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An improved physical and genetic map of the genome of alkaliphilic *Bacillus* sp. C-125

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Abstract Among alkaliphilic bacteria reported so far, *Bacillus* sp. C-125 is the strain most thoroughly characterized physiologically, biochemically, and genetically. A physical map of the chromosome of this strain was constructed to facilitate further genome analysis, and the genome size was revised from 3.7 to 4.25 Mb. Complete digestion of the chromosomal DNA with two rare cut restriction endonucleases, *Asc*I and *Sse*8387I, each yielded 20 fragments ranging in size from 20 to 600 kb. Seventeen linking clones were isolated in each instance to join the adjacent *Asc*I or *Sse*8387I fragments in the chromosomal map. All *Asc*I linking clones isolated were sequenced and analyzed by comparison with the BSORF database to map the genes in the chromosome of strain C-125. Several ORFs showing significant similarities to those of *B. subtilis* in the *Asc*I linking clones were positioned on the physical map. The *oriC* region of the C-125 chromosome was identified by southern blot analysis with a DNA probe containing the *gyrB* region.

Key words Alkaliphile · *Bacillus* sp. C-125 · Physical mapping · Gene mapping · Pulsed-field gel electrophoresis

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Introduction

The facultative alkaliphilic *Bacillus* sp. strain C-125 can grow well at pH 7 to 10.5. This strain was isolated as a β-galactosidase producer (Ikura and Horikoshi 1979). We have a specific interest in the mechanisms of adaptation of alkaliphilic bacteria to highly alkaline environments and have been using molecular biological approaches especially to determine how many genes are involved in maintenance of the alkaliphilic phenotype. We have also focused on industrial applications of these bacteria as it has been reported that many alkaliphilic *Bacillus* strains, including strain C-125, produce unique alkaline enzymes such as xylanases (Honda et al. 1985), proteases, and amylases (Horikoshi 1996).

Detailed genetic and physical maps are available for the *Bacillus subtilis* 168 chromosome (Itaya and Tanaka 1991; Biaudet et al. 1996), and physical maps of *Bacillus cereus* (Kolsto et al. 1990) and *Bacillus thuringiensis* (Carlson and Kolsto 1993) chromosomes have been constructed. In addition, a partial physical map of the chromosome of alkaliphilic *Bacillus* sp. C-125 has been proposed (Southerland et al. 1993). These physical maps will be helpful for comparative studies, including exploration of the substantial differences in genome structure among bacilli.

Recently, whole genome analysis of *B. subtilis*, which is closely related to strain C-125 except for the alkaliphilic phenotype, has been completed in a project involving collaboration between Japan and the European Community (Kunst et al. 1997). Knowledge of the complete nucleotide sequence of the *B. subtilis* genome will definitely facilitate identification of common functions in bacilli, and such data will help us in analysis of the C-125 genome.

In the present study, we attempted to construct a physical and genetic map of the chromosome of strain C-125 to facilitate further genome analysis, and we examined differences in genome structure between strain C-125 and *B. subtilis*. We report revision of genome size from 3.7 Mb to 4.25 Mb and a complete *Asc*I/*Sse*8387I physical map of the chromosome of *Bacillus* sp. C-125.

Materials and methods

Bacterial strains and media

Bacillus sp. C-125 was used as a standard alkaliphilic *Bacillus* strain. *E. coli* DH5α was used as the host strain for preparation of the DNA library from *Bacillus* sp. C-125. The cells were grown aerobically at 37°C in N-II medium (pH 7.5) consisting of 1% soluble starch, 0.5% polypeptone, 0.5% yeast extract, 0.1% K_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, and 2% NaCl (Takami et al. 1992). *E. coli* was grown under conditions described previously (Sambrook et al. 1989).

Preparation of chromosomal DNA

Bacillus sp. strain C-125 was grown in 100 ml of N-II medium for 4–5 h until reaching mid-logarithmic phase. The cells from 500 μl of culture were harvested by centrifugation, washed once in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA), resuspended in the same volume of TE buffer (50°C), and mixed with 500 μl of 2% Pulsed Field Certified (PFC) agarose prewarmed at 50°C. The resulting suspension was poured into a mold chamber (BioRad, Hercules, CA, USA). Solidified blocks were immersed in 10 ml of TE buffer containing 40 mg of lysozyme and incubated at 37°C for 2 h. After washing the blocks in TE buffer twice, they were incubated at 50°C in 10 ml of TE buffer containing 8 mg of Proteinase K (Gibco BRL, Gaithersburg, MD, USA) overnight. The blocks were washed once in TE buffer and then incubated in TE buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF) for 1 h at room temperature. They were then washed three times more in TE buffer. The blocks prepared as above were stored immediately in TE buffer at 4°C until required for digestion.

Digestion of chromosomal DNA with restriction endonuclease

Agarose blocks containing the chromosomal DNA of *Bacillus* sp. strain C-125 were washed in 50 ml of 0.1× TE buffer twice and then equilibrated with the corresponding restriction buffer at 4°C for 1 h. DNA was digested with 100–200 units of *Asc*I or *Sse*8387I (Takara Shuzo, Kyoto, Japan) or *Sfi*I or *Not*I at 37°C overnight in 500 μl of the restriction buffer recommended by the manufacture. Restriction endonuclease *Asc*I, *Sfi*I, *Not*I, and the vector pNEB193 were obtained from New England Biolabs (Beverly, MA, USA).

Electrophoretic analysis

Pulsed-field gel electrophoresis (PFGE) in 1% PFC agarose was performed using the CHEF Mapper system (BioRad) in 0.5× TBE buffer at 14°C. To resolve the shorter fragments (5–75 kb), pulse times were ramped from 0.22 to 5.10 s with a ramping factor of 0.107 for 15 h at 6 V cm^{-1}. For separation of fragments in the range of 50–600 kb, PFGE was performed using a pulse time of 6.75–54.17 s, a ramping factor of −1.357, a voltage of 6 V cm^{-1}, and a run time of 28 h. PFC agarose and DNA size markers [8–48 kb mixed digest of λ, λ ladder (λ C1857Sam7, 0.05–1 Mb concatemers), and *Saccharomyces cerevisiae* 0.2–2.2 Mb DNA] were from Japan BioRad Laboratories (Tokyo, Japan).

Hybridization analysis

Agarose gels were blotted onto Hybond N^+ membranes using a vacuum blotting system (VacuGene XL; Pharmacia Biotech, Tokyo, Japan). Hybridization was performed using a digoxigenin- (DIG-) labeled DNA probe in a 40% (v/v) formamide hybridization solution at 42°C. Subsequent washing and detection steps were carried out according to the instructions provided with the DIG labeling and detection kit (Boehringer, Mannheim, Germany).

Isolation of linking clones

An aliquot of approximately 10 μg of genomic DNA from *Bacillus* sp. C-125 prepared by the conventional liquid isolation method (Saito and Miura 1963) was digested with *Hin*dIII or *Eco*RI or *Eco*RV. The digested DNA was circularized using a DNA Ligation Kit Ver. 1 (Takara Shuzo) at a DNA concentration of 5–40 ng per microliter at 16°C overnight. The circularized DNA was recovered by ethanol precipitation and digested with *Asc*I or *Sse*8387I. It was ligated to the *Asc*I or *Sse*8387I site of vector pNEB193, which had been previously treated with bacterial alkaline phosphatase (BAP), and then introduced into competent *E. coli* DH5α cells by the standard method (Sambrook et al. 1989).

Sequencing and analysis of linking clones

Sequencing of *Asc*I-linking clones was performed by means of a DNA sequencer ABI PRISM 377 using a Taq Dye Primer and Dye Terminator Cycle Sequencing Kit (Perkin Elmer, Norwalk, CT, USA). Several primers were prepared and used to sequence the whole PCR fragment. The sequences were analyzed for the locations of possible ORFs using the GeneWorks program (version 2.5.1N) from IntelliGenetics (Campbell, CA, USA). The deduced amino acid sequences of the identified ORFs were compared with sequences reported previously in a search of the BSORF database (http://bacillus.tokyo-center.genome.ad.jp:8008/) and the non-redundant protein databank using the FASTA and BLAST network service (GenomeNet WWW server, http://www.genome.ad.jp). The sequences of the 17 DNA fragments sequenced in this report were deposited at DDBJ with the accession numbers AB013362 for *Asc*I-A clone, AB013363 for *Asc*I-B clone, AB013364 for *Asc*I-C clone, AB013365 for *Asc*I-D clone, AB013366 for *Asc*I-E clone, AB013367 for *Asc*I-F clone, AB013368 for *Asc*I-G clone, AB013369 for *Asc*I-H clone, AB013370 for *Asc*I-I clone, AB013371 for *Asc*I-J clone, AB013372 for *Asc*I-K clone, AB013373 for *Asc*I-L clone, AB013374 for *Asc*I-M clone, AB013375 for *Asc*I-N clone, AB013376 for *Asc*I-O clone, AB013377 for *Asc*I-P clone, and AB013378 for *Asc*I-Q clone.

Preparation of the DNA probe for gene mapping

Five genes, *pALK* (accession number D00087), *groEL* (Xu et al. 1996), *secY* (Kang et al. 1992), *hag* (Sakamoto et al. 1992), and *xylA* (Hamamoto et al. 1987), were amplified from the chromosomal DNA of *Bacillus* sp. strain C-125 by PCR using specific primer sets designed from the sequences reported. The *trpS* (Chow and Wong 1988) gene was amplified by PCR from *B. subtilis* chromosomal DNA using specific primers, 5′-GTCGAACTGCAGCATGATT-3 and 5′-CAATGCCTTCAGAATCCGT-3′. Other genes, *sigA* (5′-AAAATMAATGACCCAGT-TCGTATG-3′ and 5′-TTCWCKATCAGTTAAYGTATCAAG-3′), *rpoA* (5′-RTCGAYTCKATCTAYWCGCCRGT-3′ and 5′-CTTKARGCAGTTGTAMGAMCGRAC-3′), and *gyrB* (5′-GGNAAYATHGGNYTNATG-3′ and 5′-RTTDAT-NGTYTCDATCAT-3′), were also amplified by PCR from the C-125 chromosomal DNA using degenerated primer sets for each gene. Each gene amplified was labeled by means of the dioxygenin (DIG) labeling kit (Boehringer, Mannheim, Germany).

Results and discussion

Digestion pattern of the C-125 genome with rare cut restriction endonucleases

Restriction endonucleases that recognize an 8 bp sequence were tested for their ability to digest the chromosome of strain C-125. *Pac*I (5′-TTAAT/TAA-3′) and *Pme*I (5′-GTTT/AAAC-3′) generated more than 50 fragments, each less than 50 kb in size (data not shown). *Not*I (5′-GC/GGCCGCC-3′) did not digest the C-125 chromosome as described in the previous paper (Southerland et al. 1993). *Sfi*I (5′-GGCCNNNN/NGGCC-3′) generated approximately 20 fragments ranging in size from 20 to 700 kb. The restriction pattern was, however, uneven, and smearing made it hard to identify fragments and their sizes accurately as observed in the previous paper (Southerland et al. 1993).

*Asc*I (5′-GG/CGCGCC-3′) and *Sse*8387I (5′-CCTGCA/GG-3′) generated 18 and 19 resolvable fragments, respectively (Fig. 1 and Table 1). The intensity of each resolvable band was analyzed by means of the *pdi* Desk Top Scanner System (*pdi* Inc., Huntington Station, NY, USA), and consequently it was found that two fragments of the same size overlapped at positions 3A, 12A, and 3S (Table 1). The sizes of these fragments were determined by comparison with size standards on a series of PFGE gels (Fig. 1 and Table 1). The mean total size of the genome of *Bacillus* sp. strain C-125, estimated by totaling the *Asc*I or *Sse*8387I fragments, was 4.25 Mb. It appeared that the genome size had been underestimated in the previous paper (Southerland et al. 1993) for the reason described. Thus the size of the genome was revised from 3.75 Mb to 4.25 Mb in this study. Carlson et al. have determined the sizes of the chromosomes of six strains of *B. cereus* (2.4–5.3 Mb) (Carlson and Kolsto 1994) and one strain of *B. thuringiensis* (5.4–6.3 Mb) (Carlson and Kolsto 1993, Carlson et al. 1996). The 4.25-Mb size of the chromosome of strain C-125 is almost the same as that of *B. subtilis* (4.21 Mb) (Kunst et al. 1997) and smaller than that of *B. thuringiensis* (5.4–6.3 Mb).

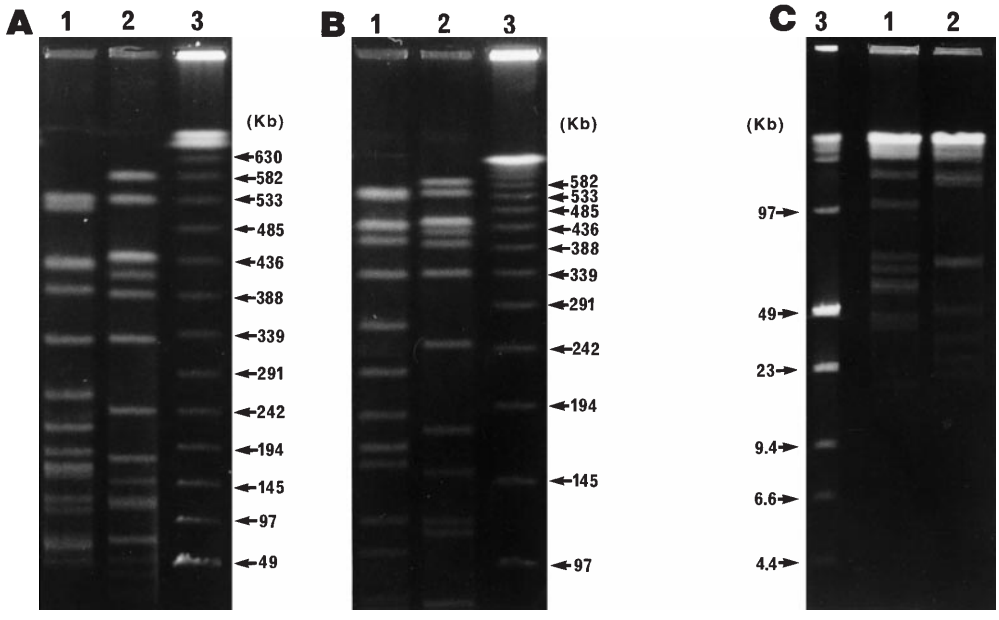

Fig. 1A–C. Digestion patterns of the chromosomal DNA of alkaliphilic *Bacillus* sp. strain C-125 obtained with *Asc*I and *Sse*8387I. **A** Separation of fragments ranging in size from 50 from 600 kb using a pulsed time of 20–50 s at 6 V cm^{-1} for 26 h. *Lane 1*, complete *Sse*8387I digestion; *lane 2*, complete *Asc*I digestion; *lane 3*, λ DNA ladder. **B** Separation of fragments ranging in size from 50 to 600 kb using a pulsed time of 6.75–54.17 s at 6 V cm^{-1} for 28 h. *Lane 1*, complete *Sse*8387I digestion; *lane 2*, complete *Asc*I digestion; *lane 3*, λ DNA ladder. **C** Separation of fragments ranging in size from 5 to 75 kb using a pulsed time of 0.22–5.1 s at 6 V cm^{-1} for 15 h. *Lane 1*, complete *Sse*8387I digestion; *lane 2*, complete *Asc*I digestion; *lane 3*, low range PFG marker (2.03–194 kb)

Isolation and sequencing analysis of linking clones

We decided to construct a physical map of *Asc*I and *Sse*8387I sites on the *Bacillus* sp. C-125 chromosome because enzymatic digestion with *Asc*I or *Sse*8387I resulted in 20 resolvable fragments. A linking clone contains DNA, which overlaps two adjacent restriction fragments and can be used as a hybridization probe to establish the identity of these two contiguous fragments (Smith and Condemine 1990). Isolation of *Asc*I- and *Sse*8387I-linking clones was attempted to join adjacent fragments generated by *Asc*I or *Sse*8387I digestion in mapping the chromosome. Linking clone libraries were constructed using *Hin*dIII or *Eco*RI or *Eco*RV as the R enzyme. Plasmid DNA was isolated from individual clones and screened for the presence of two *Asc*I or *Sse*8387I sites and two R enzyme sites. Seventeen possible *Asc*I-linking clones (A–Q) were identified and screened for authenticity by southern blot analysis of R enzyme-digested *Bacillus* sp. C-125 chromosomal DNA and partial sequencing of each *Asc*I-linking clone to identify the *Asc*I site in the clone, just in case (Table 2). Similarly, 17 possible *Sse*8387I-linking clones (a–q) were identified in the same manner as in the case of the *Asc*I-linking clones.

All *Asc*I-linking clones obtained in this study were sequenced for further characterization. The determined sequence of each linking clone was searched for open reading frames (ORFs), and putative whole or partial ORFs were identified as shown in Fig. 2. Three partial ORFs identified in the linking clones A, C, and D were similar to *B. subtilis resB* (cytochrome C biogenesis protein), *rnh* (ribonuclease H), and *tuaG* (a protein involved in biosynthesis of

Table 1. Sizes of restriction fragments generated by *Asc*I and *Sse*8387I cleavage of the alkaliphilic *Bacillus* sp. strain C-125 chromosome

Band number	Size of generated fragments (kb)		Band number
	*Asc*I	*Sse*8387I	
1A	599	552	1S
2A	549	537	2S
3A*	445	433	3S*
4A	417	394	4S
5A	388	329	5S
6A	329	273	6S
7A	254	222	7S
8A	174	185	8S
9A	144	162	9S
10A	119	150	10S
11A	112	119	11S
12A*	70	104	12S
13A	49	72	13S
14A	33	67	14S
15A	26	61	15S
16A	23	58	16S
17A	3.8	43	17S
18A	0.7	39	18S
		20	19S
Total	4250	4253	

Asterisk indicates two bands overlapped at the same position

Table 2. *Asc*I-linking clones sequenced in this study

*Asc*I-linking clones	Length of the nucleotide (bp)	Position of the *Asc*I site(s)
A	1059	159/984
B	1445	1258
C	1584	989
D	1688	185
E	2504	2007
F	3638	545
G	2429	24
H	6865	2775
I	3933	380
J	555	417
K	589	76
L	6503	4024
M	5703	1588
N	4209	1244
O	2778	24
P	2207	2067
Q	3476	255

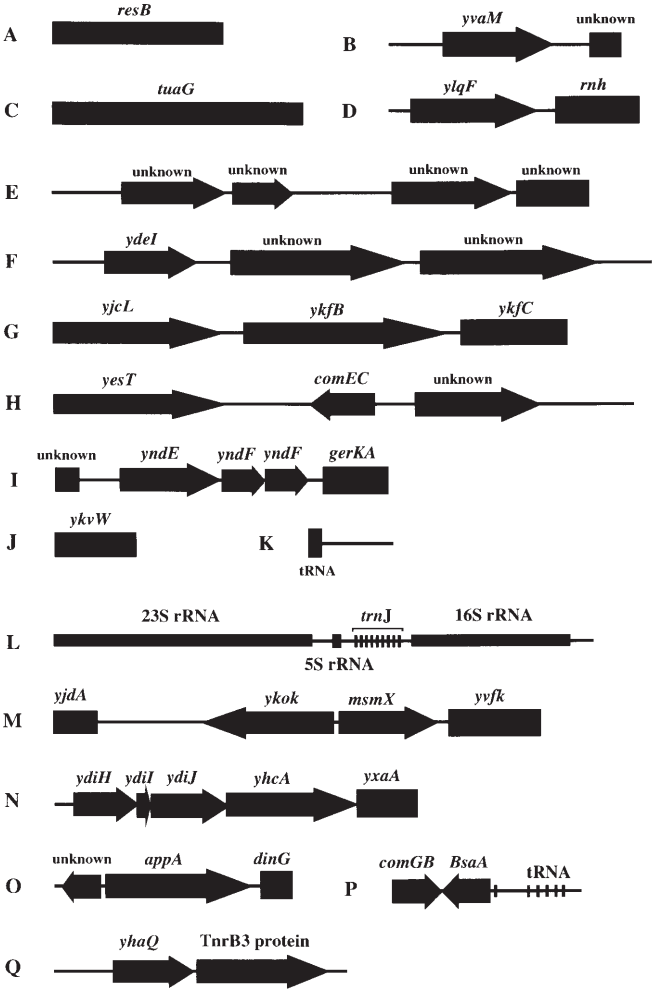

Fig. 2. Open reading frame (ORF) analysis of *Asc*I-linking clones (A–Q). The determined sequence of each linking clone was searched for ORFs consisting of more than 100 codons. The deduced amino acid sequences of these ORFs were used for homology analysis in searches of the BSORF, SWISS-PROT, and PIR protein databases using the FASTA program. For ORFs showing similarity to the protein sequences deposited in the databases, the name of the ORF is indicated for each ORF identified. An *arrow* denotes a complete ORF; a *truncated arrow* means a partial one

teichuronic acid), respectively (Fig. 2). Three putative ORFs identified in the clones B, D, and F showed similarity to *B. subtilis yvaM*, *ylqF*, and *ydeI*, respectively. In the linking clone H, there were two ORFs that were found to be similar to *B. subtilis yesT* and *comEC* (putative integral membrane protein). In the linking clone L, there was a partial ribosomal operon (Fig. 2). Several whole or partial ORFs were also identified in the linking clones M to Q, as shown in Fig. 2.

Constructing an *Asc*I/*Sse*8387I physical map

All *Asc*I- and *Sse*8387I-linking clones listed in Table 4 were used as hybridization probes in southern blot analyses of *Asc*I and *Sse*8387I digests of C-125 chromosomal DNA in PFGE gels. In most cases, the linking clones for a particular enzyme hybridized to two different fragments obtained after digestion with the enzyme. As shown in Fig. 3S and Table 3, *Sse*8387I-linking clone k hybridized to 3S′ and 15S and *Sse*8387I-linking clone l hybridized to 4S and 14S. *Asc*I-linking clone D (5A and 7A) and F (5A and 10A) hybridized to two different fragments (Fig. 3A and Table 3). *Asc*I-linking clone A containing two *Asc*I sites in the *Hin*dIII fragment hybridized to three different fragments, 1A, 18A, and 3A′, respectively. Two DNA fragments that overlapped at the 3A position in the PFGE gel (see Fig. 1 and Table 1) were distinguished one (3A) from the other (3A′) by comparing the hybridization patterns obtained for chromosomal DNA doubly digested with *Asc*I and *Sfi*I when hybridized to a series of *Asc*I-linking clones. It was found that there were at least two *Sfi*I sites in the 3A′ fragment (Fig. 4) but no *Sfi*I site in the 3A fragment. Another two DNA fragments that overlapped at the 12A position were also distinguished as 12A and 12A′ in the same manner, except that *Sse*8387I was employed rather than *Sfi*I.

*Asc*I-M hybridized to 4A and 16A, *Asc*I-N to 3A and 15A, *Asc*I-K to 3A and 17A, *Asc*I-E to 13A and 17A, *Asc*I-Q to 1A and 13A, *Asc*I-I to 3A′ and 10A, *Asc*I-B to 7A and 14A, *Asc*I-O to 12A and 14A, *Asc*I-G to 2A and 8A, *Asc*I-C to 6A and 11A, *Asc*I-H to 6A and 12A′, and *Asc*I-J to 9A and 12A′ (Table 3). A series of hybridization experiments resulted in four *Asc*I contiguous fragments as follows: 4A-16A, 15A-3A-17A-13A-1A-18A-3A′-10A-5A-7A-14A-12A, 8A-2A, and 11A-6A-12A′-9A. In mapping, the contiguous fragments were joined by means of hybridization and cross-hybridization patterns obtained on analysis of chromosomal DNA digested with *Asc*I or *Sse*8387I when hybridized to DNA probes specific for *trpS* or *Sse*8387I- or *Asc*I-linking clones (Table 3).

Similarly, *Sse*8387I-a hybridized to 8S and 17S, *Sse*8387I-b to 17S and 18S, *Sse*8387I-c to 6S and 14S, *Sse*8387I-d to 10S and 12S, *Sse*8387I-e to 2S and 13S, *Sse*8387I-f to 3S′ and 9S, *Sse*8387I-g to 1S and 16S, *Sse*8387I-h to 11S and 19S, *Sse*8387I-i to 13S and 19S, *Sse*8387I-j to 12S and 18S, *Sse*8387I-m to 10S and 11S, *Sse*8387I-n to 1S and 9S, and *Sse*8387I-o to 3S and 16S. The hybridization experiments resulted in the following three *Sse*8387I contiguous fragments not containing 5S and 7S; 4S-14S-6S, 2S-13S-19S-11S-10S-12S-18S-17S-8S, and 3S-16S-1S-9S-3S′-15S. Thus, in mapping, the *Sse*8387I contiguous fragments and another two fragments (5S and 7S) were joined by means of the hybridization and cross-hybridization patterns obtained on

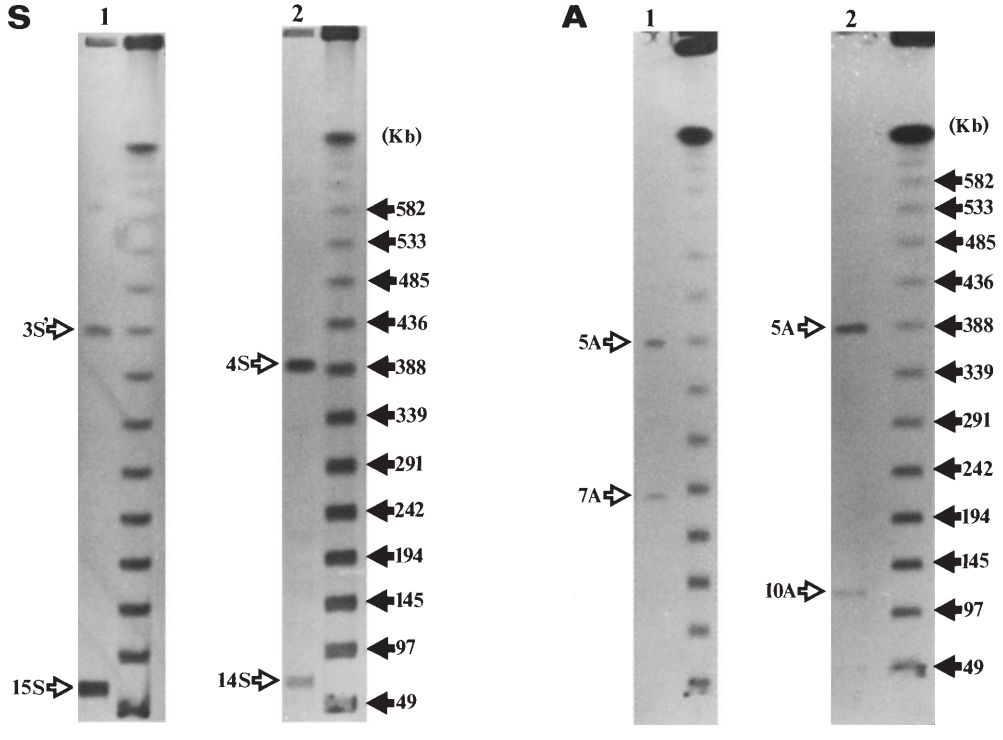

Fig. 3. Southern blot analysis of *Bacillus* sp. strain C-125 genomic DNA hybridized to *Asc*I- or *Sse*8387I-linking clones. ***S***, *Sse*8387I digest: *lane 1*, *Sse*8387I-linking clone k; *lane 2*, *Sse*8387I-linking clone l. ***A*** *Lane 1*, *Asc*I-linking clone D; *lane 2*, *Asc*I-linking clone F. *Numbers on the right* show sizes of fragments in the λ DNA ladder; *arrows on the left* show the bands that hybridized to the linking clone labeled with digoxigenin

Table 3. Hybridization of linking clones and other genes to *Asc*I and *Sse*8387I fragments of *Bacillus* sp. strain C-125 chromosome

Probe	Fragment number hybridized	
	*Asc*I	*Sse*8387I
*Asc*I-linking clone		
A	1A/18A/3A′	2S
B	7A/14A	7S
C	6A/11A	1S
D	5A/7A	5S
E	13A/17A	6S
F	5A/10A	18S
G	2A/8A	3S
H	6A/12A′	9S
I	3A′/10A	12S
J	9A/12A′	3S′
K	3A/17A	n.t.
L	n.t.	n.t.
M	4A/16A	4S
N	3A/15A	4S
O	12A/14A	7S
P	n.t.	n.t.
Q	1A/13A	6S
*Sse*8387I-linking clone		
a	5A	8S/17S
b	5A	17S/18S
c	3A	6S/14S
d	3A′	10S/12S
e	3A′	2S/13S
f	12A′	3S′/9S
g	2A	1S/16S
h	3A′	11S/19S
i	3A′	13S/19S
j	10A	12S/18S
k	4A	3S′/15S
l	3A	4S/14S
m	3A′	10S/11S
n	6A	1S/9S
o	2A	3S/16S
p	n.t.	n.t.
q	n.t.	n.t.
Other genes		
gyrB	9A	3S′
rpoA	4A	3S′
sigA	1A	2S
secY	4A	3S′
hag	11A	1S
pALK	1A	2S
xyl (A)	5A	17S
groEL	3A	4S
recA	5A	5S
trpS (B. subtilis)	8A	7S

n.t., not tested

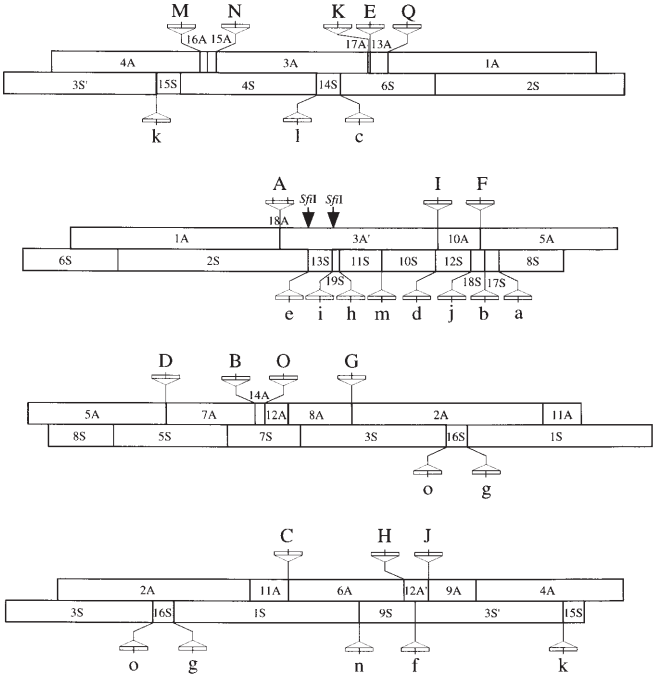

Fig. 4. Linkage map of *Bacillus* sp. strain C-125 *Asc*I fragments (*A*) and corresponding *Sse*8387I (*S*) fragments. *Capital letters*, *Asc*I-linking clones; *small letters*, *Sse*8387I-linking clones. *Fragment numbers* correspond to their assigned band numbers as described in Table 1

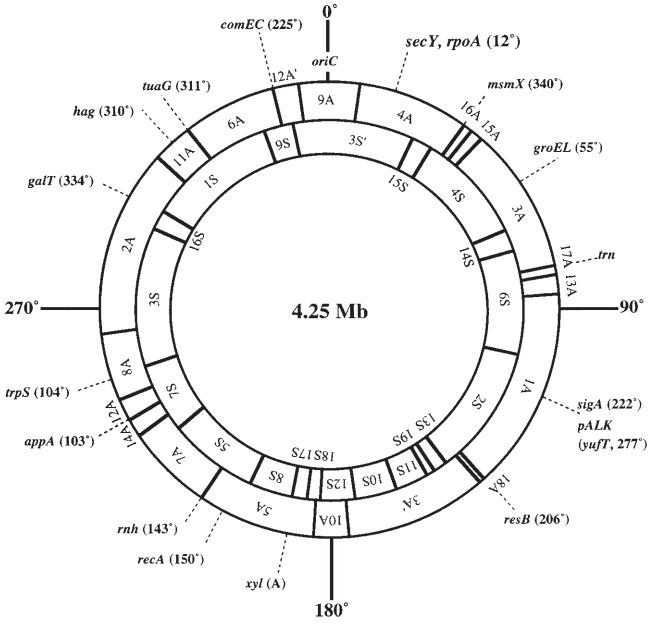

Fig. 5. *Asc*I/*Sse*8387I physical and genetic map of the chromosome of *Bacillus* sp. strain C-125. *Outer* and *inner circles* show the *Asc*I and *Sse*8387I physical map, respectively. The locations of several housekeeping genes are indicated on the map. A *dashed line* indicates the approximate position of the gene

analysis of chromosomal DNA digested with *Sse*8387I or *Asc*I when hybridized to *Sse*8387I- or *Asc*I-linking clones, the same as in the case of the *Asc*I fragments (Fig. 4, Table 3).

Positioning of the *oriC* region on the physical map

A single 400 bp DNA fragment containing a part of the *gyrB* gene was amplified by PCR from the chromosomal DNA of strain C-125 using the primers described in Materials and methods, and used as a DNA probe. A 9.8-kb *Hin*dIII fragment containing a part of the region extending from the *dnaN* gene to the *gyrA* gene hybridized to the DNA probe and the *Hin*dIII fragment was amplified by inverse PCR (Triglia et al. 1988) using the DNA primer set, 5′-AAGCTTAATCGTTCTTCTTGTGTA-3′, and 5′-AAGCTTGAATGAATTGAGCAAAATT-3′ (data not

Table 4. DNA probes used for the construction of the physical map

Probe	Size	Characteristics	Source or reference
*Asc*I-A	1.1	C-125 *Asc*I-linking clone	This study
*Asc*I-B	1.5	C-125 *Asc*I-linking clone	This study
*Asc*I-C	1.6	C-125 *Asc*I-linking clone	This study
*Asc*I-D	1.7	C-125 *Asc*I-linking clone	This study
*Asc*I-E	2.5	C-125 *Asc*I-linking clone	This study
*Asc*I-F	3.6	C-125 *Asc*I-linking clone	This study
*Asc*I-G	2.4	C-125 *Asc*I-linking clone	This study
*Asc*I-H	6.9	C-125 *Asc*I-linking clone	This study
*Asc*I-I	3.9	C-125 *Asc*I-linking clone	This study
*Asc*I-J	0.6	C-125 *Asc*I-linking clone	This study
*Asc*I-K	0.6	C-125 *Asc*I-linking clone	This study
*Asc*I-L	6.5	C-125 *Asc*I-linking clone	This study
*Asc*I-M	5.7	C-125 *Asc*I-linking clone	This study
*Asc*I-N	4.2	C-125 *Asc*I-linking clone	This study
*Asc*I-O	2.8	C-125 *Asc*I-linking clone	This study
*Asc*I-P	2.2	C-125 *Asc*I-linking clone	This study
*Asc*I-Q	3.5	C-125 *Asc*I-linking clone	This study
*Sse*8387I-a	0.5	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-b	2.2	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-c	4.5	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-d	2.4	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-e	3.2	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-f	6.3	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-g	1.5	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-h	1.3	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-i	2.0	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-j	4.0	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-k	2.8	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-l	2.2	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-m	4.0	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-n	4.0	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-o	2.1	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-p	8.0	C-125 *Sse*8387I-linking clone	This study
*Sse*8387I-q	6.0	C-125 *Sse*8387I-linking clone	This study
C-125*gyrB*	0.4	DNA gyrase subunit B	This study
C-125*rpoA*	1.0	RNA polymerase α-subunit	This study
C-125*sigA*	1.1	RNA polymerase major sigma factor (σ^A)	This study
C-125*secY*	1.3	Preprotein translocase subunit	Kang et al. (1992)
C-125*hag*	0.5	Flagellin	Sakamoto et al. (1992)
C-125*pALK*	2.4	Alkaline resistance	Kudo et al. (1990)
C-125*xyl* (A)	1.8	Alkaline xylanase	Hamamoto et al. (1987)
C-125*groEL*	1.5	Heat-shock protein (chaperonin)	Xu et al. (1996)
C-125*recA*	0.8	Homologous recombination and DNA repair	Takami (unpublished)
B. subtilis trpS	1.0	*B. subtilis* tryptophanyl-tRNA synthetase	Chow and Wong (1988)

shown). In addition, the *Hin*dIII fragment was amplified by long accurate PCR using LA PCR kit Ver. 2 (Takara Shuzo) from the chromosomal DNA and used as a probe to determine the position of the *oriC* region on the physical map. This DNA probe hybridized to fragments 9A and 3S′, and it was found that the *oriC* region was located in the region of overlap between 9A and 3S′ (Fig. 5, Table 3). If the *oriC* region is assigned the position at 12 o'clock as 0°, the 13A fragment is at approximately 90°, the region of overlap between 10A and 12S is at 180°, and the border region between 8A and 2A is at 270° on the map (Fig. 5).

Mapping of genetic loci

The DNA probes listed in Table 4 were used for mapping of genetic loci on the physical map. In addition, the ORFs identified and annotated in the linking clones (Fig. 4) were also used for mapping. The assigned positions of the genes on the physical map of *Bacillus* sp. strain C-125 were compared with those on the genetic and physical maps of *B. subtilis* (Piggott and Hoch 1991; Itaya and Tanaka 1991; Kunst et al. 1997). DNA probes for *secY* (Kang et al. 1992) and *rpoA* from strain C-125 hybridized to the same fragments 4A and 3S′ (Table 3). The genetic loci on the physical map of strain C-125 were within approximately 12° of their positions on the *B. subtilis* physical map (Fig. 5). It was also found that probes for *groEL* (Xu et al. 1996) and *hag* (Sakamoto et al. 1992) hybridized to 3A and 4S, and 11A and 1S, respectively. Their positions on the map were also close to those of *B. subtilis* (55° for *groEL* and 310° for *hag*), as shown in Fig. 5. On the other hand, it appeared that genes such as *trpS*, *appA*, *rnh*, and *recA* have divergent map positions, being located at 103° to 150° (Fig. 5) on the *B. subtilis*

genetic map and approximately 200°–250° on the map of strain C-125. The map positions of three genes, *sigA*, *pALK* (*yuft*), and *resB*, located at 100°–140°, also differ from the positions 206°–277° on the *B. subtilis* map. Thus it appears that the region around 200°–250° on the C-125 map may correspond to the region around 100°–140° on the genetic map of *B. subtilis*.

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