Thermus thermophilus*. *J Bacteriol* **174**, 7859–7863.
- Hidaka, Y., Hasegawa, M., Nakahara, T. & Hoshino, T. (1994). The entire population of *Thermus thermophilus* cells is always competent at any growth phase. *Biosci Biotech Biochem* **58**, 1338–1339.
- Hoshino, T., Fujii, R. & Nakahara, T. (1993a). Molecular cloning and sequence analysis of the *crtB* gene of *Thermus thermophilus* HB27, an extreme thermophile producing carotenoid pigments. *Appl Environ Microbiol* **59**, 3150–3153.
- Hoshino, T., Maseda, H. & Nakahara, T. (1993b). Plasmid marker rescue transformation in *Thermus thermophilus*. *J Ferment Bioeng* **76**, 276–279.
- Hoshino, T., Fujii, R. & Nakahara, T. (1994a). Overproduction of carotenoids in *Thermus thermophilus*. *J Ferment Bioeng* **77**, 423–424.
- Hoshino, T., Hidaka, Y., Kosuge, T., Hasegawa, M., Saito, T. & Nakahara, T. (1994b). Transformation as a tool for genetic fine mapping of closely located mutations in *Thermus thermophilus*. *FEMS Microbiol Lett* **117**, 175–180.
- Hoshino, T., Kosuge, T., Hidaka, Y., Tabata, K. & Nakahara, T. (1994c). Molecular cloning and sequence analysis of the *proC* gene encoding Δ^1 -pyrroline-5-carboxylate reductase from an extremely thermophilic eubacterium *Thermus thermophilus*. *Biochem Biophys Res Commun* **199**, 410–417.
- Hoshino, T., Yoshino, Y., Guevarra, E. D., Ishida, S., Hiruta, T., Fujii, R. & Nakahara, T. (1994d). Isolation and partial characterization of carotenoid underproducing and overproducing mutants from an extremely thermophilic *Thermus thermophilus* HB27. *J Ferment Bioeng* **77**, 131–136.
- Itaya, M. & Kondo, K. (1991). Molecular cloning of a ribonuclease H (RNase HI) gene from an extreme thermophile *Thermus thermophilus* HB8: a thermostable RNase H can functionally replace the *Escherichia coli* enzyme in vivo. *Nucleic Acids Res* **19**, 4443–4449.
- Ito, T. & Sakaki, Y. (1988). A novel procedure for selective cloning of *NotI* linking fragments from mammalian genomes. *Nucleic Acids Res* **16**, 9177–9184.
- Jahn, O., Hartmann, R. K. & Erdmann, V. A. (1991). Analysis of the *spc* ribosomal protein operon of *Thermus aquaticus*. *Eur J Biochem* **197**, 733–740.
- Kato, R. & Kuramitsu, S. (1993). RecA protein from an extremely thermophilic bacterium, *Thermus thermophilus* HB8. *J Biochem* **114**, 926–929.
- Kosuge, T., Tabata, K. & Hoshino, T. (1994). Molecular cloning and sequence analysis of the *proBA* operon from an extremely thermophilic eubacterium *Thermus thermophilus*. *FEMS Microbiol Lett* **123**, 55–62.
- Koyama, Y. & Furukawa, K. (1990). Cloning and sequence analysis of tryptophan synthetase genes of an extreme thermophile, *Thermus thermophilus* HB27: plasmid transfer from replica-plated *Escherichia coli* recombinant colonies to competent *T. thermophilus* cells. *J Bacteriol* **172**, 3490–3495.
- Koyama, Y., Hoshino, T., Tomizuka, N. & Furukawa, K. (1986). Genetic transformation of the extreme thermophile *Thermus thermophilus* and of other *Thermus* spp. *J Bacteriol* **166**, 338–340.
- Koyama, Y., Okamoto, S. & Furukawa, K. (1990). Cloning of α - and β -galactosidase genes from an extreme thermophile, *Thermus* strain T2, and their expression in *Thermus thermophilus* HB27. *Appl Environ Microbiol* **56**, 2251–2254.
- Kreutzer, R., Kruff, V., Bobkova, E. V., Lavrik, O. I. & Sprinzl, M. (1992). Structure of the phenylalanyl-tRNA synthetase genes from *Thermus thermophilus* HB8 and their expression in *Escherichia coli*. *Nucleic Acids Res* **20**, 4173–4178.
- Ladefoged, S. A. & Christiansen, G. (1992). Physical and genetic mapping of the genomes of five *Mycoplasma hominis* strains by pulsed-field gel electrophoresis. *J Bacteriol* **174**, 2199–2207.
- Lauer, G., Rudd, E. A., McKay, D. L., Ally, A., Ally, D. & Backman, K. C. (1991). Cloning, nucleotide sequence, and engineered expression of *Thermus thermophilus* DNA ligase, a homolog of *Escherichia coli* DNA ligase. *J Bacteriol* **173**, 5047–5053.
- Lee, Y.-C., Miyata, Y., Terada, I., Ohta, T. & Matsuzawa, H. (1991). Involvement of NH_2 -terminal pro-sequence in the production of active aqualysin I (a thermophilic serine protease) in *Escherichia coli*. *Agric Biol Chem* **55**, 3027–3032.
- Mather, M. W., Springer, P. & Fee, J. A. (1991). Cytochrome oxidase genes from *Thermus thermophilus*. *J Biol Chem* **266**, 5025–5035.
- Mather, M. W., Springer, P., Hensel, S., Buse, G. & Fee, J. A. (1993). Cytochrome oxidase genes from *Thermus thermophilus*. Nucleotide sequence of the fused gene and analysis of the deduced primary structures for subunits I and III of cytochrome *caa3*. *J Biol Chem* **268**, 5395–5408.
- Miyazaki, K., Eguchi, H., Yamagishi, A., Wakagi, T. & Oshima, T. (1992). Molecular cloning of the isocitrate dehydrogenase gene of an extreme thermophile, *Thermus thermophilus* HB8. *Appl Environ Microbiol* **58**, 93–98.
- Motoshima, H., Azuma, N., Kaminogawa, S., Ono, M., Minagawa, E., Matsuzawa, H., Ohta, T. & Yamauchi, K. (1990). Molecular cloning and nucleotide sequence of the aminopeptidase T gene of *Thermus aquaticus* YT-1 and its high-level expression in *Escherichia coli*. *Agric Biol Chem* **54**, 2385–2392.
- Nishiyama, M., Horinouchi, S. & Beppu, T. (1991). Characterization of an operon encoding succinyl-CoA synthetase and malate dehydrogenase from *Thermus flavus* AT-62 and its expression in *Escherichia coli*. *Mol & Gen Genet* **226**, 1–9.
- Nureki, O., Muramatsu, T., Suzuki, K., Kohda, D., Matsuzawa, H., Ohta, T., Miyazawa, T. & Yokoyama, S. (1991). Methionyl-tRNA synthetase gene from an extreme thermophile, *Thermus thermophilus* HB8. *J Biol Chem* **266**, 3268–3277.

- Nureki, O., Suzuki, K., Hara-Yokoyama, M., Kohno, T., Matsuzawa, H., Ohta, T., Shimizu, T., Morikawa, K., Miyazawa, T. & Yokoyama, S. (1992). Glutamyl-tRNA synthetase from *Thermus thermophilus* HB8. Molecular cloning of the *gltX* gene and crystallization of the overproduced protein. *Eur J Biochem* **204**, 465–472.
- Park, H.-J. & Kreutzer, R. (1994). Expression cloning of the *nox*, *mdh* and *ldh* genes from *Thermus* species encoding NADH oxidase, malate dehydrogenase and lactate dehydrogenase. *Appl Microbiol Biotechnol* **40**, 676–681.
- Park, H.-J., Kreutzer, R., Reiser, C. O. A. & Sprinzl, M. (1992). Molecular cloning and nucleotide sequence of the gene encoding a H₂O₂-forming NADH oxidase from the extreme thermophilic *Thermus thermophilus* HB8 and its expression in *Escherichia coli*. *Eur J Biochem* **205**, 875–879.
- Poterszman, A., Plateau, P., Moras, D., Blanquet, S., Mazauric, M. H., Kreutzer, R. & Kern, D. (1993). Sequence, overproduction and crystallization of aspartyl-tRNA synthetase from *Thermus thermophilus*: implications for the structure of prokaryotic aspartyl-tRNA synthetases. *FEBS Lett* **325**, 183–186.
- Sakaki, Y. & Oshima, T. (1975). Isolation and characterization of a bacteriophage infectious to an extreme thermophile, *Thermus thermophilus* HB8. *J Virol* **15**, 1449–1453.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sato, S., Nakada, Y., Kanaya, S. & Tanaka, T. (1988). Molecular cloning and nucleotide sequence of *Thermus thermophilus* HB8 *trpE* and *trpG*. *Biochim Biophys Acta* **950**, 303–312.
- Seidler, L., Peter, M., Meissner, F. & Sprinzl, M. (1987). Sequence and identification of the nucleotide binding site for the elongation factor Tu from *Thermus thermophilus* HB8. *Nucleic Acids Res* **15**, 9263–9277.
- Struck, J. C. R., Toschka, H. Y. & Erdmann, V. A. (1988). Nucleotide sequence of the 4.5S RNA gene from *Thermus thermophilus* HB8. *Nucleic Acids Res* **16**, 9042.
- Tabata, K., Kosuge, T., Nakahara, T. & Hoshino, T. (1993). Physical map of the extremely thermophilic bacterium *Thermus thermophilus* HB27 chromosome. *FEBS Lett* **331**, 81–85.
- Tabata, K., Ishida, S., Nakahara, T. & Hoshino, T. (1994). A carotenogenic gene cluster exists on a large plasmid in *Thermus thermophilus*. *FEBS Lett* **341**, 251–255.
- Terada, I., Kwon, S.-T., Miyata, Y., Matsuzawa, H. & Ohta, T. (1990). Unique precursor structure of an extracellular protease, aqualysin I, with NH₂- and COOH-terminal pro-sequences and its processing in *Escherichia coli*. *J Biol Chem* **265**, 6576–6581.
- Tsutsumi, S., Denda, K., Yokoyama, K., Oshima, T., Date, T. & Yoshida, M. (1991). Molecular cloning of genes encoding two major subunits of a eubacterial V-type ATPase from *Thermus thermophilus*. *Biochim Biophys Acta* **1098**, 13–20.
- Umeda, M. & Ohtsubo, E. (1989). Mapping of insertion elements IS1, IS2 and IS3 on the *Escherichia coli* K-12 chromosome. *J Mol Biol* **208**, 601–614.
- Windom, E., Jarris, D., LaFauci, G. & Rudner, R. (1988). Instability of rRNA operons in *Bacillus subtilis*. *J Bacteriol* **170**, 605–610. (Erratum, **170**, 2003.)
- Xu, J., Seki, M., Denda, K. & Yoshida, M. (1991). Molecular cloning of phosphofructokinase 1 gene from a thermophilic bacterium, *Thermus thermophilus*. *Biochem Biophys Res Commun* **176**, 1313–1318.
- Yanisch-Perron, C., Vieira, J. & Messing, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103–119.

Received 24 April 1995; revised 14 August 1995; accepted 21 September 1995.