JOURNAL OF BACTERIOLOGY, Dec. 1997, p. 7426–7434 Vol. 179, No. 23
0021-9193/97/$04.00+0

Physical and Genetic Map of the *Clostridium acetobutylicum* ATCC 824 Chromosome

EMMANUEL CORNILLOT, CHRISTIAN CROUX, AND PHILIPPE SOUCAILLE*

INSA, Centre de Bioingénierie G. Durand, UMR CNRS 5504, Laboratoire associé INRA, F-31077 Toulouse Cedex 04, France

Received 29 July 1997/Accepted 25 September 1997

A physical and genetic map of the *Clostridium acetobutylicum* ATCC 824 chromosome was constructed. The macrorestriction map for *Ceu*I, *Eag*I, and *Sst*II was created by ordering the 38 restriction sites by one- and two-dimensional pulsed-field gel electrophoresis (PFGE) and by using an original strategy based on the *Ceu*I enzyme and indirect end labelling by hybridization on both sides of the *Ceu*I sites with *rrs* (16S RNA) and 3′ *rrl* (23S RNA) probes. The circular chromosome was estimated to be 4.15 Mb in size, and the average resolution of the physical map is 110 kb. The chromosome contains 11 *rrn* loci, which are localized on 44% of the chromosome in a divergent transcriptional orientation regarding the presumed location of the replication origin. In addition to these 11 *rrn* operons, a total of 40 identified genes were mapped by hybridization experiments with genes from *C. acetobutylicum* and from various other clostridia as probes. The genetic map of *C. acetobutylicum* was compared to that of the three other endospore-forming bacteria characterized so far: *Bacillus subtilis*, *Clostridium beijerinckii*, and *Clostridium perfringens*. Parodoxically, the chromosomal backbone of *C. acetobutylicum* showed more similarity to that of *B. subtilis* than to those of the clostridia.

Clostridium acetobutylicum ATCC 824 is a strictly anaerobic spore-forming bacterium that produces acetone, butanol, and ethanol from various carbohydrates. Regarding this strain, which is now considered the *C. acetobutylicum* (30) type strain, considerable attention has focused on process development (34), physiological studies (21, 22, 26, 51), and the identification of the genes involved in the acid- and solvent-producing pathways (18, 20, 38, 52, 53). However, little is known about the genomic structure and organization of this organism. The development of pulsed-field gel electrophoresis (PFGE) techniques has dramatically influenced the field of chromosome physical mapping and the analysis of bacterial genomic organization. Combined with the identification of restriction enzymes with recognition sequences that occur infrequently in bacterial genomes, PFGE has facilitated the construction of new macrorestriction maps of the chromosomes of more than 100 bacteria (12).

We present here a macrorestriction map of the chromosome of *C. acetobutylicum* ATCC 824, created using PFGE, two-dimensional (2D) PFGE, and Southern blot analysis. The circular chromosome was estimated to be 4.15 Mb based on the sizes of the fragments generated from digesting whole chromosomal DNA with *Ceu*I, *Eag*I, and *Sst*II. *Ceu*I is an intron-encoded endonuclease from the chloroplast large rRNA gene of *Chlamydomonas eugametos*, which recognizes a 26-bp sequence present in the rRNA genes of a variety of bacterial species (32, 46). Digestion of *C. acetobutylicum* chromosome by *Ceu*I indicated that this species has 11 *rrn* operons organized in a divergent orientation with respect to the presumed location of the origin of replication. A physical map, with an average resolution of 110 kb, was constructed by ordering the 38 restriction sites of the three enzymes used. The genetic map has been established with 35 identified genes and 11 *rrn* operons on the 4.15-Mb chromosome and 5 genes on pSOL1, the previously identified 210-kb extrachromosomal element (13, 15).

* Corresponding author. Mailing address: INSA, Centre de Bioingénierie G. Durand, UMR CNRS 5504, Laboratoire associé INRA, Complexe Scientifique de Rangueil, F-31077 Toulouse Cedex 04, France. Phone: (33) 05 61 55 94 52. Fax: (33) 05 61 55 94 02. E-mail: soucaille@insa-tlse.fr.

MATERIALS AND METHODS

Bacterial strains and plasmids. All the strains and plasmids used in this study are listed in Table 1. *C. acetobutylicum* ATCC 824 was grown anaerobically at 37°C in 2× YTG (1.6% Bacto Tryptone, 1% yeast extract, 0.4% NaCl, 0.5% glucose [pH 5.2]; flushed with N_2). *C. acetobutylicum* ATCC 824 was stored in spore form after sporulation in the synthetic medium described by Monot et al. (35) with modifications of the biotin and *p*-aminobenzoic acid concentrations (0.04 and 8 mg/liter, respectively) (51).

Luria-Bertani medium was used for cultures of *Escherichia coli*. When necessary, ampicillin was added to a final concentration of 100 μg/ml. Plasmids were extracted by the alkali lysis method (5).

"Intact" chromosome preparation. A modified version of the technique described by Wilkinson and Young (55) was used to prepare intact chromosome. Overnight cultures were diluted 1:50 in 50 ml of prewarmed 2× YTG and grown to an optical density at 600 nm of 0.4 to 0.6. The bacteria were chilled on ice for 15 min, harvested by centrifugation at 4°C, washed with 50 ml of 10 mM Tris/HCl–1 mM EDTA (pH 7.6) (TE buffer), and resuspended in 1/30 of the culture volume with STE buffer (50% [wt/vol] sucrose in TE buffer). The cell suspension was mixed with an equal volume of 1.5% (wt/vol) LMP agarose (SeaPlaque GTG; FMC Bioproducts, Rockland, Maine) in STE buffer equilibrated at 42°C and introduced into an insert former in up to 60 solidified plugs (80 to 100 μl per plug) of agarose. Embedded bacteria were then incubated overnight at 37°C in 50 ml of TNES buffer (10 mM Tris-HCl [pH 7.5], 1 M NaCl, 0.1 M EDTA, 50% [wt/vol] sucrose)–0.5% SLS (*N*-lauroylsarcosine [Sigma, Saint Quentin Fallavier, France])–1 mg of lysozyme (Sigma) per ml, washed for several minutes in sterile water, and treated twice at 50°C in 25 ml of ESP buffer (0.5 M EDTA, 0.5% SLS, 2 mg of proteinase K [Sigma] per ml) for 24 h. The reaction was stopped by washing the plugs three times in TE buffer at 4°C for 30 min and incubating them for 1 h at 50°C in TE buffer plus 0.4 mg of phenylmethylsulfonyl fluoride (Sigma) per ml. The plugs were then stored at 4°C in 0.2 M EDTA (pH 8).

Digestion of DNA in agarose plugs. Plug slices were washed three times at 4°C for 30 min in 5 ml of TE buffer. Digestion was performed in Eppendorf tubes at 37°C. The plug was transferred in 260 μl of sterile water, and 40 μl of 10× restriction enzyme reaction buffer was added. We used buffers made in our laboratory from recipes provided by the suppliers (excepted for *Ceu*I, where no bovine serum albumin was added) for *Eag*I, *Ceu*I (New England Biolabs Inc. [NEB], Beverly, Mass.) and *Sst*II (GIBCO-BRL, Inchinnan, Scotland). For digestion, 70 U of *Sst*II, 50 U of *Eag*I, and 5 U of *Ceu*I were used.

For 2D digestions, the first-dimension gel was made in ultrapure agarose (SeaKem GTG). A 1-mm-wide slice from this gel was cut, covering the region of interest, and equilibrated three times for 30 min in 15-ml polypropylene sterile tubes containing 1× second restriction enzyme reaction buffer. Digestions were performed in the same tube with 150 U of enzyme in 2.5 ml of 1× buffer. For

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or source
Strains		
C. acetobutylicum ATCC 824		Laboratory stock
E. coli DH5α	F⁻ λ⁻ *endA1 recA1 relA1 hsdR17* (r_K^- m_K^+) *supE44* (*argF-lacZYA*) *U169 thi-1 gyrA96*(Nal^r) [Φ80d*lacZ*ΔM15]	GIBCO-BRL
Plasmids		
pBC23S	*rrnB*	19
pBC15	*rrs*	19
pEC10	3′ part of *rrl*	This study
pSU1	*recF gyrA gyrB*	50
prec1	*recA*	3
pKG3	*dnaKJ* (*hsp70* cluster)	37
pFN4	*groESL* (*hsp10-60* operon)	36
pK42	*hsp18 trna*$_{ACG}$	44
pAK	*ak* (acetate operon)	7
pTeco11	*thl* (thiolase)	48
pb37	β*hbd* (*BCS* operon)	6
pJC7	*butK ptb* (butyrate operon)	53
pEco14	*bdhAB* (two butanol DH)	52
pCoAT4	*ctfAB* (*sol* cluster)	10
pMFH1	*hydA* (complete locus)	24
pAC100	*lyc* (autolysin)	16
pFDP	*flg* (flagellin)	40
B5	Enzyme II PTS mannitol	23
prub	*rub* (rubredoxin PCR fragment)	This study
pMET13A	*flav* (flavodoxine P262 strain)	43
pCPRP32	[2Fe-2S] ferredoxin	33
PCR probes		
sigA	SigmaA factor	45
spo0A	spo factor	This study
sigE,G	Sigma factors E and G	45

*Eag*I, 33 μl of a bovine serum albumin solution at 10 mg/ml was added. The reaction was performed for 24 h at 37°C.

PFGE. Migrations were performed in the contour-clamped homogeneous electric field mode with the Bio-Rad (Ivry-sur-Seine, France) apparatus (CHEF-DRII) (11, 47). For most electrophoresis experiments, the gel and buffer conditions were 0.5× TBE and 1% (wt/vol) agarose (molecular biology grade; Eurobio, Les Ulis, France). For DNA fragments in the size range up to 600 kb, the buffer was stabilized at 9°C and concatemers of lambda DNA *c*I857 *ind*1 *Sam*7 (NEB) were used as molecular size standards. For fragments ranging from 600 kb to 2 Mb, the buffer was maintained at 13 to 14°C and isolated chromosomes from *Saccharomyces cerevisiae* YPH80 (NEB) were used as molecular size standards.

PCR. *Taq* PCR amplifications on *C. acetobutylicum* ATCC 824 total genomic DNA were performed in a reaction mixture including 500 μM deoxynucleoside triphosphates, 0.5 μM primers, 1 to 10 ng of template DNA, and 2 mM $MgCl_2$ in the 1× manufacturer buffer (NEB). A total of 25 cycles were performed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.), each consisting of denaturation at 94°C (1 min), annealing (2 min), and elongation at 72°C (2 min).

Long PCR amplifications were performed with the Expand Long Template PCR system (Boehringer Mannheim, Meylan, France) as recommended by the manufacturer.

Construction of probes. The pEC10 plasmid was constructed in two steps. A 0.5-kb *Nru*I-*Kpn*I fragment of the pBC23S plasmid containing part of the *rrl* gene of *C. perfringens* (19) was cloned in pBluescript SK⁻ (Stratagene Ltd., Cambridge, England). This intermediary plasmid was then deleted from a 0.15-kb *Not*I (pBluescript polylinker)-*Ceu*I fragment. The resulting pEC10 plasmid carried only the 3′ part of the *rrl* gene, starting from the *Ceu*I restriction site. The rubredoxin probe was obtained by *Taq* PCR amplification with two degenerate oligonucleotides (DI, 5′ATGAARAARTAYRYWTG3′; REV, 5′WRCWCC RCARABWGGRCA3′). The annealing temperature was 37°C with 5 mM $MgCl_2$ in the reaction solution. The amplified fragment was then cloned in pGEMT and sequenced, and the function was confirmed by homology searches with the BLASTX program (1) via the National Center for Biotechnology Information server. The *spo0A* probe was also obtained by PCR amplification on the ATCC 824 genomic DNA with specific oligonucleotides designed from strain ATCC 4259 gene sequence (8) (DI, 5′CTAGTAGTATTAGAAAAAC3′; REV, 5′GCTCTTTCTACTCTGC3′). An annealing temperature of 41°C was used.

Oligonucleotides for the orientation determination in the triplets of ribosomal operons. The orientation of the different oligonucleotides is shown in Fig. 1. The 16SU and 5SD primers were slightly modified from those of Okamoto et al. (39) (16SU, 5′CTGAGCSAKGATCAAACT3′; 5SD, 5′CCGTTCCCATVCCGAA C3′). In the PCR experiments with these primers, the annealing step was performed at 44°C. The 16SREV (5′ATGATTTGACGTCATCCCCACCTTCCTC C3′) and 23SDIR (5′GGCCGTAACTATAACGGTCCTAAGGTAGCGA3′) primers correspond to conserved regions of *rrs* and *rrl*, respectively, in bacterial chromosomes. The 23SDIR primer lies in the *Ceu*I restriction site. With these oligonucleotides, the PCR amplifications were performed with the Expand Long Template PCR system at an annealing temperature of 65°C and an elongation time of 8 min.

Gel drying and hybridization. The gels were stained for 1 h in a fresh solution of ethidium bromide (0.5 μg/ml) and photographed after overnight destaining at 4°C. DNA fragments in agarose gels were alkali denaturated for 40 min (0.5 M NaOH, 0.15 M NaCl) and neutralized for 40 min (0.5 M Tris-HCl [pH 7.5], 0.15 M NaCl). The gels were then dried at 60°C for 40 min on a Slab gel dryer (Hoefer Scientific Instrument, San Francisco, Calif.) under vacuum. The probes were labelled with the Redivue [α-^{32}P]dATP nucleotide (Amersham, Les Ulis, France) by random priming (Megaprime kit; Amersham). The unincorporated radionucleotides were removed on a Sephacryl S-200 HR gel microspin column (Pharmacia Biotech, Saclay, France). Hybridizations were made overnight at 65°C in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt's solution–0.5% sodium dodecyl sulfate (SDS) and were preceded by a prehybridization of 1 h in the same solution and at the same temperature. The denatured purified probe was simply added to the prehybridization solution. After hybridization, the gels were finally washed twice for 10 min in 2× SSC–0.1% SDS and twice for 30 min in 0.1× SSC–0.1% SDS at room temperature.

RESULTS

***C. acetobutylicum* ATCC 824 genome size estimation.** Like other *Clostridium* species, *C. acetobutylicum* ATCC 824 has a low G+C content of 25% (17). Therefore, restriction enzymes with GC-rich recognition sequences were screened to generate chromosomal digestions with relatively few fragments. Wilkinson and Young (54) have shown that *Sma*I and *Apa*I produced more than 20 fragments with several unresolved doublet or triplet bands in PFGE. For this reason, these enzymes were not used in this study. *Eag*I (CGGCCG), *Sst*II (CCGCGG), and *Rsr*II (CGG(A/T)CCG) produced 10, 17 (Table 2), and 4 (data not shown) fragments, respectively, while *Not*I (GCGGCCGC) did not cut *C. acetobutylicum* ATCC 824 DNA. By using a control replicative plasmid containing a *Not*I site, we have shown that *C. acetobutylicum* ATCC 824 DNA is not restricted by *Not*I due to a methylation of the site by the *Cac824*I methylase (data not shown). The 210-kb *Eag*I or *Sst*II fragments corresponding to the linearized form of plasmid pSOL1 are not presented in Table 2, since this plasmid has been described

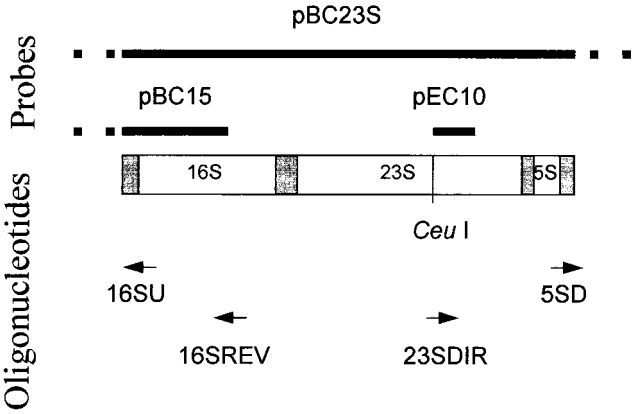

FIG. 1. Schematic representation of a ribosomal operon and position of the ribosomal probes and oligonucleotides used in this study.

TABLE 2. Sizes of *Ceu*I, *Eag*I and *Sst*II fragments of the *C. acetobutylicum* ATCC 824 chromosome after reconstruction of the physical map

*Ceu*I		*Eag*I		*Sst*II	
Fragment	Size (kb)	Fragment	Size (kb)	Fragment	Size (kb)
C1	2,330	E1	1,355	S1	570
C2	785	E2	530	S2	415
C3	675	E3	485	S3	340
C4	145	E4	440	S4	340
C5	95	E5	390	S5	330
C6	65	E6	275	S6	320
C7	30	E7	270	S7	310
C8	5	E8	200	S8	285
C9	5	E9	145	S9	235
C10	5	E10	55	S10	190
C11	5			S11	185
				S12	150
				S13	150
				S14	140
				S15	105
				S16	50
				S17	30
Total	4,145		4,145		4,145

elsewhere (13, 15). The intron-encoded endonuclease *Ceu*I (TAACTATAACGCTCCTAAGGTAGCGA) produced 8 observable fragments in PFGE (Fig. 2), but it will be shown later in this paper that there were actually 11 fragments. The *Ceu*I recognition sequence occurs in bacterial *rrl* genes, and digestion with this enzyme has been used to determine the number of *rrn* operons in a variety of bacterial genomes (32).

The fragment sizes generated by the three restriction enzymes (*Eag*I, *Sst*II, and *Ceu*I) used to construct the physical map are given in Table 2. The size of the chromosome was estimated to be 4.15 ± 0.2 Mb. Since the total number of restriction sites is 38, the average resolution of the physical map is expected to be 110 kb.

Chromosomal backbone and orientation of *rrn* loci. (i) Strategy used. The backbone of the chromosome can be defined by the positions of the *rrn* operons and their orientation with respect to the origin of replication (12). The gene order within *rrn* loci (Fig. 1) is *rrs*(16S)-*rrl*(23S)-*rrf*(5S) (27), and *Ceu*I recognizes a unique restriction site located within the *rrl* genes of all the eubacteria examined so far (32). The strategy used to construct the backbone of the *C. acetobutylicum* ATCC 824 chromosome is based on 2D PFGE with *Ceu*I in the first dimension and *Eag*I (or *Sst*II) in the second dimension, or the reverse. The order and the orientation of the *rrn* operons were determined by indirect end labelling with probes from *C. perfringens* specific to each side of the *Ceu*I site: pBC15 (19) specific to the *rrs* gene and pEC10 (this study) specific to the 3′ *rrl* gene (Fig. 1).

(ii) Eleven *rrn* operons. All the *Ceu*I fragments hybridized with the pBC23S probe (19) containing the whole *rrn* operon of *C. perfringens* (data not shown). Furthermore, all the *Eag*I or *Sst*II fragments that hybridized with this probe, and no others, were cut in the second dimension by *Ceu*I (Fig. 2; see Table 4). This demonstrates that, as in all other eubacteria examined so far, *Ceu*I cuts only within the *rrl* gene. The E1 and E4 fragments and the S4 and S6 fragments produced a 5-kb fragment when digested by *Ceu*I (Fig. 2; see Table 4). Partial *Ceu*I digestions of E1 and E4 showed a 10-kb fragment after ethidium bromide staining (data not shown) or after hybridization with the 3′ *rrl*-specific probes (Fig. 3), which indicates that the 5-kb fragments are in fact doublet bands. There are thus 11 *rrn* operons (five plus two triplets) in *C. acetobutylicum* ATCC 824. All the *Ceu*I fragments hybridized with both the *rrs* and the 3′ *rrl* probes, except for C1 and C3, which each hybridized with only one probe, 3′ *rrl* and *rrs*, respectively (Table 3).

(iii) *Ceu*I restriction map of the chromosome. The *Ceu*I restriction sites have been mapped by identification of *Eag*I or *Sst*II fragments overlapping *Ceu*I fragments. *Ceu*I end fragments were labelled with the *rrs* and 3′ *rrl* probes.

We first studied the combination of chromosomal digestions with *Ceu*I and *Eag*I enzymes. E1, E3, and E4 were the only *Eag*I fragments cut by *Ceu*I (Fig. 2A; Table 4). In the reciprocal double digestions with *Ceu*I and *Eag*I, C1, C3, and C5 were the only fragments cut by *Eag*I (Table 3). C3 was cut by *Eag*I to give two fragments of 445 and 230 kb, both hybridizing only with the *rrs* probe (Table 3). The 445-kb fragment was also obtained after *Ceu*I digestion of E3 (Table 4), while the 230-kb fragment was found in E4 after the *Ceu*I second digestion, indicating that C3 overlaps E3 and E4. E4 gave another *Ceu*I end fragment of 55 kb (Table 4) which was also obtained by *Eag*I digestion of C5. This observation shows that E4 also overlaps C5. Since the C8-C9 and C4 fragments were contained within E4 (Table 4), they are located between C3 and C5. C8-C9 were shown to be linked to C5 by using partial *Ceu*I digestions of the purified E4 fragment and hybridization with the *rrs* probe (Fig. 3) that gave, among others, a transitory signal corresponding to a size slightly larger (5 to 10 kb) than C5-E4 end fragment, corresponding to the C5-E4 end fragment plus C8-C9 (C5-E4 did not hybridize with the *rrs* probe after complete digestion). Walking further on the physical map, we demonstrated that C5 overlapped E1, because they shared a 40-kb fragment (hybridizing with the *rrs* probe) obtained after *Ceu*I digestion of E1 and *Eag*I digestion of C5. On the other hand, C1 and E1 had a 425-kb overlapping region that hybridized with 3′ *rrl* and C1 also overlapped E3, because they shared a 40-kb fragment (hybridizing with the 3′ *rrl* probe) obtained after *Ceu*I digestion of E3 and *Eag*I digestion of C1 (Tables 3 and 4). Since E3 overlaps C3, as mentioned above, this result links C1 to C3. From these data, the C1-C3-C4-C8-C9-C5 series was deduced. Since the large E1 fragment overlapped C1 and C5, it closes the map into a circular chromosome.

The order of C2, C6, C7, and C10-11 (contained within E1) was obtained from *Sst*II-*Ceu*I double digestions. S1, S4, S6, and S14 were the only *Sst*II fragments cut by *Ceu*I (Fig. 2B; Table 4). The C6 fragment, which was cut by *Sst*II to give two fragments of 50 and 15 kb (Table 3), was obtained from the 50-kb *Ceu*I end fragment of S4 that hybridized only with the 3′ *rrl* probe and the 15-kb *Ceu*I end fragment of S6 that hybridized only with the *rrs* probe (Table 4). Since S4 encompassed C4, C5, and C8-9 (Table 4), C6 and C5 were shown to be linked. C7, C10, and C11 are contained within S6, which also overlapped C2 (they share a 265-kb C2-S6 fragment hybridizing with the 3′ *rrl* probe) (Tables 3 and 4). C10-C11 were shown to be linked to C2-S6 by using partial *Ceu*I digestions of the purified S6 fragment and a similar strategy to that described above for C8 and C9 (Fig. 3). Hybridization with the *rrs* probe revealed, among others, a signal corresponding to a size slightly larger (5 to 10 kb) than C2-S6 (C2-S6 did not hybridize with the *rrs* probe), corresponding to C2-S6 plus C10-C11 (data not shown). From these data, the *Ceu*I restriction map of the chromosome showing the organization C1-C3-C4-C8-C9-C5-C6-C7-C10-C11-C2 was deduced. The region between the two triplets of *rrn* operons, which is only 355 kb in length (9% of

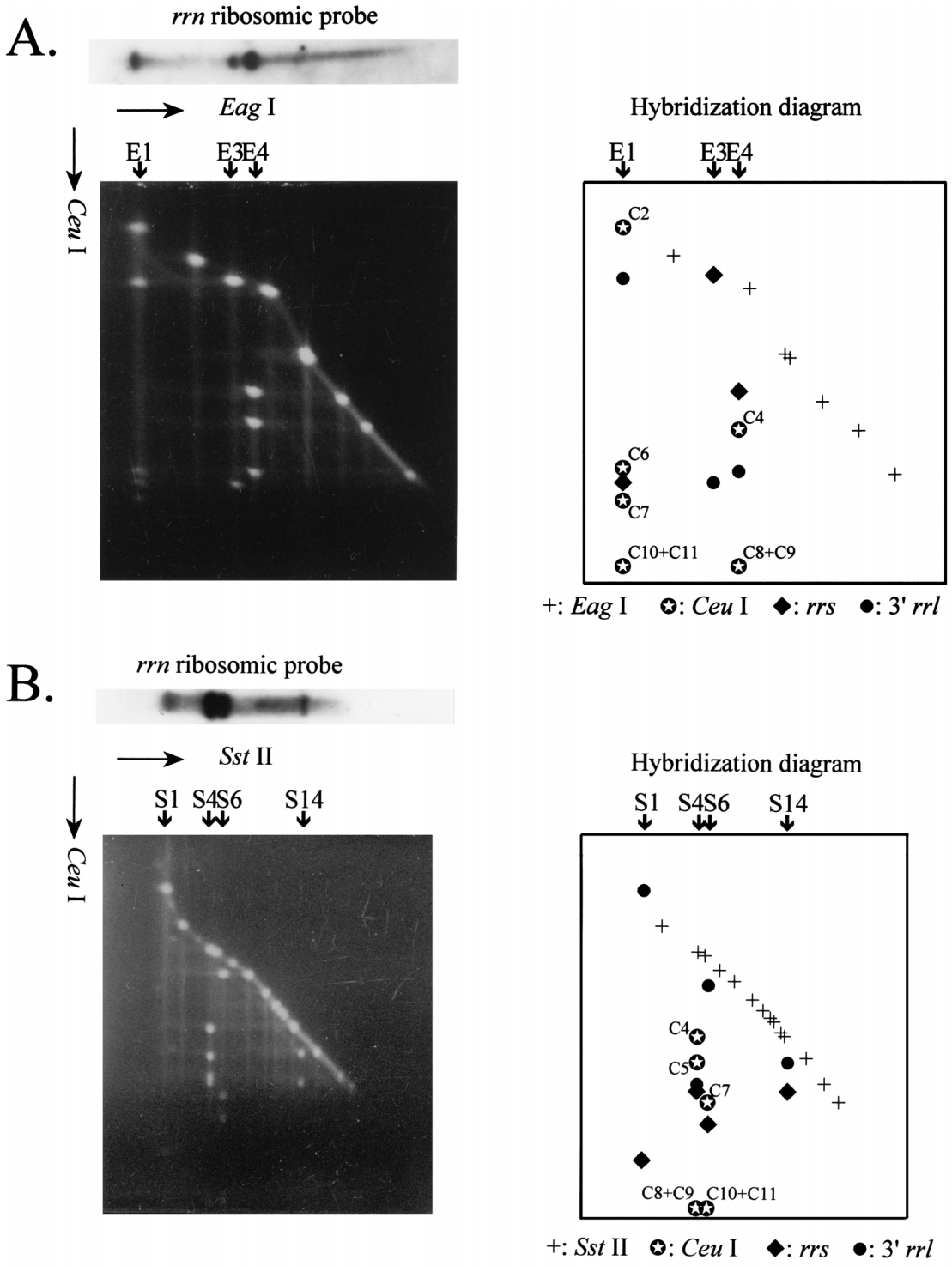

FIG. 2. The *Ceu*I restriction sites are within the *rrn* operons. *Eag*I (A)- or *Sst*II (B)-restricted DNA fragments were separated by PFGE in the first dimension, and half of the track was hybridized with the *rrn* ribosomal operon probe (pBC23S). The second half was digested with *Ceu*I and separated by PFGE in the second dimension. The gels were then hybridized with the *rrs* and the 3′ *rrl* probes. The results are summarized in the hybridization diagrams. Due to the small amount of DNA present in the gel, the 5-kb fragments C8-C9 and C10-C11 are poorly visible, if at all, on the gel after ethidium bromide staining. However, these fragments gave strong signals when hybridized with both ribosomal probes. Symbols: +, *Eag*I and *Sst*II fragments that were not cut by *Ceu*I; ✪, *Ceu*I fragments that hybridized with both ribosomal probes; ◆ and ●, *Ceu*I fragments that hybridized with the *rrs* probe only (◆) or the 3′ *rrl* probe only (●). See Table 4 for the sizes and analysis of the fragments. The PFGE conditions were as follows: first dimension, (i) 200 V, pulse time of 40 s for 20 h (A) or (ii) 200 V, pulse time of 25 s for 16.5 h and then 50 s for 1.5 h (B); second dimension, 200 V, pulse time of 25 s for 13 h and then 50 s for 6 h (A) or 200V, pulse time ramping from 10 to 50 s for 18 h (B).

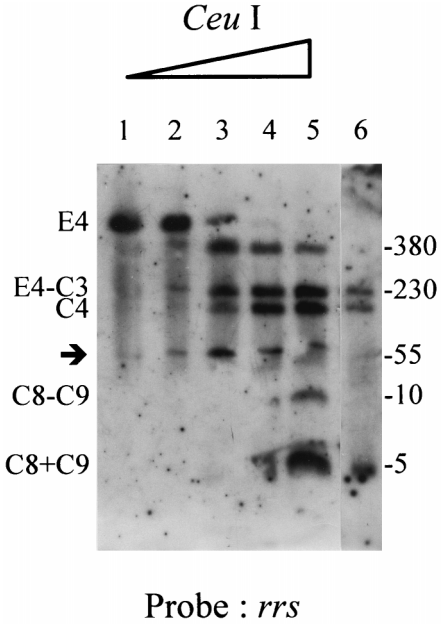

FIG. 3. Existence and position of a triplet of ribosomal operons in the E4 restriction fragment. Different PFGE-separated partial *Ceu*I digestions of the E4 fragment were hybridized with the *rrs* specific probe. PFGE conditions were as follows: 200 V and a two-step program, pulse time of 5 s for 5 h and 30 s for 12 h. The partial *Ceu*I digestions were obtained by increasing the enzyme concentration and the time of incubation (lanes 1 to 5, 4 h with 0.25, 0.5, 1, 2, and 3 U, respectively; lane 6, 48 h with 2 U). The arrow indicates the transitory signal corresponding to the C5-E4 end fragment plus C8-C9. E4-C3 indicates the E4-C3 end fragment.

the chromosome), carries 9 of the 11 *rrn* operons present in the *C. acetobutylicum* ATCC 824 chromosome.

(iv) Orientation of the ribosomal operon. The strategy used for the construction of the *Ceu*I restriction map of the chromosome gave us the orientation of most of the ribosomal operons. However, the exact organization of the two triplets of ribosomic operons and consequently the orientation of the C4 and C7 fragments remained uncertain. To resolve this problem, a strategy (based on PCR amplification) analogous to that described by Okamoto et al. (39) for the genome of *Bacillus subtilis* was used. Two sets of oligonucleotides were used as primers: 16SU and 5SD (Fig. 1) (39) for determination of the size of the region between the repeated operons, and 16SREV and 23SDIR (Fig. 1), specially designed for long-PCR amplification and identification of direct or inverted repeats.

TABLE 3. Sizes and analysis of the fragments obtained after restriction by *Ceu*I in the first dimension and by *Eag*I or *Sst*II in the second

Size of *Eag*I fragment (kb):			Size of *Sst*II fragment (kb):			
C1	C3	C5	C1	C2	C3	C6
425[b]	445[a]	55[b]	560[b]	330 (S5)	285 (S8)	50[b]
40[b]	230[a]	40[a]	100[b]	265[b]	235 (S9)	15[a]
(...)[d]			(...)[d]	150 (S12)	105 (S15)	
				40[a]	40[a]	
					8[a]	
2,330[c]	675	95	2,330	785	678	65

[a] Extremity fragment that hybridizes only with the *rrs*-specific probe (pBC15)
[b] Extremity fragment that hybridizes only with the 3′ *rrl*-specific probe (pEC10).
[c] Totals for columns are given in the bottom row.
[d] The sizes of all other fragments are not given.

TABLE 4. Sizes and analysis of the fragments obtained after restriction by *Eag*I or *Sst*II in the first dimension and by *Ceu*I in the second[a]

Size of *Ceu*I fragment (kb):						
E1	E3	E4	S1	S4	S6	S14
785 (C2)	445[b]	230[b]	560[c]	145 (C4)	265[c]	100[c]
425[c]	40[c]	145 (C4)	8[b]	95 (C5)	30 (C7)	40[b]
65 (C6)		55[c]		50[c]	15[b]	
40[b]		5 (C8)		40[b]	5 (C10)	
30 (C7)		5 (C9)		5 (C8)	5 (C11)	
5 (C10)				5 (C9)		
5 (C11)						
1,355[d]	485	440	568	340	320	140

[a] Data from Fig. 2.
[b] Extremity fragment that hybridizes only with the *rrs*-specific probe (pBC15).
[c] Extremity fragment that hybridizes only with the 3′ *rrl*-specific probe (pEC10).
[d] Totals for columns are given in the bottom row.

The template DNA for the PCR amplification consisted of either the E1 or E4 *Eag*I restriction fragments known to carry one of the two triplets (Fig. 3) or total genomic DNA. Amplification with each oligonucleotide alone was always unsuccessful. PCR amplification with the 16SU and 5SD oligonucleotides gave a product of 450 bp, while amplification with the 16SREV and 23SDIR oligonucleotides gave a fragment of 2.9 kb (data not shown). These results demonstrated that the ribosomal operons are repeated in a direct order.

The *gyrA* and *gyrB* genes of *C. acetobutylicum* DSM 1731 (ATCC 824) were cloned and sequenced recently and have been shown to be clustered with *recF* (50). The *gyrB* gene encodes the B subunit of DNA gyrase and is usually located near the origin of replication in bacterial chromosomes (42). *gyrB* hybridized with fragment C3 and was mapped to the 40-kb C3-S4 overlap (data not shown). Like that observed for *E. coli*, *B. subtilis*, and *C. perfringens* chromosomes, the *rrn* operons of *C. acetobutylicum* are therefore confluently transcribed with the direction of chromosome replication. For the final interpretation of the results obtained from our strategy associating 2D PFGE, the use of the *Ceu*I restriction enzyme, and specific end-labelling probes, see Fig. 5.

Construction of the physical map. The restriction sites of *Eag*I and *Sst*II were placed on the *Ceu*I map by using the previous data of 2D PFGE with *Ceu*I and *Eag*I (or *Sst*II) associated with new 2D PFGE experiments with *Eag*I and *Sst*II (Fig. 4; Tables 5 and 6). Most of the sites could be placed by this method, with only two uncertainties within the E1 fragment: the order of the *Sst*II sites in C2 and in the 425-kb C1-E1 fragment. The order of the *Sst*II sites within C2 was resolved by a partial *Sst*II digestion of this fragment and hybridization with the *rrs* probe (data not shown). The order of the *Sst*II sites in the 425-kb C1-E1 fragment was also determined by this approach (data not shown). The average resolution of the physical map presented in Fig. 5 is 110 kb. Due to the organization of the *rrn* operons, the resolution is higher around the putative origin of replication.

Genetic map of *C. acetobutylicum* ATCC 824. To combine a genetic map with the physical map, different chromosomal genes (Table 1) were located by hybridization experiments with probes from the type strain ATCC 824 or from very closely related strains (28, 30) like DSM 1731, DSM 792, or ATCC 4259. Except for the genes involved in the final steps of solvent

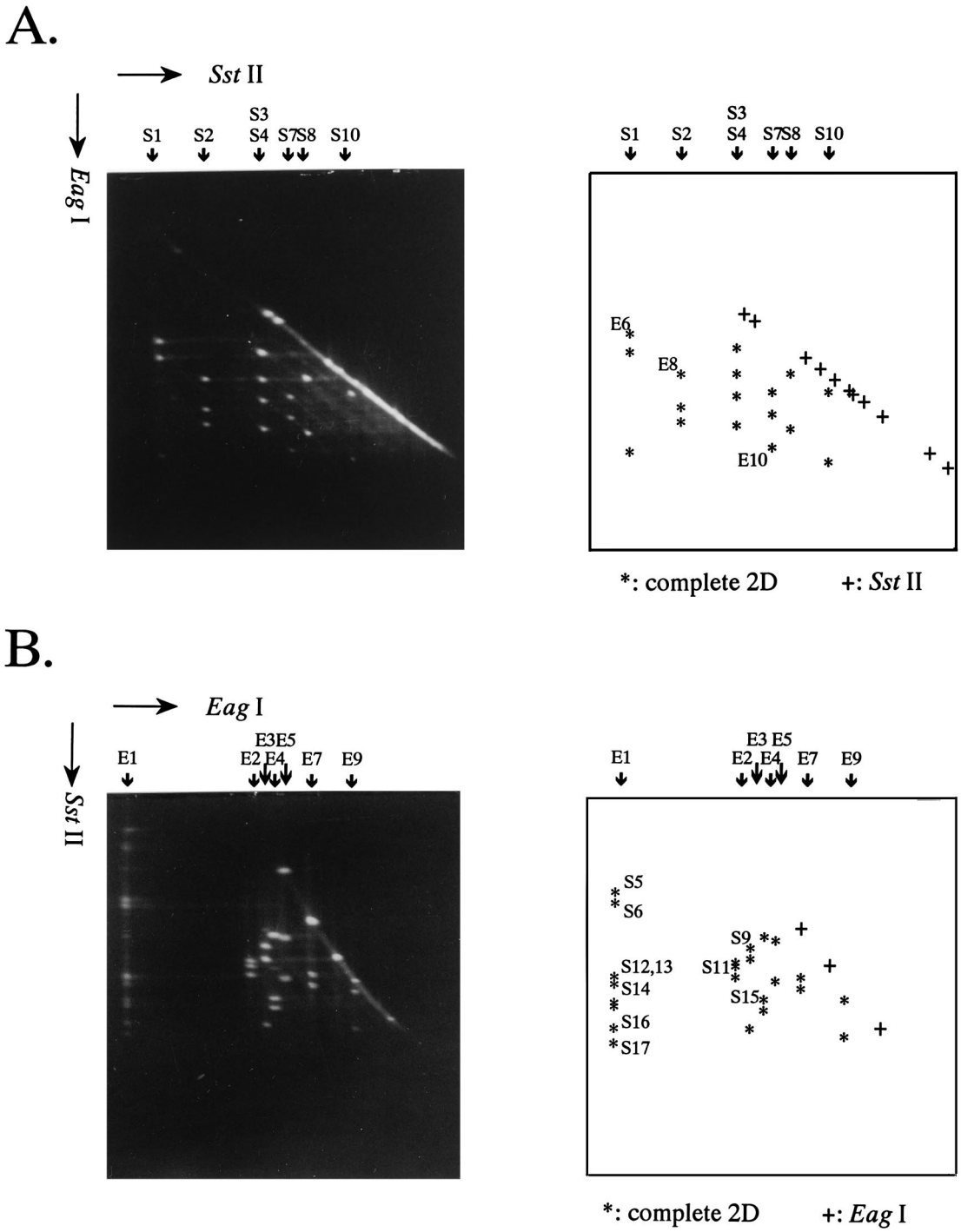

FIG. 4. 2D PFGE experiments with *Eag*I and *Sst*II. (A) *Sst*II followed by *Eag*I. The running conditions were as follows: first and second dimensions, 200 V, pulse time ramping from 40 to 60 s over 20 h. (B) *Eag*I followed by *Sst*II. The PFGE conditions were as follows: first dimension, 200 V, pulse time of 25 s for 22 h; second dimension, 200 V, pulse time ramping from 20 to 40 s over 20 h. See Tables 5 and 6 for the size and analysis of the fragments. +, unrestricted fragments in the second dimension. The spots corresponding to a complete digestion in the second dimension are represented by asterisks in the diagram. When a spot is absent from the diagram, it corresponds to a partial digestion.

formation, which are carried by pSOL1 (13), all the genes of primary metabolism cloned so far have been mapped on the chromosome (Fig. 5). The positions of several genes involved in housekeeping functions, the heat shock response, and sporulation were also determined.

DISCUSSION

To construct the physical map of the *C. acetobutylicum* ATCC 824 chromosome, an original strategy based on the *Ceu*I enzyme and indirect end labelling with the *rrs* and 3′ *rrl*

TABLE 5. Sizes and analysis of the fragments obtained after restriction by *Sst*II in the first dimension and by *Eag*I in the second[a]

Size of *Eag*I fragment (kb):						
S1	S2	S3	S4	S7	S8	S10
275 (E6)	200 (E8)	195	250	150	200	150
245	120	145	90	105	85	40
50	95			55 (E10)		
570[b]	415	340	340	310	285	190

[a] Data from Fig. 4A.
[b] Totals for columns are given in the bottom row.

probes on both sides of the *Ceu*I site was used. When used with two rare-cutting enzymes, *Sst*II and *Eag*I, and 2D PFGE, this strategy enabled the rapid construction of the physical map presented in Fig. 5. We confirmed what has been observed for most of the eubacteria examined so far: the gene order within the *rrn* loci of *C. acetobutylicum* ATCC 824 is *rrs-rrl-rrf*, and *Ceu*I recognizes a unique restriction site located within the *rrl* genes. The *rrn* locus is repeated 11 times on the chromosome. The *rrn* loci are localized on 44% of the chromosome in a divergent orientation with respect to putative origin of replication mapped by the position of the *recF gyrA gyrB* genes cluster. A similar orientation of the *rrn* operons has already been described for many bacteria, e.g., *Escherichia coli* (2), *B. subtilis* (39), *C. perfringens* (9, 29), and *Lactococcus lactis* (31, 49).

The presence of doublets or triplets of *rrn* operons is less frequent but has been described in *C. perfringens* (29), *C. beijerinckii* (55), *B. subtilis* (39), and *Streptococcus thermophilus* (41). The *B. subtilis* chromosome carries both a triplet (*rrnGHI*) and a doublet (*rrnJW*) of *rrn* operons (39). For a laboratory strain, the triplet was reported to be unstable due to the loss of one copy of the *rrn* operons (25). The presence of two triplets in *C. acetobutylicum* ATCC 824 is exceptional.

It has recently been shown that the genes involved in the final steps of solvent production are located on a 210-kb plasmid in strains ATCC 824 and ATCC 4259 (13, 15). In this study, we demonstrate that the rubredoxin gene and the genes involved in the conversion of acetyl coenzyme A (acetyl-CoA) to butyryl-CoA (6, 48) are found on the chromosome on a *Eag*I-*Sst*II (E-S) fragment contiguous to another E-S fragment

TABLE 6. Sizes and analysis of the fragments obtained after restriction by *Eag*I in the first dimension and by *Sst*II in the second[a]

Size of *Sst*II fragment (kb):						
E1	E2	E3	E4	E5	E7	E9
330 (S5)	195	235 (S9)	250	245	150	105
320 (S6)	185 (S11)	200	105 (S15)	145	120	40
150 (S12)	150	50	85			
150 (S13)						
140 (S14)						
95						
90						
50 (S16)						
30 (S17)						
1,355[b]	530	485	440	390	270	145

[a] Data from Fig. 4B.
[b] Totals for columns are given in the bottom row.

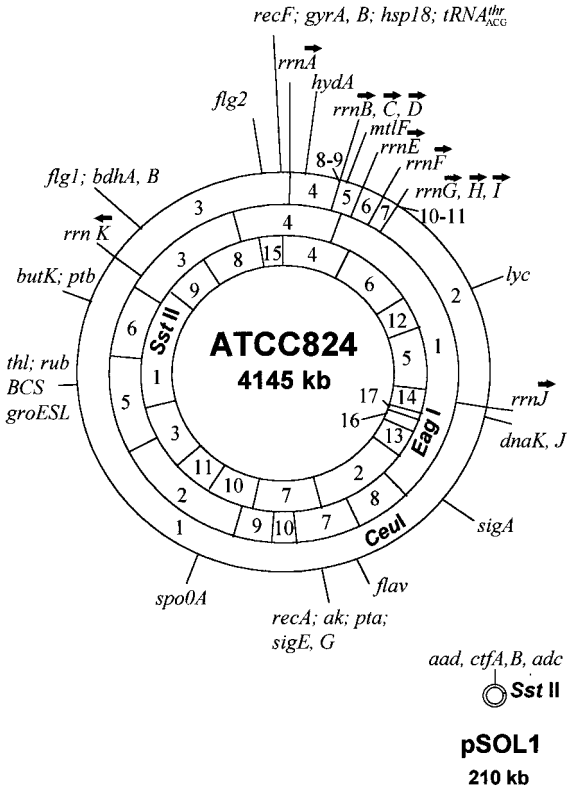

FIG. 5. Physical and genetic map of the *C. acetobutylicum* ATCC 824 genome. The arrows indicate the orientation of the ribosomal operons. The *rrn* operons corresponding to *Ceu*I sites were named from *rrnA* to *rrnK* in a clockwise direction from the putative origin of replication. The positions of the following genes are indicated: (i) *ak*, *pta*, acetate operon; *butK*, *ptb*, butyrate operon; *thl*, thiolase; *BCS*, *crt*, *bcd*, *etfA*,*B*, β*hbd* genes in the *BCS* operon; *bdhAB*, butanol dehydrogenase isogenes; *aad*, *ctfAB*, *adc*, *sol* gene cluster; *hydA*, hydrogenase; *flav*, flavodoxin; *rub*, rubredoxin; for the primary metabolic genes; (ii) *recF*, *gyrAB*, homologous recombination protein, DNA gyrase subunits, and putative origin of replication; *recA*, multifunctional protein in homologous recombination and SOS pathway induction; *spo0A*, transcription factor; *hsp18*, *tRNA*$^{thr}_{ACG}$, small heat shock gene cluster; *mtlF*, homologous fragment to the gene coding for enzyme II of the PTS mannitol; *lyc*, autolysin; *grpE*, *dnaKJ*, HSP70 coding gene cluster; *groESL*, HSP10–60 operon; *sigA*, *sigEG*, sigma factors; *flg12*, two copies of a flagellin gene. For references, see Table 1.

that carries the genes for the conversion of butyryl-CoA to butyrate (53).

The electron flow plays an important role in the regulation of *C. acetobutylicum* metabolism (21, 22, 51). Some of the genes involved in the electron flow have been mapped. The flavodoxin gene (43) is close to the acetate operon. The *hydA* gene, coding for the iron-only hydrogenase (24), is close to the origin of replication. On the other hand, the [2Fe-2S] ferredoxin gene found in *Clostridium pasteurianum* (33) did not hybridize with *C. acetobutylicum* ATCC 824 DNA under heterologous conditions.

The genetic map of *C. acetobutylicum* presented in this study is the fourth for endospore-forming bacteria and follows those for *B. subtilis* (4), *C. beijerinckii* (55), and *C. perfringens* (9, 29). We have extended the comparison (29) between these maps and the map of *C. acetobutylicum* ATCC 824 (Fig. 6). Despite their differences in size, *C. beijerinckii* and *C. perfringens* chromosomes have a similar gene organization within the *gyrAB* and *dnaK* loci, which is not found in *C. acetobutylicum*. Paradoxically, the chromosome backbone of *C. acetobutylicum* shows more similarity to that of *B. subtilis*: most of the ribo-

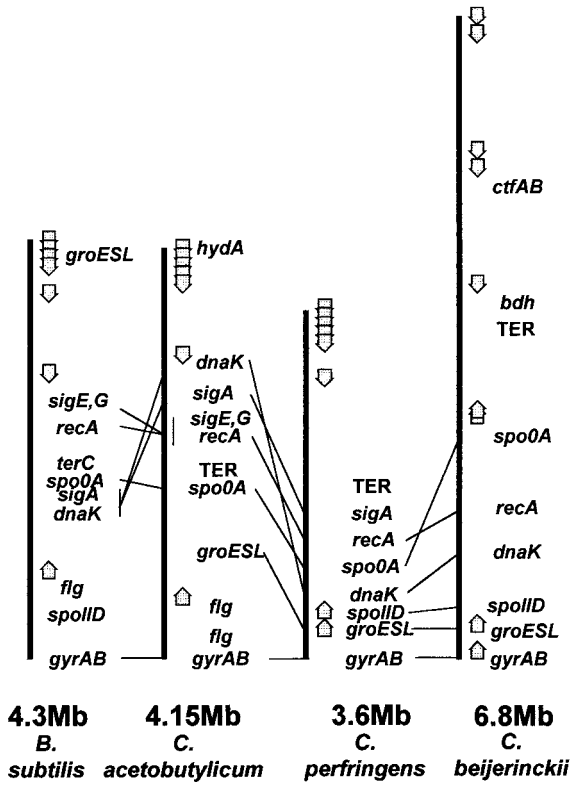

FIG. 6. Comparison of the chromosomes of *C. acetobutylicum*, *C. beijerinckii*, *C. perfringens*, and *B. subtilis*. This comparative analysis of chromosomes among low-G+C% gram-positive bacteria derives from data from this study and other sources (4, 29, 55). The alignment was done from the putative origin of replication labelled by the *gyrAB* genes. The TER regions of clostridial chromosomes have not been physically characterized. The arrows correspond to ribosomal operons or clusters of ribosomal operons. The exact number of *rrn* copies is 10 for *B. subtilis*, 11 for *C. acetobutylicum*, 14 for *C. beijerinckii*, and 10 for *C. perfringens*.

somal operons are concentrated in a small region, only one operon is in a divergent orientation with respect to the others, and the relative positions of several markers are conserved (*spo0A*, *recA*, *sigEG*, and *flg*).

The strategy used in this study should allow a rapid construction of the backbone of many bacterial chromosomes and a comparative study of genome architecture and plasticity. For example, the chromosome backbones of two different species like *E. coli* and *Salmonella typhimurium* (32) are very highly conserved. The *Ceu*I restriction profile of the *C. acetobutylicum* ATCC 4259 (Weizmann strain) chromosome shows only one difference from that of *C. acetobutylicum* ATCC 824: the integration of a 50-kb fragment in the corresponding C6 fragment (data not shown). Analysis of chromosome backbones will also give new insights into the relationships between genome structure, solventogenesis, and sporulation among solventogenic clostridia, in a similar approach to that used to study the relationship between genome structure and enterotoxicity in *C. perfringens* (14).

ACKNOWLEDGMENTS

We thank H. Bahl, G. N. Bennett, S. T. Cole, P. Dürre, J. Meyer, E. T. Papoutsakis, and J. D. Santangelo for providing plasmids; W. Philipp for assistance with 2D PFGE; and P. Lebourgeois for helpful discussions.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54:**130–197.
3. **Bennett, G. N.** Personal communication.
4. **Biaudet, V., F. Samson, C. Anagnostopoulos, S. D. Ehrlich, and P. Bessiere.** 1996. Computerized genetic map of *Bacillus subtilis*. Microbiology **142:**2669–2729.
5. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
6. **Boynton, Z. L., G. N. Bennett, and F. B. Rudolf.** 1996. Cloning, sequencing and expression of clustered genes encoding beta-hydroxybutyryl-coenzyme A (CoA) dehydrogenase, crotonase and butyryl-CoA dehydrogenase from *Clostridium acetobutylicum* ATCC 824. J. Bacteriol. **178:**3015–3024.
7. **Boynton, Z. L., G. N. Bennett, and F. B. Rudolf.** 1996. Cloning, sequencing and expression of genes encoding phosphotransacetylase and acetate kinase from *Clostridium acetobutylicum* ATCC 824. Appl. Environ. Microbiol. **178:** 2758–2766.
8. **Brown, D. P., L. Ganova-Raeva, B. D. Green, S. R. Wilkinson, M. Young, and P. Youngman.** 1994. Characterization of *spo0A* homologues in diverse *Bacillus* and *Clostridium* species identifies a probable DNA-binding domain. Mol. Microbiol. **14:**411–426.
9. **Canard, B., and S. T. Cole.** 1989. Genome organization of the anaerobic pathogen *Clostridium perfringens*. Proc. Natl. Acad. Sci. USA **86:**6676–6680.
10. **Cary, J. W., D. J. Petersen, E. T. Papoutsakis, and G. N. Bennett.** 1990. Cloning and expression of *Clostridium acetobutylicum* ATCC 824 acetoacetyl-coenzyme A:acetate/butyrate:coenzyme A-transferase in *Escherichia coli*. Appl. Environ. Microbiol. **56:**1576–1583.
11. **Chu, G., D. Vollrath, and R. W. Davis.** 1986. Separation of large DNA molecules by countour-clamped homogeneous electric fields. Science **234:** 1582–1585.
12. **Cole, S. T., and I. Saint-Girons.** 1994. Bacterial genomics. FEMS Microbiol. Rev. **14:**139–160.
13. **Cornillot, E., R. V. Nair, E. T. Papoutsakis, and P. Soucaille.** 1997. The genes for butanol and acetone formation in *Clostridium acetobutylicum* ATCC 824 reside on a large plasmid whose loss leads to strain degeneration. J. Bacteriol. **179:**5442–5447.
14. **Cornillot, E., B. Saint-Joanis, G. Daube, S. Katayama, P. E. Granum, B. Canard, and S. T. Cole.** 1995. The enterotoxin gene (*cpe*) of *Clostridium perfringens* can be chromosomal or plasmid-borne. Mol. Microbiol. **15:**639–647.
15. **Cornillot, E., and P. Soucaille.** 1996. Solvent-forming genes in clostridia. Nature **380:**489.
16. **Croux, C., and J. L. Garcia.** 1992. Reconstruction and expression of the autolytic gene from *Clostridium acetobutylicum* ATCC 824 in *Escherichia coli*. FEMS Microbiol. Lett. **95:**13–20.
17. **Cumins, C. S., and J. L. Johnson.** 1971. Taxonomy of the *Clostridia*: wall composition and DNA homologies in *Clostridium butyricum* and other butyric acid producing *Clostridia*. J. Gen. Microbiol. **67:**33–46.
18. **Fisher, R. J., J. Helms, and P. Dürre.** 1993. Cloning, sequencing and molecular analysis of the *sol* operon of *Clostridium acetobutylicum*, a chromosomal locus involved in solventogenesis. J. Bacteriol. **175:**6959–6969.
19. **Garnier, T., B. Canard, and S. T. Cole.** 1991. Cloning, mapping and molecular characterization of the rRNA operons of *Clostridium perfringens*. J. Bacteriol. **173:**5431–5438.
20. **Gerischer, U., and P. Dürre.** 1990. Cloning, sequencing and molecular analysis of the acetoacetate decarboxylase gene region from *Clostridium acetobutylicum*. J. Bacteriol. **172:**6907–6918.
21. **Girbal, L., C. Croux, I. Vasconcelos, and P. Soucaille.** 1995. Regulation of metabolic shifts in *Clostridium acetobutylicum* ATCC 824. FEMS Microbiol. Rev. **17:**287–297.
22. **Girbal, L., and P. Soucaille.** 1994. Regulation of *Clostridium acetobutylicum* metabolism as revealed by mixed-substrate steady-state continuous cultures: role of NADH/NAD ratio and ATP pool. J. Bacteriol. **176:**6433–6438.
23. **Gorwa, M.-F.** Ingénierie métabolique des enzymes impliquées dans la distribution du flux d'électrons chez *Clostridium acetobutylicum*. Ph.D. dissertation. National Institute of Applied Sciences, Toulouse, France.
24. **Gorwa, M.-F., C. Croux, and P. Soucaille.** 1996. Molecular characterization and transcriptional analysis of the putative hydrogenase gene of *Clostridium acetobutylicum* ATCC 824. J. Bacteriol. **178:**2668–2675.
25. **Gottlieb, P., G. LaFauci, and R. Rudner.** 1985. Alteration in the number of rRNA operons within the *Bacillus subtilis* genome. Gene **33:**259–268.
26. **Grupe, H., and G. Gottschalk.** 1992. Physiological events in *Clostridium acetobutylicum* during the shift from acidogenesis to solventogenesis in continuous culture and presentation of a model for shift induction. Appl. Environ. Microbiol. **58:**3896–3902.
27. **Jinks-Robertson, S., and M. Nomura.** 1987. Ribosomes and tRNA, p. 1358–1379. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.

28. **Johnson, J. L., and J.-S. Chen.** 1995. Taxonomic relationships among strains of *Clostridium acetobutylicum* and other phenotypically similar organisms. FEMS Microbiol. Rev. **17:**233–240.
29. **Katayama, S., B. Dupuy, T. Garnier, and S. T. Cole.** 1995. Rapid expansion of the physical and genetic map of the chromosome of *Clostridium perfringens* CPN50. J. Bacteriol. **177:**5680–5685.
30. **Keis, S., C. F. Bennett, V. K. Ward, and D. T. Jones.** 1995. Taxonomy and phylogeny of industrial solvent-producing clostridia. Int. J. Syst. Bacteriol. **45:**693–705.
31. **Le Bourgeois, P., M. Lautier, M. Mata, and P. Ritzenthaler.** 1992. Physical and genetic map of the chromosome of *Lactococcus lactis* subsp. *lactis* IL403. J. Bacteriol. **174:**6752–6762.
32. **Liu, S., A. Hessel, and K. E. Sanderson.** 1993. Genomic mapping with I-*Ceu*I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc. Natl. Acad. Sci. USA **90:**6874–6878.
33. **Meyer, J.** 1993. Cloning and sequencing of the gene encoding the [2Fe-2S] ferredoxin from *Clostridium pasteurianum*. Biochim. Biophys. Acta **1174:** 108–110.
34. **Minier, M., R. Grateloup, E. Blanc-Ferras, and G. Goma.** 1990. Extractive acetonobutylic fermentation by coupling ultrafiltration and distillation. Biotechnol. Bioeng. **35:**861–869.
35. **Monot, F., J. R. Martin, H. Petitdemange, and R. Gay.** 1982. Acetone and butanol production by *Clostridium acetobutylicum* in a synthetic medium. Appl. Environ. Microbiol. **44:**1318–1324.
36. **Narberhaus, F., and H. Bahl.** 1992. Cloning, sequencing and molecular analysis of the *groESL* operon of *Clostridium acetobutylicum*. J. Bacteriol. **174:**3282–3289.
37. **Naberhaus, F., K. Giebeler, and H. Bahl.** 1992. Molecular characterization of the *dnaK* gene region of *Clostridium acetobutylicum*, including *grpE*, *dnaJ*, and a new heat shock gene. J. Bacteriol. **174:**3290–3299.
38. **Nair, V. R., G. N. Bennett, and E. T. Papoutsakis.** 1994. Molecular characterization of an aldehyde/alcohol dehydrogenase gene from *Clostridium acetobutylicum* ATCC 824. J. Bacteriol. **176:**871–885.
39. **Okamoto, K., P. Serror, V. Azevedo, and B. Vold.** 1993. Physical mapping of stable RNA genes in *Bacillus subtilis* using polymerase chain reaction amplification from a yeast artificial chromosome library. J. Bacteriol. **175:**4290–4297.
40. **Petersen, D. J., and G. N. Bennett.** Personal communication.
41. **Roussel, Y., M. Pebay, G. Guedon, J. Simonet, and B. Decaris.** 1994. Physical and genetic map of *Streptococcus thermophilus* A054. J. Bacteriol. **176:**7413–7422.
42. **Salazar, L., H. Fsihi, E. de Rossi, G. Riccardi, C. Rios, S. T. Cole, and H. E. Takiff.** 1996. Organization of the origins of replication of the chromosomes of *Mycobacterium smegmatis*, *Mycobacterium leprae* and *Mycobacterium tuberculosis* and isolation of a functional origin from *M. smegmatis*. Mol. Microbiol. **20:**283–293.
43. **Santangelo, J. D., D. T. Jones, and D. R. Woods.** 1991. Metronidazole activation and isolation of *Clostridium acetobutylicum* electron transport genes. J. Bacteriol. **173:**1088–1095.
44. **Sauer, U., and P. Dürre.** 1993. Sequence and molecular characterization of a DNA region encoding a small heat shock protein of *Clostridium acetobutylicum*. J. Bacteriol. **175:**3394–3400.
45. **Sauer, U., A. Treuner, M. Buchholtz, J. D. Santangelo, and P. Dürre.** 1994. Sporulation and primary sigma factor homologous genes in *Clostridium acetobutylicum*. J. Bacteriol. **176:**6575–6582.
46. **Schmidt, K. D., B. Tümmler, and U. Römling.** 1996. Comparative genome mapping of *Pseudomonas aeruginosa* PAO with *P. aeruginosa* C, which belongs to a major clone in cystic fibrosis patients and aquatic habitats. J. Bacteriol. **178:**85–93.
47. **Schwartz, D. C., and C. R. Cantor.** 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell **37:**67–75.
48. **Stim-Herndon, K. P., D. J. Petersen, and G. N. Bennett.** 1995. Characterization of acetyl-CoA C-acetyltransferase (thiolase) gene from *Clostridium acetobutylicum* ATCC 824. Gene **154:**81–85.
49. **Tulloch, D. L., L. R. Finch, A. J. Hillier, and B. E. Davidson.** 1991. Physical map of the chromosome of *Lactococcus lactis* subsp. *lactis* DL11 and localization of six putative rRNA operons. J. Bacteriol. **173:**2768–2775.
50. **Ullmann, S., and P. Dürre.** 1996. Nucleotide sequence and molecular characterization of the DNA gyrase genes from *Clostridium acetobutylicum*. Anaerobe **2:**239–248.
51. **Vasconcelos, I., L. Girbal, and P. Soucaille.** 1994. Regulation of carbon and electron flow in *Clostridium acetobutylicum* grown in chemostat culture at neutral pH on mixtures of glucose and glycerol. J. Bacteriol. **176:**1443–1450.
52. **Walter, K. A., G. N. Bennett, and E. T. Papoutsakis.** 1992. Molecular characterization of *Clostridium acetobutylicum* ATCC 824 butanol dehydrogenase isozyme genes. J. Bacteriol. **174:**7149–7158.
53. **Walter, K. A., R. V. Nair, J. W. Cary, G. N. Bennett, and E. T. Papoutsakis.** 1993. Sequence and arrangement of genes encoding the two butyrate-formation enzymes of *Clostridium acetobutylicum* ATCC824. Gene **134:**107–111.
54. **Wilkinson, S. R., and M. Young.** 1993. Wide diversity of genome size among different strains of *Clostridium acetobutylicum*. J. Gen. Microbiol. **139:**1069–1076.
55. **Wilkinson, S. R., and M. Young.** 1995. Physical map of the *Clostridium beijerinckii* (formerly *Clostridium acetobutylicum*) NCIMB 8052 chromosome. J. Bacteriol. **177:**439–448.