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Sporulation and Primary Sigma Factor Homologous Genes in *Clostridium acetobutylicum*

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Using a PCR-based approach, we have cloned various σ factor homologous genes from *Clostridium acetobutylicum* DSM 792. The nucleotide sequence of the *dnaE-sigA* operon has been determined and predicts two genes encoding 69- and 43-kDa proteins. The deduced DnaE amino acid sequence has approximately 30% amino acid identity with protein sequences of other primases. The putative *sigA* gene product shows high homology to primary σ factors of various bacteria, most significantly to *Bacillus subtilis* and *Staphylococcus aureus*. Northern (RNA) blot analysis revealed that both genes form an operon, which is clearly expressed under conditions that allow for cell division. A promoter sequence with significant homology to the σ^H-dependent *Bacillus* promoters preceded the determined transcriptional start point, 182 bp upstream of the GUG start codon of *dnaE*. The homologous genes to *Bacillus* spp. sporulation σ factors G, E, and K have been cloned and sequenced. Indirect evidence for the existence of σ^F was obtained by identification of a DNA sequence homologous to the respective *Bacillus* consensus promoter. Southern hybridization analysis indicated the presence of σ^D and σ^H homologous genes in *C. acetobutylicum*. A new gene group conserved within the eubacteria, but with yet unspecified functions, is described. The data presented here provide strong evidence that at least some of the complex regulation features of sporulation in *B. subtilis* are conserved in *C. acetobutylicum* and possibly *Clostridium* spp.

Sigma (σ) factors are transcription factors that operate global switches in gene expression by conferring promoter-specific transcription initiation on bacterial RNA polymerase. Many σ factors, controlling the transcription from housekeeping promoters as well as several coordinately regulated sets of genes, have been identified in the last decade (for reviews, see references 30, 40, and 50). On the basis of sequence information, σ factors seem to fall into two major classes, of which only the σ^{70} homologous group will be considered here. A great deal of information has been obtained on the major vegetative σ factors, including nucleotide sequences from various bacteria (40), conservation and evolution of the operon structure (72), as well as functional domains of the protein (30, 40). Alternative σ factors are involved in regulation of many postexponential-phase phenomena, such as sporulation in *Bacillus* spp. (20, 44), stationary phase in enterobacteria, and motility and chemotaxis in many bacteria. However, with the exception of *Bacillus* spp., only limited information is currently available on σ factors in gram-positive organisms. Nucleotide sequences of vegetative σ factors have been determined only for *Streptomyces* spp. (36, 67) and *Staphylococcus aureus* (5) in addition to a sporulation-specific σ factor (WhiG) from *Streptomyces coelicolor* (12).

The orchestral expression of stage-specific gene sets during sporulation in *Bacillus subtilis*, governed by specific σ factors, has attracted much scientific interest (20, 44). On the basis of morphological studies, sporulation in *Bacillus* spp. and sporulation in *Clostridium* spp. have long been suggested to employ similar mechanisms (34). Nevertheless, almost no attention has been paid to the genetic analysis of sporulation in the spore-forming, gram-positive, anaerobic genus *Clostridium*, which is of considerable biotechnological potential (77). Hitherto, sequence information of clostridial sporulation genes is available only for two *ssp* genes from *Clostridium perfringens* (31) and the *spoIID* homolog of *C. acetobutylicum* (28).

Among the more than 120 clostridial species, *C. acetobutylicum*, *C. perfringens*, *C. botulinum*, *C. pasteurianum*, and *C. thermocellum* have been studied at the genetic level (3, 77, 79, 80). The first and second species are now accepted as model organisms of apathogenic and pathogenic clostridia, respectively (3, 80). *C. acetobutylicum* is characterized by its metabolic ability to shift from a predominantly acidogenic to a solventogenic fermentation pattern (4, 34). Along with the shift to solventogenesis, the cells undergo a series of morphological and physiological changes in motility, shape, and granulose content. These accumulated data suggest that the solventogenic phase is an important feature for sporulation in *C. acetobutylicum* but not a sporulation-specific event (33, 41–43, 49, 57), as has been shown for the solventogenic *C. thermosaccharolyticum* (39).

An understanding of the developmentally regulated sporulation process and its impacts on product formation might be helpful for the development of an optimal fermentation process. In this report, we provide the first description of the molecular mechanisms involved in sporulation and transcriptional control mediated by σ factors in *Clostridium* spp.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Chromosomal DNA of *C. acetobutylicum* DSM 792 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH,

Braunschweig, Germany) was used for recombinant DNA library construction and PCR amplifications. Total RNA of batch and continuous cultures was obtained from strain DSM 1731. Genomic DNA for comparative analysis of *C. acetobutylicum* strains was isolated from ATCC 824, DSM 1731, NCIMB 8052, and P262 (laboratory stocks). *B. subtilis* 168 (DSM 402) chromosomal DNA served as a control template in the PCR experiments. *Escherichia coli* JM83 (78) and SURE (Stratagene GmbH, Heidelberg, Germany) were used as hosts, and pUC9 (78) and pEcoR252 (kindly supplied by D. R. Woods, University of Cape Town, Cape Town, South Africa) were used as vectors for genomic library construction and cloning experiments.

In batch culture, *C. acetobutylicum* was routinely grown under strictly anaerobic conditions at 37°C in clostridial basal medium (51). The growth experiment in batch culture was done in CAMM medium, which has been described as being particularly useful for induction of sporulation (41). Continuous culture experiments with strain DSM 1731 were performed as described by Bahl et al. (2). *E. coli* was cultivated at 37°C in LB medium (58) supplemented with ampicillin (50 μg/ml), isopropyl-β-D-thiogalactopyranoside (IPTG; 50 μg/ml), or 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; 40 μg/ml) as required.

Physiological and morphological determinations. Numbers of total bacterial cells as well as percentages of motile cells, clostridial stages, forespores, and spores were determined in a counting chamber with a phase-contrast microscope (Standard RA; Carl Zeiss, Oberkochen, Germany). Product formation was measured by gas chromatography (model 439 gas chromatograph; Packard Instruments GmbH, Frankfurt, Germany) as described by Bahl et al. (2).

DNA isolation and manipulation. Chromosomal DNA of *C. acetobutylicum* and *B. subtilis* was isolated by the method of Marmur (47), with the modifications described by Bertram and Dürre (6). For isolation of plasmids from *E. coli*, the Quiagen Midi kit (Diagen GmbH, Düsseldorf, Germany) was used. DNA was manipulated by standard methods (58) with restriction enzymes and T4 DNA ligase obtained from GIBCO/BRL GmbH (Eggenstein, Germany) according to the manufacturer's instructions.

PCR. PCR amplifications were performed in 100-μl volumes containing primers (0.5 μM each), deoxynucleoside triphosphates (200 μM each), chromosomal DNA (< 0.5 μg), and *Taq* polymerase (2.5 U; GIBCO/BRL) in 50 mM KCl–10 mM Tris HCl (pH 7.5)–1.5 $MgCl_2$–10 μg of gelatin, covered with light mineral oil. Temperature cycling was performed by a programmable thermocycler (Trio-Thermoblock; Biometra biomedizinische Analytik GmbH, Göttingen, Germany) with the following conditions: 94°C for 1 min, 37°C for 30 s, and 72°C for 1 min/1,000 bp for 35 cycles. The conditions were adjusted by raising the annealing temperature and lowering the primer concentrations if unspecific fragments were amplified. Synthetic oligonucleotides for PCR and sequencing reactions were prepared by a Gene Assembler Plus (Pharmacia LKB GmbH, Freiburg, Germany) as instructed by the manufacturer. The following heterologous primers (in 5′-to-3′ direction) for PCR amplification of σ factor fragments were used (nucleotides at wobble positions are indicated in parentheses): for *sigA*, GGCTCGAGAT(ACT)GC(ACGT)AA(AG)(AC)(AG)(ACGT)TA (SA2.1), containing an *Xho*I linker at the 5′ end, and GT(AGT)AT(ACGT)GC(CT)TG(ACGT)C(GT)(AGT)ATCCACC (SA2.3); for *sigD*, TTGGATCCTGGAA(AG)GA(AG)TGGAA(AG)GA (SD2.1), containing a *Bam*HI linker at the 5′ end, and AAGAATTCGC(AG)TA(AT)GT(AG)TC(AG)AA(CT)TT (SD2.3), containing an *Eco*RI linker at the 5′ end; for *sigE*, TTGGATCCAA(AG)TT(CT)GA(AG)AA(CT)AC(ACGT)GG (SE2.1), containing a *Bam*HI linker at the 5′ end, and AAGAATTCCC(AG)TCCCA(AG)TC(AGT)AT(AG)TT (SE2.3), containing an *Eco*RI linker at the 5′ end; and for *sigH*, TTGGATCCAA(AG)TA(CT)(AC)G(ACGT)AA(CT)TT(CT)GT (SH2.1), containing a *Bam*HI linker at the 5′ end, and AAGAATTCTC(ACGT)GC(AG)AA(ACGT)GC(CT)TT(AG)AA (SH2.3), containing an *Eco*RI linker at the 5′ end. These oligonucleotides correspond to the amino acid sequences of subregions 2.1 (core binding region) and 2.3 (RpoD box), known to be conserved in many σ factors (30, 40).

Partial genomic libraries of *C. acetobutylicum*. Chromosomal DNA of strain DSM 792 was digested with *Hin*dIII and subjected to sucrose density centrifugation (10 to 40% [wt/vol]). Fractions containing fragments of 0.8 to 3.5 kbp were dialyzed against TE buffer (10 mM Tris HCl [pH 8.0], 1 mM EDTA), ethanol precipitated, and ligated into *Hin*dIII-digested pUC9. Ligation mixtures were directly used for transformation of *E. coli* cells by means of electroporation with a Gene Pulser (Bio-Rad Laboratories GmbH, München, Germany) as described by Dower et al. (17). Different libraries resulted from partial digestion of chromosomal DNA with *Sau*3AI and ligation into pEcoR252 or pUC9 (21, 22). Colonies from agar plates were transferred to Hybond N nylon filters (Amersham Buchler GmbH, Braunschweig, Germany), lysed with NaOH, washed as previously described (7), and subjected to the hybridization procedure described below.

Southern hybridization. Chromosomal DNA of *C. acetobutylicum* was digested to completion with the appropriate restriction enzymes, separated on 1% agarose gels, and transferred to nylon membranes (GeneScreen Plus; Dupont NEN Research Products, Dreieich, Germany) by capillary transfer in 10× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate). Prehybridization was performed for 1 to 3 h at 60°C in 10 ml of the following solution: 0.2% (wt/vol) polyvinylpyrrolidone, 0.2% (wt/vol) bovine serum albumin, 0.2% (wt/vol) Ficoll 400, 0.1% (wt/vol) sodium pyrophosphate, 10% (wt/vol) dextran sulfate, 1 M NaCl, 1% (wt/vol) sodium dodecyl sulfate (SDS), 50 mM Tris HCl (pH 7.5), and 100 μg of denatured salmon sperm DNA per ml. α-^{32}P-labeled PCR-generated DNA probes were prepared as previously described (60) and denatured for 10 min at 95°C immediately prior to hybridization. Hybridization temperatures were 60°C for homologous and 40°C for heterologous DNA probes. Following hybridization for 10 to 15 h, the membranes were washed twice in 2× SSC and once in 2× SSC–1% (wt/vol) SDS for 5 min at room temperature and then subjected to autoradiography.

DNA sequencing and sequence analysis. The nucleotide sequence was determined by primer walking with the dideoxy-chain termination method, using the T7 sequencing kit from Pharmacia LKB. All nucleotide data presented were confirmed by sequencing the opposite strand. The dideoxy-terminated fragments were separated on 55-cm wedge-shaped thickness gradient gels (0.2 to 0.4 mm, 6% [wt/vol] polyacrylamide) with a Macrophor sequencing unit (Pharmacia LKB) as recommended by the manufacturer. The computer-assisted analysis was done with the GeneWorks program (IntelliGenetics, Inc., Mountainview, Calif.) on a Macintosh IIsi computer (Apple Computer, Inc., Cupertino, Calif.). Sequence comparison results were obtained by utilization of the Bestfit program (Genetics Computer Group Inc. sequence analysis software package, version 6.0 [14]) on a VAX 9000 computer.

RNA isolation and analysis. Samples (8 ml each) of *C. acetobutylicum* cells were harvested from batch cultures at various time points, and total RNA was prepared by the hot phenol-chloroform method (52), with recently described mod-

ifications (23). For concentration determinations, the optical density was measured at 260 nm (optical density of 1 corresponds to 40 μg/ml) in a model 601 spectrophotometer (Milton Roy, Rochester, N.Y.). Total RNA for Northern (RNA) blots was separated in denaturing formaldehyde agarose gels and transferred to nylon membranes (GeneScreen Plus) as described by Sambrook et al. (58). Fragment sizes were estimated by comparison with an RNA ladder (GIBCO/BRL). Hybridization and washing were performed as described above for the Southern hybridization procedure. After the probes were stripped off by heating the membranes for 30 to 120 s at 95°C in 0.1× SSC–0.1% SDS, Northern blots could be reused. Primer extension analysis was performed as described by Gerischer and Dürre (23) except that SuperScript reverse transcriptase (GIBCO/BRL) was used.

Nucleotide sequence accession number. The DNA sequences reported in this paper have been submitted to EMBL and GenBank databases and assigned accession numbers Z23079 (*sigE/G*), Z23080 (*dnaE-sigA*), and L23317 (*sigK*).

RESULTS

Cloning and sequencing of the *dnaE-sigA* operon from *C. acetobutylicum*. Sequence information from the highly conserved region 2 of the vegetative σ factor of *B. subtilis* (RpoD) (30, 40) was used for preparation of degenerate oligonucleotides. The oligonucleotides were employed as primers in a subsequent PCR amplification using genomic DNA from *C. acetobutylicum* DSM 792 as the template source, according to the approach of Engel and Ganem (18). A 156-bp PCR fragment was obtained with *B. subtilis* as well as *C. acetobutylicum* genomic DNA, subcloned, and partially sequenced. The deduced amino acid sequence of the clostridial fragment showed significant homology to RpoD of *B. subtilis* (24) and was therefore radiolabeled for screening a partially digested *Sau*3AI genomic library of *C. acetobutylicum* (21). A positive clone with a 4,435-bp insert was identified, and the respective plasmid was designated pSA21. The cloned fragment contained seven internal *Sau*3AI sites but was originally contiguous on the chromosome, as proved by Southern hybridization and PCR (59).

Figure 1 shows 3,720 bp of the nucleotide sequence from the 4,435-bp insert and the deduced amino acid sequences of two large open reading frames. The first open reading frame exhibited significant homology to the *dnaE*(*G*) genes of enterobacteria, *Buchnera aphidicola*, *Rickettsia prowazekii*, and *B. subtilis* (Table 1), encoding primases, and was designated accordingly. On the basis of amino acid sequence comparison of DNA primases from other organisms, we propose that *C. acetobutylicum dnaE* encodes a protein with a calculated mass of 69 kDa. All known DNA primases are 50 to 60% homologous to each other, as judged by pairwise alignment, except for the enterobacterial primases, which exhibit 86% identity to each other. By means of sequence comparison, the second open reading frame was identified as the *sigA* homologous gene of *C. acetobutylicum*, presumably encoding the primary σ factor (Table 2). The putative clostridial SigA protein is of the smaller, *B. subtilis*-like type with a calculated molecular mass of 43 kDa. The overall similarity as determined by multiple sequence alignment was highest to the major vegetative σ factors of gram-positive bacteria, especially to the respective proteins of *B. subtilis* and *S. aureus* (data not shown). Highest variability between SigA from *C. acetobutylicum* and *B. subtilis* was found among the first 100 amino acids, whereas the sequences of regions 2.4 and 4.2 (30) were identical in both proteins. These two regions are believed to mediate recognition of −10 and −35 regions from cognate promoters (10, 16, 30), and certain amino acids within these regions are known to suppress mutations in specific promoter nucleotides (35). The helix-turn-helix motif of region 4.2 is known to be highly conserved exclusively among the primary σ factors (40).

A putative ribosome-binding site (5′-AGGAGG-3′) is located 8 bp upstream of the *sigA* start codon. However, no such site was detected upstream of *dnaE*, which is in agreement with data reported for *R. prowazekii* (46). Immediately downstream of *sigA* is a 10-bp inverted repeat with the potential to form a stable stem-loop structure (ΔG, −69 kJ/mol) (71) followed by a run of U's, resembling a rho-independent transcriptional terminator (13). Several possible, partly overlapping stem-loop structures are located between the GUG start codon of *dnaE* and the transcription start point, as discussed below.

RNA analysis of the *dnaE-sigA* operon. During batch cultivation of *C. acetobutylicum* DSM 1731 in CAMM medium, several morphological parameters, such as motility and appearance of phase-bright swollen clostridial forms, proposed to be involved in sporulation and solventogenesis (33), were monitored in addition to the product formation pattern (Fig. 2). We conducted Northern blot experiments to investigate a possible operon structure of *dnaE* and *sigA*, as has been shown for many gram-negative bacteria as well as *B. subtilis* (72). Total RNA was isolated from cells harvested at different time points during the CAMM batch culture and from acidogenic and solventogenic continuous cultures of *C. acetobutylicum* DSM 1731. Three signals (3.0, 2.2, and 1.2 kb) were observed with a PCR-generated radiolabeled DNA probe, complementary to region 2 of the clostridial *sigA* gene (positions 2917 to 3192) (Fig. 3). In continuous culture under acidogenic (Fig. 3) as well as solventogenic conditions (data not shown), only the 3.0-kb transcript was detected. With RNA from *E. coli* containing pSA21, a slightly smaller transcript of approximately 2.8 kb was detected, suggesting that the clostridial *dnaE-sigA* operon is transcribed from a different promoter in *E. coli*. RNA harvested from overnight cultures of *C. acetobutylicum* prior and after a heat shock (30 to 42°C for 10 min) showed identical hybridization signals with the *sigA* gene probe (Fig. 3).

A transcription start point of the *dnaE-sigA* operon was determined by primer extension analysis, using oligonucleotides σ4336 (5′-ATAAGACATTCCACCCT-3′) and σ4342 (5′-AACAACTAAATTCCCAT-3′), complementary to the 5′ region of *dnaE* (Fig. 4). A single transcriptional start site, obtained with both oligonucleotides, was mapped to position 438, 182 bp upstream of the *dnaE* start codon (Fig. 1). Additional signals appeared with both oligonucleotides, representing most likely nonspecific break-off products of the reverse transcriptase reaction, since they differed in size. Preceding the mapped transcription start point, a sequence (5′-AAGGATTTTA-12 bp-GAATTAA-4 bp-transcription start point-3′) with significant homology to the σ^H-dependent consensus promoter of *B. subtilis* (68), showing only one mismatch, was identified. Known *C. acetobutylicum* promoter sequences (21, 23, 61, 73) could not be detected within the appropriate distance. RNA from batch culture cells yielded weaker signals with fewer nonspecific bands, possibly because only 3 μg of RNA was applied, as opposed to 10 μg from continuous culture cells. The 17-bp prolonged RNA transcript from the 46-h batch culture cells, obtained with oligonucleotide σ4342, is most likely an artifact, since it was detected in only one case. No signals were obtained with RNA isolated from batch culture cells older than 60 h. Attempts to identify an internal transcription start point upstream of *sigA* by primer extension,

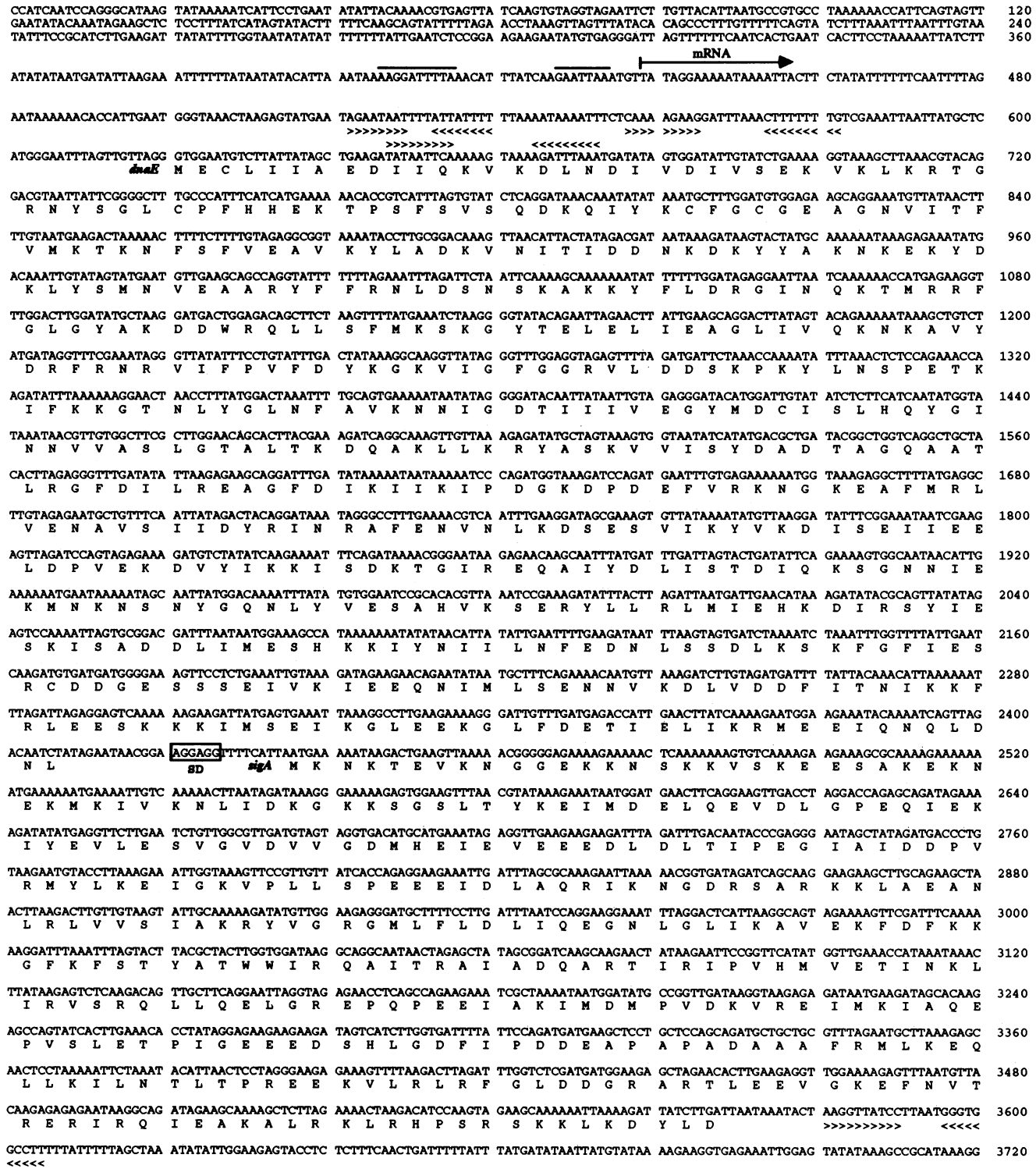

FIG. 1. Nucleotide sequence of the *dnaE-sigA* operon region. The deduced amino acid sequences for both genes are shown below the DNA sequence. The putative ribosome-binding (Shine-Dalgarno [SD]) site is boxed, and potential stem-loop structures are marked by arrowheads below the sequence. The start point of the transcript as identified by primer extension analysis is marked by an horizontal arrow. Putative promoter regions are overlined.

TABLE 1. Amino acid similarity of DnaE from *C. acetobutylicum* to corresponding proteins of other bacteria[a]

Organism	Protein	Identity (%)	Similarity (%)	Reference
Escherichia coli	DnaG	33	56	9
Salmonella typhimurium	DnaG	31	54	19
Rickettsia prowazekii	DnaG	29	53	46
Buchnera aphidicola	DnaG	24	51	38
Bacillus subtilis	DnaE	30	55	74

[a] Similarity data were derived from pairwise alignments with the complete amino acid sequence of DnaE from *C. acetobutylicum*. The symbol comparison table of Gribskov and Burgess (25) was used to calculate percentage identity and similarity.

which could give rise to the 1.2-kb transcript, failed. Even though the clostridial *dnaE-sigA* operon is highly expressed in *E. coli* (Fig. 3), we were unable to obtain primer extension signals with the above-mentioned oligonucleotides and RNA from the respective *E. coli* clone. This fact further strengthens the implication that a different promoter is utilized for expression of the cloned operon in *E. coli*.

Cloning and sequencing of the *sigE* and *sigG* genes from *C. acetobutylicum*. Degenerate oligonucleotides, complementary to region 2 of the *B. subtilis* sporulation σ factor E (30, 40), were employed as primers in a PCR amplification using genomic DNA of *C. acetobutylicum* DSM 792 as a template. A 235-bp DNA fragment was amplified and subsequently radiolabeled for screening a *Hin*dIII partial gene bank of *C. acetobutylicum* DSM 792. The nucleotide sequence of a single positive clone (pSE1) with a 3,450-bp *Hin*dIII insert was determined, revealing six open reading frames (Fig. 5), the first of which was truncated and showed significant homology to σ^E of *Bacillus* spp. A probe consisting of the first 159 bp of pSE1 was then used to screen two different *Sau*3AI partial genomic libraries of *C. acetobutylicum* (21, 22). In the second library, a positive clone (pT22) with a 2,416-bp *Sau*3AI insert could be detected. Its nucleotide sequence was determined and found to encode three open reading frames (Fig. 5). Computer-assisted sequence comparison for the putative products of the first three open reading frames of the complete DNA region showed high similarity to the *Bacillus* spp. sporulation-specific protease SpoIIGA and the sporulation σ factors E and G (Table 3). Therefore, these genes were classified as the *C. acetobutylicum* homologous genes and designated *spoIIGA*, *sigE*, and *sigG*, accordingly. The respective DNA region and

TABLE 2. Amino acid similarity of SigA from *C. acetobutylicum* to selected corresponding proteins of other bacteria[a]

Organism	Protein	Identity (%)	Similarity (%)	Reference
Bacillus subtilis	RpoD	69	83	24
Staphylococcus aureus	PlaC	69	81	5
Salmonella typhimurium	SigA	58	76	19
Escherichia coli	RpoD	58	76	8
Streptomyces coelicolor	HrdB	54	68	62
Chlamydia trachomatis	SigA	53	72	18
Streptomyces coelicolor	HrdA	48	68	67
	HrdC	47	64	67
	HrdD	45	67	67

[a] Similarity data were derived from pairwise alignments with the complete amino acid sequence of SigA from *C. acetobutylicum*. The symbol comparison table of Gribskov and Burgess (25) was used to calculate percentage identity and similarity.

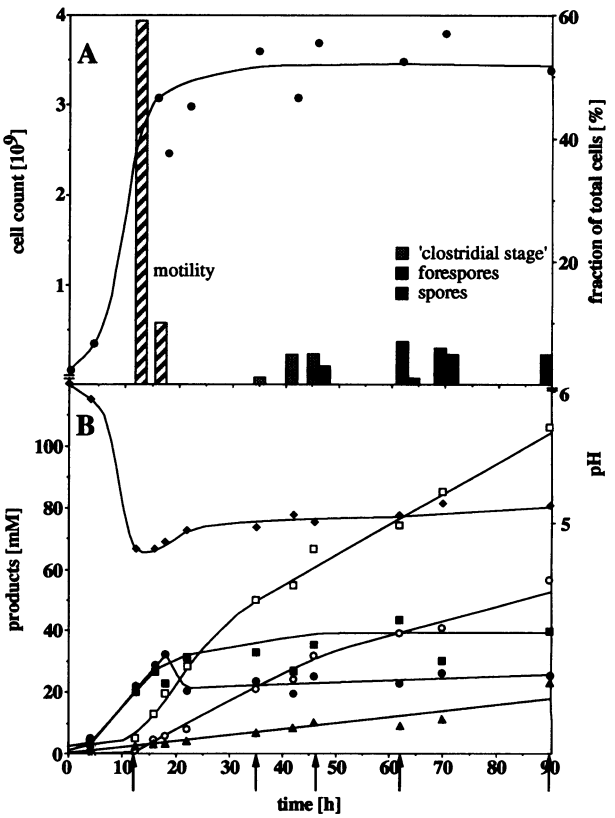

FIG. 2. Time course of cell count and the occurrence of motility, forespores, and spores (A) and pattern of product formation and pH (B) during a batch cultivation of *C. acetobutylicum* DSM 1731 in CAMM medium. (A) ●, cell count. (B) ■, acetate; ○, acetone; □, butanol; ●, butyrate; ▲, ethanol; ◆, pH. Arrows indicate the time points of cell harvest for subsequent RNA isolation.

the deduced amino acid sequence is presented in Fig. 6. On the basis of homology to σ^G from *Bacillus* spp., the translation of the clostridial *sigG* starts at a GTG codon. The conservation of methionine residues in the sequence context of the α-helical segment in region 2.4 is striking, as this is known to suppress mutations in the promoter −13 and −12 positions of *B. subtilis* (15). A possible stem-loop structure (Δ*G*, −30 kJ/mol [71]) without the features of a prokaryotic rho-independent transcriptional terminator (13) was identified between *sigE* and *sigG*. Overlapping this possible secondary structure is an identifiable promoter sequence (5′-AGTATA-15 bp-GGAATAAT-3′) with reasonable homology to the σ^F consensus sequence (66), which is known to control expression of *sigG* in *B. subtilis* (20). Ribosome-binding sites could be identified upstream of the *spoIIGA* (5′-AGGTGG-3′), *sigE* (5′-GGGGGG-3′), and *sigG* (5′-AGGGGC-3′) genes (Fig. 6). The putative transcript of *sigG* has a potential of forming a strong stem-loop structure (Δ*G*, −82.4 kJ/mol [71]), again lacking typical features of a rho-independent terminator. The deduced amino acid sequence of the *spoIIGA* gene product showed considerably lower homology to its *Bacillus* analog than the σ factors (Table 3). However, the hydropathy profile of the *B. subtilis* SpoIIGA (63) was almost identical to that of the clostridial SpoIIGA (data not shown).

The deduced amino acid sequence derived from open reading frame V exhibited significant similarity (46 to 59% identity) to three hypothetical bacterial proteins of equal size

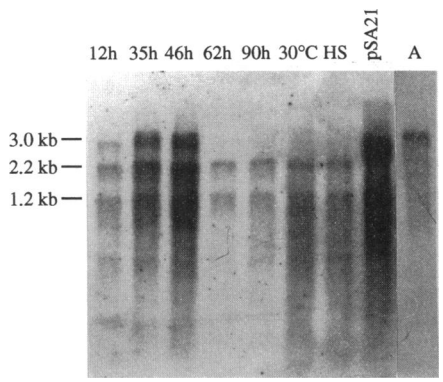

FIG. 3. Northern hybridization with the radioactively labeled PCR-generated fragment complementary to *sigA*. The lanes marked 12h to 90h contained 10 μg each of total RNA isolated from *C. acetobutylicum* DSM 1731 at different time points during a batch cultivation in CAMM medium (Fig. 2). Total RNA (10 μg) for the next two lanes was isolated from strain DSM 792 prior (30°C) and after (HS) a heat shock from 30 to 42°C for 10 min in batch culture. The lane marked pSA21 contained 10 μg of total RNA isolated from the *E. coli* clone with plasmid pSA21. In lane A, 7 μg of total RNA of an acidogenic continuous culture of *C. acetobutylicum* DSM 1731 was applied. Transcript sizes, as judged by comparison with size markers, are indicated at the left.

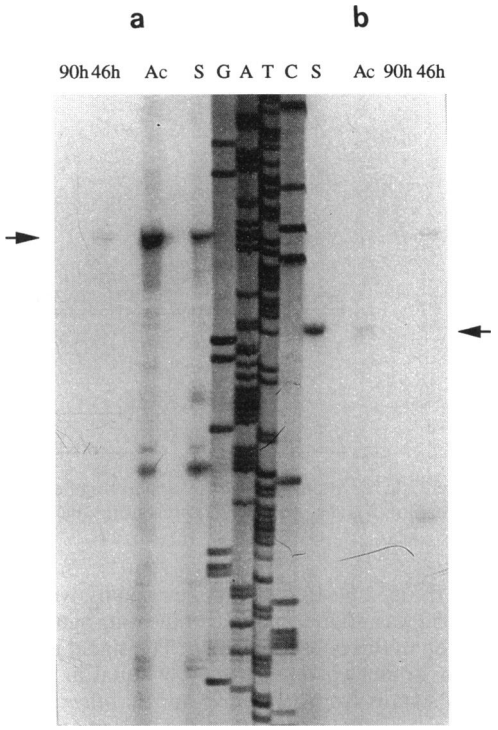

FIG. 4. Identification of the 5′ end of the *C. acetobutylicum dnaE-sigA* operon by primer extension analysis. Oligonucleotides σ4336 (a) and σ4342 (b), complementary to the 5′ region of *dnaE*, were annealed to 10 μg of total RNA from acidogenic (lanes Ac) and solventogenic (lanes S) continuous culture and 3 μg of batch culture (Fig. 2) *C. acetobutylicum* DSM 1731 cells. The primer-extended products were coelectrophoresed with a sequencing ladder generated with σ4336 as the primer. The arrows on each side indicate the positions of the products obtained with both primers.

with unknown function (Fig. 7). Particularly striking is a run of four arginines at the 5′ end, found in the putative products of all of these completely sequenced genes. The respective open reading frames have been found in all cases in the vicinity of genes involved in DNA-RNA-protein interaction: *nusB* gene region of *E. coli* (antitermination factor) (69), *dnaB* gene of *B. subtilis* (involved in replication) (32), 16S rRNA region of *Thermus thermophilus* (translation) (29), and *sigE-sigG* gene region of *C. acetobutylicum* (regulation of sporulation). The conserved sequence of these proteins among phylogenetically distinct bacteria indicates an important and similar function within the eubacteria. No significant homologies were detected for any of the other open reading frames encoded in the sequenced DNA region.

Cloning and sequencing of the *sigK* gene from *C. acetobutylicum*. A 159-bp PCR-generated DNA fragment (positions 971 to 1129; Fig. 6) was originally designed for detection of *sigE* in a partially *Sau*3AI-digested genomic library of *C. acetobutylicum* DSM 792 in vector pEcoR252 (21). A positive clone (pT4) that carried an approximately 4,350-bp insert was identified. However, sequencing of the first 900 bp of this insert revealed that pT4 did not contain the *sigE* gene but contained a gene homologous to σ^K of *Bacillus* spp. Only the 3′ end of the respective open reading frame was encoded on the *Sau*3AI fragment. A 372-bp probe (positions 276 to 647; Fig. 8) was then used to screen a different, partially *Sau*3AI-digested genomic library of *C. acetobutylicum* DSM 792 in pUC9 (22). A positive clone (pT41) that carried an approximately 7-kbp insert was found. The nucleotide sequence (960 bp) of the DNA region encoding the *sigK* gene on this insert was determined, and computer-assisted analysis revealed an open reading frame encoding the putative σ factor K (Fig. 8). On the basis of homology to σ^K from *Bacillus* spp., the translation of the clostridial *sigK* starts at a GTG codon. A ribosome-binding site matching the consensus sequence (5′-AGGAGG-3′) was identified 8 to 13 bp upstream of *sigK* (Fig. 8). The deduced amino acid sequence similarity between σ^K from *C. acetobutylicum*, *B. subtilis*, and *B. thuringiensis* is shown in Table 3. The similarity was slightly higher to the respective protein of *B. thuringiensis*, as in the case of σ^G. As in *B. thuringiensis* (1), the *sigK* gene of *C. acetobutylicum* was not interrupted by insertion of an unrelated DNA segment as known for *sigK* of *B. subtilis* (65). Two possible stem-loop structures, both lacking homology to rho-independent transcriptional terminators, were detected 19 and 108 bp downstream of the stop codon (13).

Southern blot analysis of σ factor genes in various *C. acetobutylicum* strains. Earlier reports showed major differences between *C. acetobutylicum* strains (61, 76, 80). Therefore, possible sequence variations of σ factor genes among these strains were checked by Southern hybridizations. The *sigA* probe used was complementary to parts of region 2 (positions 2917 to 3192; Fig. 1). The probe for *sigE* detection was identical to the PCR-generated fragment used for screening of the *Hin*dIII genomic library (positions 1049 to 1267; Fig. 6). Both probes were obtained by PCR amplification with genomic DNA of strain DSM 792 as the template. With the *sigE*-specific probe, fragments of identical size were detected in strains DSM 792 and ATCC 824, whereas genomic DNA of P262 and NCIMB 8052 gave only very weak signals of different size, possibly due to unspecific hybridization (data not shown). Essentially the same result was obtained with the *sigA*-specific probe. Southern hybridization revealed only a single signal, indistinguishable in size and intensity, with genomic DNA of strains ATCC 824, DSM 792, and DSM 1731, suggesting that *sigA* is the only gene encoding a primary σ factor. However,

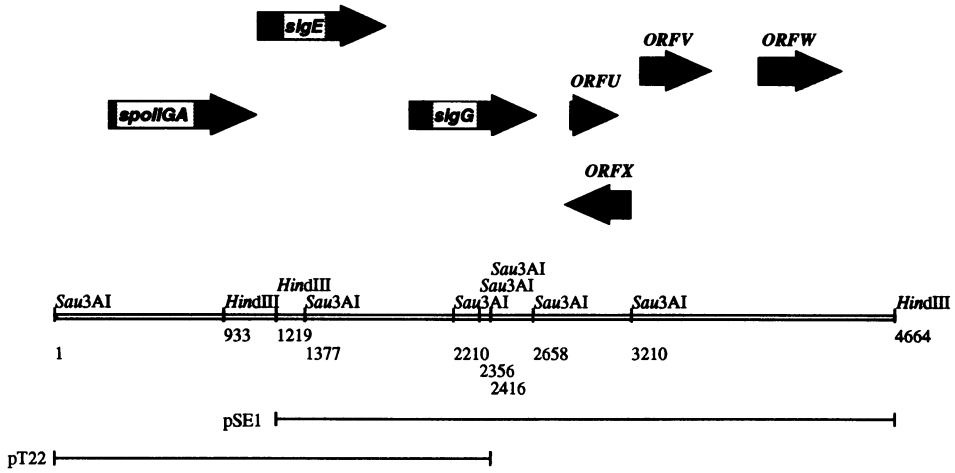

FIG. 5. Schematic representation of the cloned fragment in plasmids pSE1 and pT22. Arrows indicate directions of transcription, positions, and sizes of open reading frames. Numbers representing the nucleotide positions and restriction endonuclease sites are marked below the bar.

P262 and NCIB 8052 gave much weaker signals, which in case of NCIMB 8052 was of different size, indicating again a genetic difference, even in this conserved gene. The signal intensity for the latter two strains was approximately equal in intensity to control DNA signals from *B. subtilis* and *C. butyricum*. These results are in good agreement with earlier observations (61, 76, 80), providing strong evidence for a specific grouping of different *C. acetobutylicum* strains.

In addition to the above-mentioned σ factors, we looked for homologous genes to the *B. subtilis* σ factors H and D. Applying the approach of Engel and Ganem (18), we were unable to obtain specific PCR fragments with clostridial DNA and degenerate primers (see Materials and Methods). However, when genomic DNA of *B. subtilis* was used, PCR fragments of the expected size were obtained, subsequently radiolabeled, and employed for Southern hybridization. In both cases, single signals were obtained in the hybridization with chromosomal DNA of *C. acetobutylicum* DSM 792. The size of these signals was distinct from those of the other σ factors, thus indicating the presence of σ^H and σ^D homologous genes in *C. acetobutylicum*.

DISCUSSION

This report presents the first analysis on σ factor genes in the gram-positive genus *Clostridium*. The operon encoding the putative principal σ factor and primase of *C. acetobutylicum* was cloned and sequenced. The *dnaE* gene is specified by an 1.8-kbp open reading frame, whose putative product is similar to other primases, and we therefore conclude that the cloned gene encodes the primase of *C. acetobutylicum*. The putative product of the *sigA* gene exhibits significant homology to primary σ factors of other bacteria and is of the smaller, gram-positive type. Amino acid sequence identity of the α helix in region 2.4 and the helix-turn-helix motif in region 4.2 between *C. acetobutylicum* and *B. subtilis*, believed to mediate recognition of −10 and −35 regions from cognate promoters (10, 30), provides strong evidence for identical −10 and −35 consensus promoter regions in both organisms, as was suggested earlier by sequence comparison of promoter regions (21, 23, 59, 61, 73). The predicted molecular mass for the clostridial σ factor A of 43 kDa is in good agreement with the value of 46 kDa calculated from the migration of the protein in SDS-polyacrylamide gels (54).

On the basis of Northern blot analysis, the two genes were shown to form an operon, which, however, is less complex than the analogous operons of gram-negative bacteria and *B. subtilis* (72), since it comprises only two genes. In contrast to its counterpart in *E. coli* (70), our experiments could not demonstrate any increase of *sigA*-specific mRNA under conditions of heat shock. However, no 3.0-kbp transcript was detected in this case; therefore, the presence of a heat-inducible promoter cannot be completely ruled out. The transcriptional start point of the *dnaE-sigA* operon was mapped 182 bp upstream of the *dnaE* start codon. This site was not preceded by a typical σ^A-dependent promoter structure; thus, a different promoter must have been utilized by *E. coli* for transcription of the cloned operon. However, we cannot rule out the possibility that a closer transcription start point exists, since the primers used were complementary to the sequence directly upstream of *dnaE*, including the first 14 nucleotides of the structural gene. Transcription of this operon may be regulated during different growth phases in *C. acetobutylicum*. In batch culture, the mRNA transcript comprising both genes was observed only in

TABLE 3. Amino acid similarity of SpoIIGA, SigE, SigG, and SigK from *C. acetobutylicum* to corresponding proteins of bacilli[a]

Organism	SpoIIGA		SigE		SigG		SigK		Reference(s)
	Identity (%)	Similarity (%)	Identity (%)	Similarity (%)	Identity (%)	Similarity (%)	Identity (%)	Similarity (%)	
B. subtilis	23	52	72	86	74	88	57	76	48, 63–65
B. thuringiensis	24	51	69	84	80	88	58	77	1

[a] Similarity data were derived from pairwise alignments of the respective amino acid sequences. The symbol comparison table of Gribskov and Burgess (25) was used to calculate percentage identity and similarity.

FIG. 6. Nucleotide sequence of the *spoIIGA-sigE-sigG* DNA region. The predicted amino acid sequences for the three genes are shown below the DNA sequence. Potential stem-loop structures are marked by arrowheads below the sequence. The sequence marked by the bars above the nucleotides exhibits significant homology to σ^F-dependent promoters of *Bacillus* spp. Putative ribosome-binding (Shine-Dalgarno [SD]) sites are boxed.

exponential and early postexponential growth phases, while two smaller transcripts, possibly encoding *sigA* only, were detected throughout all growth phases. In continuous cultures, however, the longer transcript was exclusively detected. This could indicate a permanent expression of *sigA*, while transcription of the *dnaE* gene, specifying the replication protein primase, would occur only during growth phases that are characterized by cell division. On the other hand, we cannot exclude the possibility that the two shorter transcripts are the result of less specific hybridization, since no respective transcription start points could be detected in the primer extension experiments at the time points when only these transcripts were found. The lack of a suitable ribosome-binding site preceding *dnaE* might be a regulatory feature in control of primase protein levels, as was also suggested for *E. coli* (45). A transcript of the *dnaE-sigA* operon has the potential of forming several stem-loop structures in its 5′ noncoding region, which might serve three possible functions, (i) stabilization of the 3.0-kbp transcript, (ii) binding site for endoribonuclease attack, or (iii) inhibition of RNA polymerase read-through, and thus serve as a means of regulation. The identification of a σ^H-dependent promoter upstream of *dnaE* in *C. acetobutylicum* reveals similarities to the transcriptional regulation of the analogous operon in *B. subtilis*, in which three σ^H-dependent promoters in addition to two σ^A-dependent promoters have been shown to participate in initiation of transcription (11, 55, 56, 75). The Southern hybridization data for *sigA*, its sequence homology to other primary σ factors, and the similar operon structure support the hypothesis that *sigA* encodes the major vegetative σ factor of *C. acetobutylicum*.

In *B. subtilis*, four sporulation-specific σ factors play crucial regulatory roles within this developmental process. The two early sigma factors σ^E and σ^F determine the establishment of a distinct cell-specific program of gene expression, whereas σ^G and σ^K are synthesized at later stages in the prespore compartment and the mother cell, respectively (20, 44). In this paper, we reported the cloning and sequencing of homologous genes for three of these σ factors from *C. acetobutylicum*. The

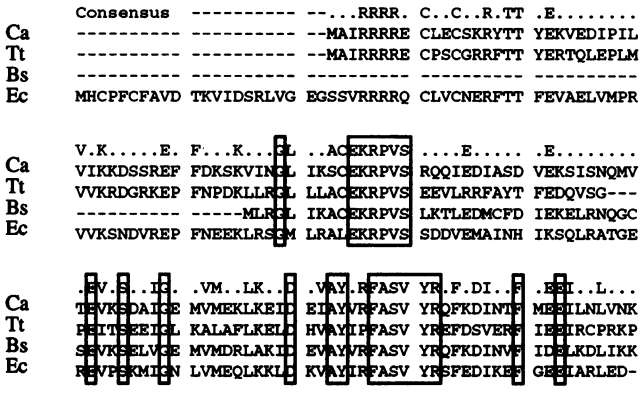

FIG. 7. Amino acid sequence alignment of the putative open reading frame V gene product of *C. acetobutylicum* (Ca) with putative proteins encoded in the following DNA regions: Tt, *T. thermophilus* 16S rRNA region (29); Bs, *B. subtilis dnaB* region (partly sequenced) (32); Ec, *E. coli nusB* region (69). Identical amino acids in all four proteins are boxed. A consensus sequence is given above the alignment if the respective amino acid occurs in at least three proteins.

identification of a DNA sequence upstream of *sigG*, with homology to σ^F-specific consensus promoters of *Bacillus* spp. (66), provides indirect evidence for the existence of the fourth σ factor in *C. acetobutylicum*, σ^F. The sequential arrangement of the genes encoding SpoIIGA, σ^E, and σ^G is conserved between *Bacillus* spp. and *C. acetobutylicum*, with the interesting feature that the former σ factor is active only in the mother cell during early sporulation, while the latter is synthesized specifically in the prespore compartment at later stages (20, 44). The conservation of this arrangement might indicate a yet unknown selective advantage. The *sigE* gene of *C. acetobutylicum* is the first gene known to be terminated by an UGA stop codon in this organism. Another sequenced sporulation-specific gene of *C. acetobutylicum*, *spoIID* (28), known to be under transcriptional control of σ^E in *B. subtilis*, is preceded by a sequence with homology to the *B. subtilis* consensus sequence for σ^E-dependent promoters (15). This fact together with the conservation of region 2.4, presumed to specify −10 promoter region binding (15), provides strong evidence for the conclusion that similar promoters are employed in the two organisms. Furthermore, the promoter recognition regions (2.4 and 4.2) in the SigE, SigG, and SigK sequences are 100% conserved compared with *B. subtilis*. The gene encoding the mother cell-specific σ^K analog of *C. acetobutylicum* is specified by a single undisrupted open reading frame, as in *B. thuringiensis* (1). This observation confirms the demonstration that the chromosomal rearrangement of *sigK* in *B. subtilis* is not an essential feature for sporulation (37). However, the 5-bp recombination site sequence (5′-AATGA-3′) (65) is 100% conserved. On the basis of Northern blot analysis, we were unable to demonstrate differential expression of the sequenced σ factor homologous genes, which might be due to cross-hybridization of these conserved genes with the probes used. Furthermore, only a low sporulation efficiency could be achieved in CAMM medium.

The data presented here provide strong evidence that many of the complex regulation features of sporulation in *B. subtilis* seem to be conserved in *C. acetobutylicum* and possibly *Clostridium* spp., despite different induction patterns of sporulation in *Bacillus* spp. (nutrient starvation) (20) and *C. acetobutylicum* (excess nutrients required; induction of solvent production is essential) (42). Thus, regulation of sporulation in these two genera seems to differ from the mycelial, gram-positive genus *Streptomyces*, in which a σ factor with significant homology to the motility σ factor D of *B. subtilis* is required for early sporulation (12).

A number of investigators have previously suggested common features in regulation of solvent formation and sporulation in *C. acetobutylicum* (33, 41–43, 49, 57). These two processes are not inextricably linked, though their mechanisms of induction do have features in common, as indicated by the loss of spore-forming potential and the loss of solvent formation ability in some mutants (33, 42, 43, 57). Furthermore, motility (highest motility is observed during acidogenesis and declines with the onset of solventogenesis) and solventogenesis appear to be linked to some extent (26, 27, 53). The findings reported here, that hybridization signals were obtained in strain DSM 792 with a probe complementary to the motility σ factor D of *B. subtilis*, indicate the presence of such a σ factor in *C. acetobutylicum*. Currently, only vague speculations are possible about the regulatory network controlling motility, solventogenesis, and sporulation. However, the data presented here suggest that at least sporulation and possibly motility are regulated by alternative σ factors. Solventogenic genes are most likely not regulated by a similar mechanism since their promoters have considerable sequence homology with the σ^A-dependent promoters (21, 23, 59, 73). Previously reported

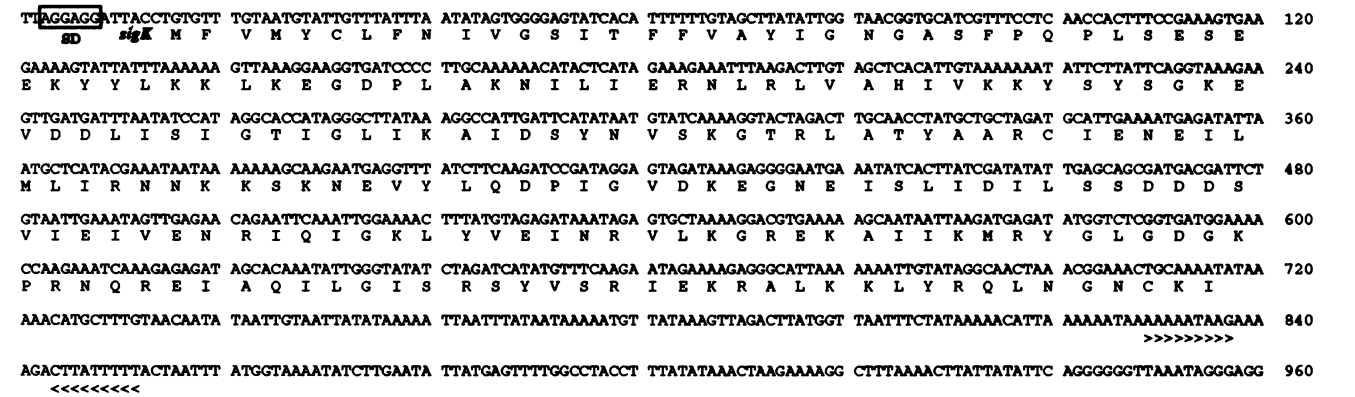

FIG. 8. Nucleotide sequence of the *sigK* DNA region. The predicted amino acid sequence for the gene is shown below the DNA sequence. Potential stem-loop structures are marked by arrowheads below the sequence. A putative ribosome-binding (Shine-Dalgarno [SD]) site is boxed.

data indicated that $tRNA^{Thr}_{ACG}$ may play a role in the translational control of several inducible *C. acetobutylicum* metabolic networks (60). However, the nucleotide sequences of the *dnaE* and *sigA* genes contain three and one ACG codon, respectively. It is highly probable that the *dnaE* and *sigA* genes are constitutively expressed in *C. acetobutylicum*, thus rendering the foregoing translational control hypothesis less likely. Work currently performed in several laboratories is aimed at elucidating the molecular events leading to initiation of solventogenesis and sporulation.

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REFERENCES

1. **Adams, L. F., K. L. Brown, and H. R. Whiteley.** 1991. Molecular cloning and characterization of two genes encoding sigma factors that direct transcription from a *Bacillus thuringiensis* crystal protein gene promoter. J. Bacteriol. **173**:3846–3854.
2. **Bahl, H., W. Andersch, and G. Gottschalk.** 1982. Continuous production of acetone and butanol by *Clostridium acetobutylicum* in a two-stage phosphate limited chemostat. Eur. J. Appl. Microbiol. Biotechnol. **15**:201–205. (Erratum, **17**:73, 1983.)
3. **Bahl, H., and P. Dürre.** 1993. Clostridia, p. 285–323. *In* H. Sahm (ed.), Biotechnology, 2nd ed., vol. 1. VCH Verlagsgesellschaft mbH, Weinheim, Germany.
4. **Bahl, H., and G. Gottschalk.** 1988. Microbial production of butanol/acetone, p. 1–30. *In* H. J. Rehm and G. Reed (ed.), Biotechnology, vol. 6b. VCH Verlagsgesellschaft mbH, Weinheim, Germany.
5. **Basheer, R., and S. Iordanescu.** 1991. The *Staphylococcus aureus* chromosomal gene *plaC*, identified by mutations amplifying plasmid pT181, encodes a sigma factor. Nucleic Acids Res. **19**:4921–4924.
6. **Bertram, J., and P. Dürre.** 1989. Conjugal transfer and expression of streptococcal transposons in *Clostridium acetobutylicum*. Arch. Microbiol. **151**:551–557.
7. **Buluwela, L., A. Forster, T. Boehm, and T. H. Rabbitts.** 1989. A rapid procedure for colony screening using nylon filters. Nucleic Acids Res. **17**:452.
8. **Burton, Z., R. R. Burgess, J. Lin, D. Moore, S. Holder, and C. A. Gross.** 1981. The nucleotide sequence of the cloned *rpoD* gene for the RNA polymerase sigma subunit from *E. coli* K 12. Nucleic Acids Res. **9**:2889–2903.
9. **Burton, Z. F., C. A. Gross, K. K. Watanabe, and R. R. Burgess.** 1983. The operon that encodes the sigma subunit of RNA polymerase also encodes ribosomal protein S21 and DNA primase in *E. coli* K12. Cell **32**:335–349.
10. **Chang, B.-Y., and R. H. Doi.** 1993. Effects of amino acid substitutions in the promoter −10 binding region of the σ^A factor on growth of *Bacillus subtilis*. J. Bacteriol. **175**:2470–2474.
11. **Carter, H. L., III, L.-F. Wang, R. H. Doi, and C. P. Moran, Jr.** 1988. *rpoD* operon promoter used by σ^H-RNA polymerase in *Bacillus subtilis*. J. Bacteriol. **170**:1617–1621.
12. **Chater, K., C. J. Bruton, K. A. Plaskitt, M. J. Buttner, C. Méndez, and J. D. Helmann.** 1989. The developmental fate of *S. coelicolor* hyphae depends upon a gene product homologous with the motility σ factor of *B. subtilis*. Cell **59**:133–143.
13. **d'Aubentor-Carafa, Y., E. Brody, and C. Thermes.** 1990. Prediction of rho-independent *Escherichia coli* transcription terminators. A statistical analysis of their RNA stem-loop structures. J. Mol. Biol. **216**:835–858.
14. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
15. **Diederich, B., K. M. Tatti, C. H. Jones, B. Beall, and C. P. Moran, Jr.** 1992. Genetic suppression analysis of σ^E interaction with three promoters in sporulating *Bacillus subtilis*. Gene **121**:63–69.
16. **Dombroski, A. J., W. A. Walter, M. T. Record, Jr., D. A. Siegele, and C. A. Gross.** 1992. Polypeptides containing highly conserved regions of transcription initiation factor σ^{70} exhibit specificity of binding to promoter DNA. Cell **70**:501–512.
17. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16**:6127–6145.
18. **Engel, J. N., and D. Ganem.** 1990. A polymerase chain reaction-based approach to cloning sigma factors from eubacteria and its application to the isolation of a sigma-70 homolog from *Chlamydia trachomatis*. J. Bacteriol. **172**:2447–2455.
19. **Erickson, B. D., Z. F. Burton, K. K. Watanabe, and R. R. Burgess.** 1985. Nucleotide sequence of the *rpsU-dnaG-rpoD* operon from *Salmonella typhimurium* and a comparison of this sequence with the homologous operon of *Escherichia coli*. Gene **40**:67–78.
20. **Errington, J.** 1993. *Bacillus subtilis* sporulation: regulation of gene expression and control of morphogenesis. Microbiol. Rev. **57**:1–33.
21. **Fischer, 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.
22. **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.
23. **Gerischer, U., and P. Dürre.** 1992. mRNA analysis of the *adc* gene region of *Clostridium acetobutylicum* during the shift to solventogenesis. J. Bacteriol. **174**:426–433.
24. **Gitt, M. A., L.-F. Wang, and R. H. Doi.** 1985. A strong sequence homology exists between the major RNA polymerase σ factors of *Bacillus subtilis* and *Escherichia coli*. J. Biol. Chem. **260**:7178–7185.
25. **Gribskov, M., and R. R. Burgess.** 1986. Sigma factors from *E. coli*, *B. subtilis*, phage SP01, and phage T4 are homologous proteins. Nucleic Acids Res. **14**:6745–6763.
26. **Gutierrez, N. A., and I. S. Maddox.** 1987. Role of chemotaxis in solvent production by *Clostridium acetobutylicum*. Appl. Environ. Microbiol. **53**:1924–1927.
27. **Gutierrez, N. A., and I. S. Maddox.** 1990. Isolation and partial characterization of a non-motile mutant of *Clostridium acetobutylicum*. Biotechnol. Lett. **12**:853–856.
28. **Hancock, K. R., S. J. Reid, J. D. Santangelo, and D. R. Woods.** Cloning and sequencing of a gene from *Clostridium acetobutylicum* that shows homology to the *Bacillus* sporulation gene, *spoIID*. Submitted for publication. GenBank accession no. M87835.
29. **Hartmann, R. K., and V. A. Erdmann.** 1989. *Thermus thermophilus* 16S rRNA is transcribed from an isolated transcription unit. J. Bacteriol. **171**:2933–2941.
30. **Helmann, J. D., and M. J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57**:839–872.
31. **Holck, A., H. Blom, and P. E. Granum.** 1990. Cloning and sequencing of the genes encoding acid-soluble spore proteins from *Clostridium perfringens*. Gene **91**:107–111.
32. **Hoshino, T., T. McKenzie, S. Schmidt, T. Tanaka, and N. Sueoka.** 1987. Nucleotide sequence of *Bacillus subtilis dnaB*: a gene essential for DNA replication initiation and membrane attachment. Proc. Natl. Acad. Sci. USA **84**:653–657.
33. **Jones, D. T., A. van der Westhuizen, S. Long, E. R. Allock, S. J. Reid, and D. R. Woods.** 1982. Solvent production and morphological changes in *Clostridium acetobutylicum*. Appl. Environ. Microbiol. **43**:1434–1439.
34. **Jones, D. T., and D. R. Woods.** 1986. Acetone-butanol fermentation revisited. Microbiol. Rev. **50**:484–524.
35. **Kenney, T. J., and C. P. Moran, Jr.** 1991. Genetic evidence for interaction of σ^A with two promoters in *Bacillus subtilis*. J. Bacteriol. **173**:3282–3290.
36. **Kormanec, J., M. Farkasovsky, and L. Potúcková.** 1992. Four genes in *Streptomyces aureofaciens* containing a domain characteristic of principal sigma factors. Gene **122**:63–70.
37. **Kunkel, B., R. Losick, and P. Stragier.** 1990. The *Bacillus subtilis* gene for the developmental transcription factor σ^K is generated by excision of a dispensable DNA element containing a sporulation

recombinase gene. Genes Dev. **4**:525–535.
38. **Lai, C.-Y., and P. Baumann.** 1992. Sequence analysis of a DNA fragment from *Buchnera aphidicola* (an endosymbiont of aphids) containing genes homologous to *dnaG*, *rpoD*, *cysE*, and *secB*. Gene **119**:113–118.
39. **Landuyt, S. L., and E. J. Hsu.** 1992. Preparation of refractile spores of *Clostridium thermosaccharolyticum* involves a solventogenic phase. Appl. Environ. Microbiol. **58**:1797–1800.
40. **Lonetto, M., M. Gribskov, and C. A. Gross.** 1992. The σ^{70} family: sequence conservation and evolutionary relationships. J. Bacteriol. **174**:3843–3849.
41. **Long, S., D. T. Jones, and D. R. Woods.** 1983. Sporulation of *Clostridium acetobutylicum* P262 in a defined medium. Appl. Environ. Microbiol. **45**:1389–1393.
42. **Long, S., D. T. Jones, and D. R. Woods.** 1984. Initiation of solvent production, clostridial stage and endospore formation in *Clostridium acetobutylicum* P262. Appl. Microbiol. Biotechnol. **20**:256–261.
43. **Long, S., D. T. Jones, and D. R. Woods.** 1984. The relationship between sporulation and solvent production in *Clostridium acetobutylicum* P262. Biotechnol. Lett. **6**:529–534.
44. **Losick, R., and P. Stragier.** 1992. Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis*. Nature (London) **355**:601–604.
45. **Lupski, J. R., B. L. Smiley, and G. N. Godson.** 1983. Regulation of the *rpsU-dnaG-rpoD* macromolecular synthesis operon and the initiation of DNA replication in *Escherichia coli* K-12. Mol. Gen. Genet. **189**:48–57.
46. **Marks, G. L., and D. O. Wood.** 1993. Characterization of the gene coding for the *Rickettsia prowazekii* DNA primase analogue. Gene **123**:121–125.
47. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**:208–218.
48. **Masuda, E. S., H. Anaguchi, K. Yamada, and Y. Kobayashi.** 1988. Two developmental genes encoding σ factor homologs are arranged in tandem in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **85**:7637–7641.
49. **Meinecke, B., H. Bahl, and G. Gottschalk.** 1984. Selection of an asporogenous strain of *Clostridium acetobutylicum* in continuous culture under phosphate limitation. Appl. Environ. Microbiol. **48**:1064–1065.
50. **Moran, C. P., Jr.** 1993. RNA polymerase and transcription factors, p. 653–667. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics. American Society for Microbiology, Washington, D.C.
51. **O'Brien, R. W., and J. G. Morris.** 1971. Oxygen and the growth and metabolism of *Clostridium acetobutylicum*. J. Gen. Microbiol. **68**:307–318.
52. **Oelmüller, U., N. Krüger, A. Steinbüchel, and C. G. Friedrich.** 1990. Isolation of prokaryotic RNA and detection of specific mRNA with biotinylated probes. J. Microbiol. Methods **11**:73–84.
53. **Petersen, D. J., and G. N. Bennett.** 1991. Enzymatic characterization of a nonmotile, nonsolventogenic *Clostridium acetobutylicum* ATCC 824 mutant. Curr. Microbiol. **23**:253–258.
54. **Pich, A., and H. Bahl.** 1991. Purification and characterization of the DNA-dependent RNA polymerase from *Clostridium acetobutylicum*. J. Bacteriol. **173**:2120–2124.
55. **Qi, F.-X., and R. H. Doi.** 1990. Localization of a second SigH promoter in the *Bacillus subtilis sigA* operon and regulation of *dnaE* expression by the promoter. J. Bacteriol. **172**:5631–5636.
56. **Qi, F.-X., X.-S. He, and R. H. Doi.** 1991. Localization of a new promoter, P5, in the *sigA* operon of *Bacillus subtilis* and its regulation in some *spo* mutant strains. J. Bacteriol. **173**:7050–7054.
57. **Rogers, P., and N. Palosaari.** 1987. *Clostridium acetobutylicum* mutants that produce butyraldehyde and altered quantities of solvents. Appl. Environ. Microbiol. **53**:2761–2766.
58. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
59. **Sauer, U.** 1992. Ph. D. thesis. University of Göttingen, Göttingen, Germany.
60. **Sauer, U., and P. Dürre.** 1992. Possible function of $tRNA^{Thr}_{ACG}$ in regulation of solvent formation in *Clostridium acetobutylicum*. FEMS Microbiol. Lett. **100**:147–154.
61. **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.
62. **Shiina, T., K. Tanaka, and H. Takahashi.** 1991. Sequence of *hrdB*, an essential gene encoding sigma-like transcription factor of *Streptomyces coelicolor* A3(2): homology to principal sigma factors. Gene **107**:145–148.
63. **Stragier, P., C. Bonamy, and C. Karmazyn-Campelli.** 1988. Processing of a sporulation sigma factor in *Bacillus subtilis*: how morphological structure could control gene expression. Cell **52**:697–704.
64. **Stragier, P., J. Bouvier, C. Bonamy, and J. Szulmajster.** 1984. A developmental gene product of *Bacillus subtilis* homologous to the sigma factor of *E. coli*. Nature (London) **312**:376–378.
65. **Stragier, P., B. Kunkel, L. Kroos, and R. Losick.** 1989. Chromosomal rearrangement generating a composite gene for a developmental transcription factor. Science **243**:507–512.
66. **Sun, D., P. Fajardo-Cavazos, M. D. Sussman, F. Tovar-Rojo, R.-M. Cabrera-Martinez, and P. Setlow.** 1991. Effect of chromosome location of *Bacillus subtilis* forespore genes on their *spo* gene dependence and transcription by $E\sigma^F$: identification of features of good $E\sigma^F$-dependent promoters. J. Bacteriol. **173**:7867–7874.
67. **Tanaka, K., T. Shiina, and H. Takahashi.** 1991. Nucleotide sequence of genes *hrdA*, *hrdC*, and *hrdD* from *Streptomyces coelicolor* A3(2) having similarity to *rpoD* genes. Mol. Gen. Genet. **229**:334–340.
68. **Tatti, K. M., H. L. Carter III, A. Moir, and C. P. Moran, Jr.** 1989. Sigma H-directed transcription of *citG* in *Bacillus subtilis*. J. Bacteriol. **171**:5928–5932.
69. **Taura, T., C. Ueguchi, K. Shiba, and K. Ito.** 1992. Insertional disruption of the *nusB* (*ssyB*) gene leads to cold-sensitive growth of *Escherichia coli* and suppression of the *secY24* mutation. Mol. Gen. Genet. **234**:429–432.
70. **Taylor, W. E., D. B. Straus, A. D. Grossman, Z. F. Burton, C. A. Gross, and R. R. Burgess.** 1984. Transcription from a heat-inducible promoter causes heat shock regulation of the sigma subunit of *E. coli* RNA polymerase. Cell **38**:371–381.
71. **Tinoco, I., Jr., P. N. Borer, B. Dengler, M. D. Levine, O. C. Uhlenbeck, D. M. Crothers, and J. Gralla.** 1973. Improved estimation of secondary structure in ribonucleic acids. Nature (London) New Biol. **246**:40–41.
72. **Versalovic, J., T. Koeuth, R. Britton, K. Geszvain, and J. R. Lupski.** 1993. Conservation and evolution of the *rpsU-dnaG-rpoD* macromolecular synthesis operon in bacteria. Mol. Microbiol. **8**: 343–355.
73. **Walter, K. A., G. N. Bennett, and E. T. Papoutsakis.** 1992. Molecular characterization of two *Clostridium acetobutylicum* ATCC 824 butanol dehydrogenase isozyme genes. J. Bacteriol. **174**: 7149–7158.
74. **Wang, L.-F., and R. H. Doi.** 1986. Nucleotide sequence and organization of *Bacillus subtilis* RNA polymerase major sigma (σ^{43}) operon. Nucleic Acids Res. **14**:4293–4307.
75. **Wang, L.-F., and R. H. Doi.** 1987. Promoter switching during development and the termination site of the σ^{43} operon of *Bacillus subtilis*. Mol. Gen. Genet. **207**:114–119.
76. **Wilkinson, S. R., and M. Young.** 1993. Wide diversity of genome size among different strains of *Clostridium acetobutylicum*. J. Gen. Microbiol. **139**:1069–1076.
77. **Woods, D. R. (ed.).** 1993. The clostridia and biotechnology. Butterworth-Heinemann, Stoneham, Mass.
78. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
79. **Young, M., and S. T. Cole.** 1993. *Clostridium*, p. 35–52. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics. American Society for Microbiology, Washington, D.C.
80. **Young, M., N. P. Minton, and W. L. Staudenbauer.** 1989. Recent advances in the genetics of the clostridia. FEMS Microbiol. Rev. **63**:301–326.