A Clostridium acetobutylicum regulator gene (regA) affecting amylase production in Bacillus subtilis

Sean P. Davison, Joseph D. Santangelo, Sharon J. Reid and David R. Woods

INTRODUCTION

Clostridium acetobutylicum is a Gram-positive, endospore-forming, obligate anaerobe which produces acetone, butanol and ethanol from a variety of carbohydrate substrates including starch and molasses. In batch culture, C. acetobutylicum produces hydrogen, carbon dioxide, acetate and butyrate during the initial growth phase (acidogenic phase). The onset of solvent production involves a switch in the carbon flow from the acid-producing pathways to the solvent-producing pathways (solventogenic phase), and is accompanied by morphological changes and the appearance of solvent-forming enzymes (Jones et al., 1982). A second major morphological and physiological differentiation step occurs at the end of the solventogenic phase with the initiation of sporulation. Although the physiological conditions required to trigger solventogenesis (Bahl & Gottschalk, 1988; Jones & Woods, 1989; Rogers, 1984) and sporulation (Long et al., 1984a, b) have been investigated, the molecular mechanisms controlling differentiation or substrate utilization remain obscure. C. acetobutylicum grows very well on starch but little is known about the starch utilization system(s) or the regulation of the amylase enzymes. Glucose repression of amylase has been reported in C. acetobutylicum (Annous & Blaschek, 1990).

Plasmid pMET7C was isolated as part of a study on the molecular characterization of genes from C. acetobutylicum involved in the electron-transfer system which are able to activate the drug metronidazole in Escherichia coli (Santangelo et al., 1991). The presence of pMET7C rendered E. coli F19 sensitive to metronidazole. In the work described here, nucleotide sequencing and database analysis of the deduced protein sequence of the region affecting metronidazole sensitivity in E. coli indicated that it contained a regulator gene, regA, which had 40% identity to the ccpA gene of Bacillus subtilis. The regA gene complemented a ccpA mutant of B. subtilis and repressed the expression of a C. acetobutylicum gene capable of degrading starch.

Keywords: Clostridium acetobutylicum, Bacillus subtilis, amylase, metronidazole susceptibility
METHODS

Bacterial strains and growth conditions. The bacterial strains used in this study are listed in Table 1. *C. acetobutylicum* P262 was grown under strict anaerobic conditions in modified *Clostridium* basal medium (CBM) (O'Brien & Morris, 1971) as described by Allcock et al. (1982). *E. coli* and *B. subtilis* were grown aerobically in Luria broth (LB) (Sambrook et al., 1989). Anaerobic growth of *E. coli* was carried out in an anaerobic glove box (Forma Scientific). To support growth of *E. coli* via anaerobic respiration or via fermentation, bacteria were grown respectively in LB supplemented with 0.2% (w/v) NaNO₃ and 0.1% (w/v) glucose, or in the complex medium (pH 8.0) described by Clark (1982), basal medium (CBM) (O'Brien et al., 1983), or glucose, or in the complex medium (pH 8.0) described by Clark & Cronan (1980) supplemented with 1% (w/v) glucose.

Plasmid pMET7C. Plasmid pMET7C, isolated by Santangelo et al. (1991), contained a 6.05 kb DNA insert from *C. acetobutylicum* in pEcoR251 (Zabeau & Stanley, 1982). *E. coli* FI9(pMET7C) and was sensitive to 20 pg metronidazole ml⁻¹, giving plasmids pCar4-8 (Fig. 1).

Cloning of genes encoding *C. acetobutylicum* starch-degrading enzymes. Gene libraries in pEcoR251 were constructed with DNA isolated from *C. acetobutylicum* P262 and NCIMB 8052 (Zappe et al., 1987). Recombinant pEcoR251 plasmids were selected on LB agar containing starch azure (0.4%, w/v) and ampicillin (100 μg ml⁻¹). Three colonies that were surrounded by clear zones and were resistant to ampicillin were isolated.

Recombinant DNA procedures. Restriction endonucleases were obtained from several sources (Amersham, Boehringer Mannheim, New England Biolabs and Promega) and were used as specified by the manufacturers. The enzymes exonuclease III and S1 nuclease were obtained from Boehringer Mannheim. α-³²P- and α-³⁵S-labelled nucleotides were obtained from Amersham. Plasmid DNA was prepared by the alkaline-hydrolysis method of Ish-Horowicz & Burke (1981). *C. acetobutylicum* chromosomal DNA was prepared by the method of Marmur (1961), which was modified (Zappe et al., 1986) to overcome the high nuclease activity exhibited by *C. acetobutylicum* (Urano et al., 1983).

For exonuclease III digestion, the *C. acetobutylicum* insert-DNA from pMET7C was subcloned into the PstI site of the phagemid Bluescript KS (Stratagene). Progressive deletions of this clone from both the 5′ and the 3′ ends of the insert were generated by unidirectionally digesting XhoI-ApaI and BamHI-SacI fragments with exonuclease III (Henikoff, 1984), respectively. The deletions were transformed into *E. coli* JM105 and transformants were selected on LB agar containing 100 μg ampicillin ml⁻¹, giving plasmids pCar4–8 (Fig. 1).

Nucleotide sequencing. The nucleotide sequence of both strands of pCar5 containing the metronidazole-active region was determined using overlapping DNA fragments generated by exonuclease III digestion as described above. Nucleotide sequencing was carried out by the deoxy chain-termination method (Sanger et al., 1977), according to the protocol outlined by Tabor & Richardson (1987), using the Sequenase DNA sequencing kit (USB). The DNA chains were radiolabelled with [³²P]dATP αS (1200 Ci mmol⁻¹, 44 Tbmol⁻³; Amersham). The nucleotide and deduced amino acid sequences were analysed on a VAX 6000–330 computer using the GCG suite of sequence analysis programs (Devereux et al., 1984).

Metronidazole sensitivity. An overnight culture of *E. coli* FI9 was diluted 10⁻⁴ with sterile water, and 100 μl was spread onto pre-reduced LB agar supplemented with 0.2% NaNO₃, 0.1% glucose and various concentrations of metronidazole (Sigma). The plates were incubated anaerobically. The MIC was taken to be the amount of metronidazole per ml that totally inhibited growth.

Enzyme assays. Amylase activity from the culture supernatant of *B. subtilis* cells grown in liquid culture was determined by measuring the increase in reducing sugars released from starch by the 3,5-dinitrosalicilic acid method of Miller (1959) as previously described by Rumbak et al. (1991). Amylase activities

---

**Table 1. Bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characteristics</th>
<th>Reference/source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. acetobutylicum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P262</td>
<td>Wild-type</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td>8052</td>
<td>Wild-type</td>
<td>NCIMB</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DH5α</td>
<td>supE44 ΔlacU169 (q80lacZAM15) bsdR17 recA1 endA1 gyrA96 thi-1 recA1</td>
<td>Hanahan (1983)</td>
</tr>
<tr>
<td>GD40</td>
<td>supFI leu</td>
<td>Leclerc et al. (1990)</td>
</tr>
<tr>
<td>F19</td>
<td>Nitroreductase-deficient</td>
<td>Santangelo et al. (1991)</td>
</tr>
<tr>
<td>JM105</td>
<td>endA bsdR supE sbeI thy1 strA (Δlac–proAB) λ lac122 λ : relA1 spoT1 thi-1</td>
<td>Yanisch-Perron et al. (1985)</td>
</tr>
<tr>
<td>CGSC 808</td>
<td></td>
<td>Pardee et al. (1959)</td>
</tr>
<tr>
<td>CGSC 5674</td>
<td>ΔlacB6 thi-1 ΔlacY1 gellR61 strA129 λ : supE44</td>
<td>Buttin (1963)</td>
</tr>
<tr>
<td>CGSC 5060</td>
<td>lacZ2286(Am) purR97 rpoL150 relA1 spoT1 λ</td>
<td>Kilstrup et al. (1989)</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>168</td>
<td>trpC2</td>
<td>Henkin et al. (1991)</td>
</tr>
<tr>
<td>WLN-29</td>
<td>araG932 trpC2 cepA::Tn917lac</td>
<td>Henkin et al. (1991)</td>
</tr>
<tr>
<td>ZB307A</td>
<td>spB2de1::Tn917::pSK10A6</td>
<td>Zuber et al. (1987)</td>
</tr>
<tr>
<td>ZB449</td>
<td>trpC2 pheA1 abrB703 SP7 cured</td>
<td>Zuber et al. (1987)</td>
</tr>
</tbody>
</table>
were expressed as μmol reducing sugar produced per min per OD unit. Amylase production by B. subtilis on agar was tested by plating on brain heart infusion agar (BHIA) containing 1% (w/v) potato starch; glucose-resistant amylase production was tested by the addition of 2% (w/v) glucose. After overnight incubation, plates were flooded with a solution of 0.5% L/50% (w/v) KI for detection of starch hydrolysis. Starch-degrading activity by E. coli cells containing recombinant plasmids was tested by plating on LB agar containing starch-azure and ampicillin. The degradation and solubilization of starch by E. coli cells was assayed in LB medium containing 1% insoluble starch (Unilab). Samples (8 ml) were removed at different time intervals and centrifuged at 4000 g for 5 min and the remaining insoluble starch was measured by wet weight.

**RESULTS**

**Plasmid pMET7C and metronidazole sensitivity**

Plasmid pMET7C containing a 6.05 kb insert of *C. acetobutylicum* DNA, was isolated in *E. coli* F19 by screening for transformants sensitive to 20 μg metronidazole ml⁻¹ (Santangelo et al., 1991). pMET7C cells overproduced a 100 kDa protein which was easily observed on SDS-polyacrylamide gels, and was further characterized to determine the role that this large protein might play in metronidazole sensitivity. Exonuclease III shortening of pMET7C indicated that the large protein and the metronidazole sensitivity were determined by different regions of the 6.05 kb insert DNA on pMET7C (Fig. 1). The metronidazole sensitivity region was localized on a 2.6 kb derivative of pMET7C (pCar5). Sequencing of this subclone revealed a truncated ORF of 892 bp (bases 278–1169; Fig. 2). Colony hybridization, using pMET7C as a probe, was used to isolate a 6.5 kb clone from *C. acetobutylicum* pEcoR251 plasmid-pools constructed by Zappe et al. (1986). This newly isolated recombinant plasmid contained the entire ORF of the gene coding for metronidazole sensitivity and was named pCar1 (summarized in Fig. 1).

**Nucleotide sequence of the *C. acetobutylicum* gene conferring metronidazole sensitivity**

The nucleotide sequence of the region of *C. acetobutylicum* DNA conferring metronidazole sensitivity in *E. coli* F19 revealed a complete ORF of 972 bp, regA, which encoded a protein of 324 amino acids with a calculated M₆ of 35 600 (Fig. 2). The ATG start codon was preceded by a ribosome-binding site (GGAGGA) 8 bp upstream. This Shine–Dalgarno sequence resembles that reported for the *Staphylococcus aureus* β-lactamase (Mclaughlin et al., 1981) and the *C. acetobutylicum* endoglucanase gene (Zappe et al., 1988). A putative promoter region showing extensive homology to the Gram-positive promoters regulating the *C. acetobutylicum* *glnA* gene was detected at nucleotide positions 212–217 and 237–242 (Fig. 2) (Janssen et al., 1990). Another relevant feature in the DNA sequence was the presence of two stem–loop structures downstream of the *regA* gene. This dyad symmetry region is indicative of factor-independent terminators (Brendel & Trifonov, 1984). Just downstream from the first of these regions is a stretch of eight consecutive thymine residues. The dyad symmetry allows for the formation of stable hairpin structures in the RNA transcript, and has been implicated in slowing down the polymerase, whereas the thymine-rich region allows for the formation of an rU–dA hybrid that facilitates release of the transcript (Martin & Tinoco, 1980). Partial DNA sequencing of ORF2, which lies upstream of the *regA* gene and encodes the 100 kDa protein on the pMET7C, indicated that it has homology to a tRNA synthetase.

Sequence comparisons at the amino acid level suggested that the deduced protein encoded by the *regA* gene was a DNA-binding protein (Fig. 3). The deduced amino acid sequence encoded by *regA* showed 40% identity to the *B. subtilis* *espA* gene product, and 38% identity to the *B. megaterium* *espA* gene product (Fig. 3). The RegA protein also showed considerable homology to PurR (34% amino acid identity), LacI (24% identity) and Shl, a tRNA repressor from *E. coli* (21% identity). The amino terminal region of the RegA protein contained an amino acid sequence which has been shown to form a helix–turn–helix structure and was very similar to the DNA-binding domains of other repressor proteins (Fig. 3) (Adler et al., 1972; Sauer et al., 1982).

**Complementation of *E. coli* regulatory mutants**

*E. coli* supH is a suppressor mutation that relieves the auxotrophies associated with the presence of *leu* and *ih* mutations. However, when *E. coli* contains the *shl* gene (suppressor H-linked phenotype) on a multicopy plasmid, the growth of *supH* cells is prevented on minimal medium (Leclerc et al., 1990) while there is no inhibition of growth of an *E. coli* wild-type host. Since the *C. acetobutylicum regA* gene product showed homology to the *E. coli* *shl* gene product, the ability of the *regA* gene to complement the *shl* gene was determined. Transformation of the *E. coli* GD40 *leu supH* mutant with pCar1 containing the *C. acetobutylicum regA* gene resulted in the inability of the *E.
**S. P. DAVISON and OTHERS**

**Fig. 1.** Restriction map of *C. acetobutylicum* DNA cloned in pCar1. The *C. acetobutylicum* DNA in pMET7C originally isolated by Santangelo et al. (1991) was cloned in the vector pEcoR251 (Zabeau & Stanley, 1982). The vector in plasmids pCar1, pCar2 and pCar3 is the integrative shuttle vector, pDH88 (Henner, 1990). Plasmids pCar4-8 are deletions of the BglII-Sau3A insert DNA subcloned in Bluescript SK. The metronidazole-sensitive phenotypes conferred on *E. coli* by the various constructs are indicated by M+ or M-. The 100 kDa protein expressed in *E. coli* by the various constructs and determined by SDS-PAGE are indicated by P+ or P-. A, Accl; B, BamHI; Bg, BglII; C, CiaI; El, EcoRI; EV, EcoRV; H, HindIII; P, PstI; X, XbaI.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Restriction Sites</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCar1</td>
<td>Bg, H, El, B, A, El, Xc, H, X</td>
<td>P+ M+</td>
</tr>
<tr>
<td>pMET7C</td>
<td></td>
<td>P+ M+</td>
</tr>
<tr>
<td>pCar2</td>
<td></td>
<td>P+ M+</td>
</tr>
<tr>
<td>pCar3</td>
<td></td>
<td>P+ M-</td>
</tr>
<tr>
<td>pCar4</td>
<td></td>
<td>P+ M-</td>
</tr>
<tr>
<td>pCar5</td>
<td></td>
<td>P+ M+</td>
</tr>
<tr>
<td>pCar6</td>
<td></td>
<td>P+ M-</td>
</tr>
<tr>
<td>pCar7</td>
<td></td>
<td>P+ M-</td>
</tr>
<tr>
<td>pCar8</td>
<td></td>
<td>P+ M-</td>
</tr>
</tbody>
</table>

*coli* GD40 *leu supH* mutant to grow on minimal medium without leucine. The addition of leucine (100 μg ml⁻¹) to the minimal medium enabled the *E. coli* GD40 *leu supH* (pCar1) transformant to grow. The *C. acetobutylicum* regA gene was unable to complement the lacI, galR or purR mutations in the various *E. coli* strains.

**Complementation of the *B. subtilis* ccpA gene**

The *B. subtilis* WLN-29 ccpA mutant is unable to mediate glucose repression of amylase synthesis (Henkin et al., 1991). Since the *C. acetobutylicum* regA gene product showed the highest amino acid homology to the ccpA gene product, the ability of the regA gene to complement ccpA was determined. The *C. acetobutylicum* regA gene, within a 6·5 kb DNA fragment, was inserted into a SPβ-specialized transducing phage vector, and transduced into the *B. subtilis* ccpA mutant. Plasmids pCar2 and pCar3 containing the DNA regions flanking the regA ORF were transduced into the *B. subtilis* ccpA mutant as controls. Transductants were selected for chloramphenicol resistance conferred by the transducing phages and tested for production of amylase on BHIA medium containing 1% starch (Table 2). Two transductants containing an incomplete regA gene, pCar2 and pCar3, produced levels of amylase activity similar to that of the *B. subtilis* ccpA mutant, that is 59, 50 and 58 U amylase activity, respectively. The transductant with the intact regA gene, pCar1, showed marked repression of amylase activity and only produced 9 U amylase activity. The *C. acetobutylicum* regA gene therefore appears to complement the *B. subtilis* ccpA gene, but its repressive activity is not relieved in the absence of glucose.

**Effect of the *C. acetobutylicum* regA gene on acetoin production in *B. subtilis***

The ccpA gene in *B. subtilis* also controls the production of acetoin in the presence of glucose by regulating the expression of the alsA gene responsible for the synthesis of acetolactate synthase. The *B. subtilis* 168 wild-type strain produced acetoin in LB medium in the presence of 2% glucose (Table 2). The *B. subtilis* ccpA mutant produced acetoin in LB + 2% glycerol medium (data not shown), although it did not produce acetoin in either LB or LB + glucose medium. Transductants of the *B. subtilis*
**regA regulator gene from C. acetobutylicum**

**Fig. 2.** Nucleotide sequence of the DNA fragment containing the 972 bp regA ORF. The nucleotide sequence is numbered throughout. The deduced amino acid sequence of the repressor is shown in single-letter code below the coding sequence. The putative promoter sequences (−35 and −10) and Shine-Dalgarno sequence (SD) are shown in bold type. Dyad symmetries are indicated by horizontal lines.

cepA mutant with the recombinant SPβ phage containing the intact C. acetobutylicum regA gene restored the ability to produce acetoin in the LB + glucose medium, but both the transductants with the truncated regA genes (Table 2) were unable to produce acetoin.

**Cloning of C. acetobutylicum starch-degrading enzyme genes**

Since the regA gene from C. acetobutylicum P262 complemented a B. subtilis cepA mutant, the ability of the regA gene to regulate the expression of a C. acetobutylicum gene was investigated. A C. acetobutylicum P262 gene bank was screened extensively in E. coli JM105 for the presence of an amylase-type gene, but no amylase gene was detected by this method. In contrast, the screening of a similar gene bank containing C. acetobutylicum NCIMB 8052 DNA yielded three E. coli JM105 transformants which were ampicillin-resistant and surrounded by clear zones on blue starch-azure agar plates. E. coli JM105 colonies containing an ampicillin-resistant control plasmid did not produce clear zones on the starch-azure agar containing ampicillin. One of the recombinant plasmids pEco106 was chosen for further study and was characterized by restriction endonuclease mapping and contained a 8.5 kb insert of C. acetobutylicum DNA.
Regulation of starch-degrading activity by pEco106

The ability of the *C. acetobutylicum* regA gene to affect the production of starch-degrading activity encoded by pEco106 was determined. The starch-degrading region from pEco106 was contained on a 10.7 kb *BamHI* fragment; this fragment was subcloned into pACYC184 to give plasmid pACYCStarl, which was compatible with pCar1. Starch-degrading activity was determined in *E. coli* DH5α cells containing pACYCStar1, both pACYCStar1 and pCar1, or both pACYC and Bluescript SK. *E. coli* DH5α(pACYCStar1) colonies were surrounded by clear zones on starch-azure agar plates. However, no zones were observed surrounding *E. coli* DH5α(pACYCStar1 plus pCar1) colonies or the control *E. coli*. The presence of the regA gene in *E. coli* cells containing the *C. acetobutylicum* starch-degrading region clearly repressed starch-degradation activity in liquid medium (Fig. 4).

**DISCUSSION**

The initial aim of this study was to identify *C. acetobutylicum* electron transport genes which reductively activate metronidazole. In a previous study, we reported the isolation of 25 clones which rendered *E. coli* F19 sensitive to metronidazole (Santangelo et al., 1991). One of these clones, pMET7C, was chosen for further study since it produced a 100 kDa protein in *E. coli* F19 cells. Subcloning of the *C. acetobutylicum* DNA on pMET7C indicated that the 100 kDa protein was not involved in the metronidazole sensitivity of *E. coli* transformants, but that a second gene on pMET7C rendered *E. coli* F19 sensitive to metronidazole. Surprisingly, the *C. acetobutylicum* gene product responsible for metronidazole sensitivity in *E. coli* F19 did not share any properties or amino acid homology with proteins associated with electron transport or with enzymes capable of carrying out reduction.

**Table 2. Acetoin production and extracellular amylase activity in *B. subtilis* strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Acetoin production</th>
<th>Amylase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No addition</td>
<td>Glucose</td>
</tr>
<tr>
<td>ZB449</td>
<td>Wild-type</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>WLN-29</td>
<td>apA::Tn917</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>WLN-29</td>
<td>apA::Tn917/SPj-pCar1</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>WLN-29</td>
<td>apA::Tn917/SPj-pCar2</td>
<td></td>
<td>59</td>
</tr>
<tr>
<td>WLN-29</td>
<td>apA::Tn917/SPj-pCar3</td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

Amylase activity was assayed by measuring the increase in reducing sugars produced from starch (amol per min per OD unit) by the 3,5-dinitrosalicylic method. Acetoin production was assayed by the Voges–Proskauer reaction.
reactions. Homology studies indicated that the \textit{C. acetobutylicum} gene controlling metronidazole sensitivity encoded a repressor protein that was similar to the LacI family of repressor proteins. The \textit{C. acetobutylicum} gene, which was termed \textit{regA}, appears to be the first repressor protein of this type from \textit{C. acetobutylicum} to be reported. The conserved amino acid residues associated with the LacI-type DNA-binding region were present in the amino-terminal portion of the predicted amino acid sequence of the \textit{regA} gene.

The complementation of the \textit{supH} mutation in \textit{E. coli} Leu" strains by the \textit{C. acetobutylicum} \textit{regA} gene provides a mechanism for the control of metronidazole sensitivity by the \textit{C. acetobutylicum} \textit{regA} repressor protein in \textit{E. coli}. It has been shown that a \textit{supH} mutation renders \textit{E. coli} sensitive to UV irradiation and high temperatures. It appears that a \textit{supH} mutant is therefore unable to produce a DNA-repair protein, unless the \textit{shl} repressor is present (Leclerc \textit{et al.}, 1989). We propose that the \textit{C. acetobutylicum} \textit{RegA} protein fortuitously functions as a repressor of the \textit{supH} mutation in an analogous fashion to the Shl repressor protein. It has been demonstrated that \textit{E. coli} strains which are unable to produce a fully functional complement of DNA-repair proteins show increased sensitivity to metronidazole (Jackson \textit{et al.}, 1984; Yeung \textit{et al.}, 1984). By indirectly inhibiting the production of a UV-repair protein, the presence of \textit{regA} renders \textit{E. coli} F19 sensitive to metronidazole.

The deduced amino acid sequence of the \textit{C. acetobutylicum} \textit{regA} gene showed the highest homology to the \textit{B. subtilis} CcpA and \textit{B. megaterium} CcpA proteins, which act as negative regulators controlling amylase production by the \textit{amyE} genes in response to glucose. Transductants containing the intact \textit{regA} gene on pCar1 were able to complement a \textit{B. subtilis ccpA} mutant, and showed repression of amylase activity in the presence or absence of glucose. Transductants containing truncated versions of \textit{regA} on pCar2 or pCar3 were unable to repress amylase activity, irrespective of the presence or absence of glucose, indicating that the flanking regions of the \textit{regA} gene did not contain additional genes which affected the production of amylase by the \textit{B. subtilis ccpA} mutant, and that the intact \textit{regA} gene was required for the repression of amylase activity. The other phenotype associated with the \textit{B. subtilis ccpA} gene, regulation of acetolactate synthase expression, was complemented by the \textit{C. acetobutylicum} \textit{regA} gene in liquid medium. It is concluded that the \textit{C. acetobutylicum} \textit{regA} gene is able to complement the \textit{B. subtilis ccpA} mutation, albeit somewhat differently in the case of glucose repression of amylase synthesis.

The complementation of the \textit{B. subtilis ccpA} mutant by the \textit{C. acetobutylicum} \textit{regA} gene suggested that it may be involved in the regulation of starch degradation in \textit{C. acetobutylicum}. Experiments carried out in \textit{E. coli} DH5\textalpha transformed with two plasmids, pCar1 containing the \textit{regA} gene, and pStar1, containing an uncharacterized \textit{C. acetobutylicum} gene capable of degrading and solubilizing starch, showed that the RegA protein repressed the degradation of starch. Further experiments are under way to elucidate the specific role of this starch-degrading enzyme in \textit{C. acetobutylicum}.

It is interesting that in \textit{B. subtilis} it has recently been reported that CcpA is involved in the regulation of the acetate kinase gene (Grundy \textit{et al.}, 1993). Acetate kinase is part of a pathway for the interconversion of acetyl-CoA and acetate. In this pathway, acetyl-CoA is initially converted to an acetyl phosphate intermediate by phosphotransacetylase, and then converted to acetate by acetate kinase. Since this biochemical pathway has a critical role in the physiology of \textit{C. acetobutylicum} (Jones & Woods, 1986), it will be important to establish whether RegA is involved in the regulation of acetate production as well as the utilization of carbohydrate substrates.

ACKNOWLEDGEMENTS

We thank Di James for excellent technical assistance, and acknowledge research grants from National Chemical Products and the Foundation for Research Development.

REFERENCES


Received 24 October 1994; accepted 23 November 1994.