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ORIGINAL PAPER

Activity of abrB310 promoter in wild type and spo0A-deficient strains of *Clostridium acetobutylicum*

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Abstract In *Clostridium acetobutylicum*, *abrB310* is transcribed from two transcription start sites (designated A1 and A2) forming an abundant large, and a five- to ten-fold less abundant small transcript, respectively throughout exponential, acidogenic growth and early in the transitional period to stationary, solventogenic growth. β-galactosidase reporter vectors were constructed to compare the transcriptional activity of the entire *abrB310* promoter and the A1 and A2 transcription start sites individually. In stark contrast to the primer extension data, the A2 start site was threefold more active than the entire promoter, which was threefold more active than the A1 start site in wild type *C. acetobutylicum*. The activity expressed from all three reporter vectors declined as the cultures transitioned from exponential to stationary growth. In the *spo0A*-deficient strain SKO1, reporter vector activity continued for 10 h into stationary growth. The removal of the putative Spo0A binding site from all three vectors had no significant effect on promoter activity in either wild type or SKO1. We conclude that the presence of both the A1 and A2 transcription start sites is required for the correct control of *abrB310* expression, and that AbrB310 is necessary but not sufficient for the correct transition between acidogenic and solventogenic growth.

Keywords Clostridium · Sporulation · Solvent · Metabolite · Promoter

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Introduction

Clostridium acetobutylicum is a Gram-positive obligate anaerobe capable of producing acetic and butyric acid during the exponential phase of its lifecycle, and the solvents acetone and butanol during the stationary phase. In the past 25 years, the biochemistry involved in acidogenesis and solventogenesis has been elucidated and is well understood [for a review, see 1]. Less well understood is the control of expression of the relevant genes required for acid and solvent production, and how the bacterium switches from an exponential, acidogenic growth phase to a stationary, solventogenic growth phase, and finally into sporulation.

It has been shown that the transcription factor Spo0A is a master regulator of the transition between the acidogenic and solventogenic phases. In strain SKO1, where *spo0A* has been disrupted, significantly decreased transcripts of the solvent genes *adhE*, *ctfA*, *ctfB*, *adc* are observed, correlating with extremely low concentrations of acetone and butanol. Conversely strain 824(pMSpo0A), in which *spo0A* expression is elevated using the multicopy plasmid pMSpo0A, produces marginally higher solvent concentrations [2, 3].

Broad genomic and proteomic surveys using wild type *C. acetobutylicum*, SKO1 and 824(pMspo0A) have been performed to try to identify other factors involved in the regulation of the solventogenic switch. DNA microarrays have shown significant differences in the expression of at least 123 genes between wild type and strain 824(pMspo0A), as well as significant transcriptional differences between the acidogenic and solventogenic phases in wild type *C. acetobutylicum* and the asolventogenic mutant strain, M5 [4, 5]. 2D gel analysis of the proteome of wild type, SKO1 and 824(pMspo0A) revealed significantly different levels of heat shock proteins, metabolic regulators

and translational proteins between strains [6]. Such surveys are useful in allowing us to gage the complexity of the life-cycle of *C. acetobutylicum*.

In contrast, several single-gene investigations have also been useful. The stage II sporulation protein E (SpoIIE) was found to have an indirect effect on solvent production, such that in strains downregulated for *spoIIE*, sporulation was delayed indefinitely, allowing elevated concentrations of acetone and butanol to accumulate [7]. The transcription factor AbrB310 was also identified and the corresponding gene *abrB310* was shown to be transcriptionally active in *C. acetobutylicum*. In strains where *abrB310* expression is decreased, elevated acetate and butyrate, and decreased acetone and butanol concentrations are observed, indicating a possible role for AbrB310 as a regulator of the transition between acidogenesis and solventogenesis. The effects of *abrB310* over-expression have not been determined [8].

In *Bacillus subtilis*, the interaction between AbrB and Spo0A is well documented. During vegetative growth, *abrB* is expressed and acts to repress the expression of sporulation-associated genes, thus preventing premature initiation of sporulation [9, 10] activated Spo0A accumulates during the transition from vegetative growth into the sporulation phase, *abrB* expression is repressed by Spo0A allowing the cell to progress through the process of sporulation [11–14].

Previous work using primer extension has revealed the presence of two transcription start sites (A1 and A2) of *abrB310* in *C. acetobutylicum*, both with plausible −10 and −35 consensus sequences upstream (Fig. 1). Additionally, a putative Spo0A-binding site known as the 0A box was identified downstream of the second transcription start site, but upstream of the predicted ribosome binding site and start codon [8].

In this report, we investigated the relative abundance of the *abrB310* transcript throughout the first 24 h of growth using primer extension analysis. A thermostable *β*-galactosidase reporter vector system was employed to examine the transcriptional activity of A1, A2 and the entire *abrB310* promoter in wild type and SKO1 strains of *C. acetobutylicum*, and the effect of the putative 0A box on this activity.

Materials and methods

Bacterial strains, plasmids and oligonucleotides

The bacterial strains and plasmids used in this study are listed in Table 1. All oligonucleotides used are shown in Table 2.

Growth conditions

Escherichia coli was grown in Luria–Bertani medium aerobically at 37°C [15]. For recombinant strains, liquid or agar-solidified medium was appropriately supplemented with ampicillin (100 μg/ml), chloramphenicol (35 μg/ml) or kanamycin (50 μg/ml). Strains were stored at −80°C in medium supplemented with 50% glycerol.

Clostridium acetobutylicum was grown in clostridial growth medium (CGM) with an initial glucose content of 50 g/l (∼280 mM) anaerobically at 37°C [16]. For recombinant strains, liquid or agar-solidified media was appropriately supplemented with erythromycin (40 μg/ml) or the chloramphenicol alternative, thiamphenicol (25 μg/ml). Strains were stored as horse serum-supplemented lyophilized stocks at room temperature.

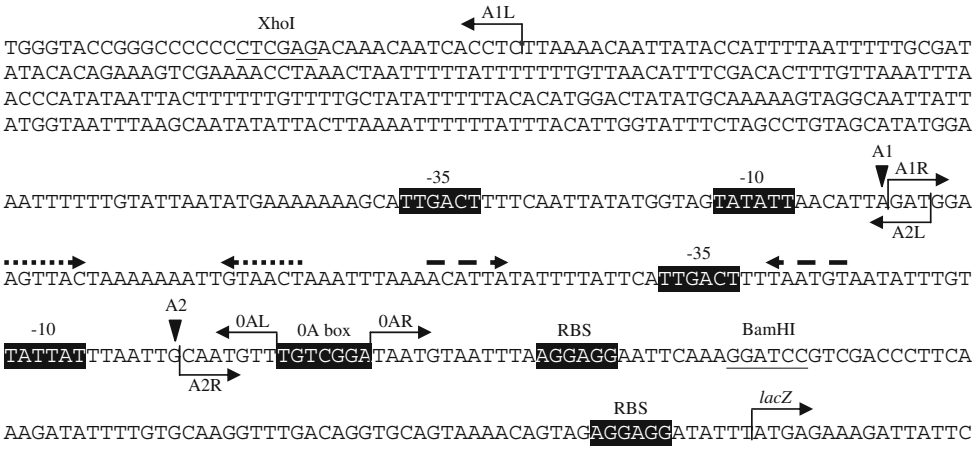

Fig. 1 The *abrB310* promoter within pTL310-1. The *Xho*I and *Bam*HI restriction sites used are shown *underlined*, with the start codon of the *β*-galactosidase open reading frame indicated by "*lacZ*". −35 and −10 consensus sequences are indicated upstream of the two transcription start sites, A1 and A2, with the predicted Spo0A-binding site (*0A box*) and ribosome binding site (*RBS*) shown downstream of A2. *Arrows* indicating the positions of the primers used in the construction of pTL310-2 through -6 are labeled with the primer name. *Dashed* and *dotted arrows* indicate possible formation of stem-loop structures in the mRNA

Table 1 Bacterial strains and plasmids

Strain/plasmid	Description	Reference/source
Strain		
Clostridium acetobutylicum		
ATCC824	Wild type	ATCC, Manassas VA
SKO1	*spo0A*, MLS^R	[3]
Escherichia coli		
DH5α	*mcrA*, *ΔmcrBC*, *recA1*	NEB, Beverly, Mass.
DH10*β*	*mcrA*, *ΔmcrBC*, *recA1*, Str^R	NEB, Beverly, Mass.
TOP10	*mcrA*, *ΔmcrBC*, *recA1*, Str^R	Invitrogen, Carlsbad, CA
Plasmid		
pTrcHisTOPO-TA	Ap^R, pBR322ori	Invitrogen, Carlsbad, CA
pTrcHis310prom	Ap^R, pBR322ori, *abrB310* promoter	This study
pDHKM	RSF1030ori, Km^R, Φ3tI	[17]
pThilac	Thi^R, OriII, ColE1ori, *lacZ*	[8]
pTL310-1 through -6	Thi^R, OriII, ColE1ori, *lacZ*	This study

spo0A Disruption of spo0A, MLS^R macrolide, lincosamide streptogramin B resistant, *mcrA*, *ΔmcrBC* methylcytosine-specific restriction system abolished, recA1 homologous recombination abolished, Str^R streptomycin resistant, Ap^R ampicillin resistant, *pBR322ori ColE1 ori* gram-negative origin of replication, *RSF1030ori* gram-negative origin of replication, Km^R kanamycin resistant, *Φ3tI* Φ3t methylase, Thi^R thiamphenicol resistant, *OriII* gram-positive origin of replication, *lacZ* functional, promoterless *β*-galactosidase gene

Table 2 Oligonucleotides used in this study

Name	Sequence
310UPS	AGCCTCGAGACAAACAATCACCTCTTAAAACAATTATAC
310DS	CCGGGATCCTTTGAATTCCTCCTTAAATTACATA
0AR	TAATGTAATTTAAGGAGGAATTCAAA
0AL	AACATTGCAATTAAATAATAACAAATATTAC
A1R	GATGGAAGTTACTAAAAAAATTGTAAC
A1L	GAGGTGATTGTTTGTCTCGAG
A2R	CAATGTTTGTCGGATAATGTAATTTAA
A2L	ATCTAATGTTAATATACTACCATATAATTG
pTLcheck	CAGTGAATTGTAATACGACTCACTATAG

Underlined regions correspond to a restriction site for the restriction endonucleases *Xho*I (CTCGAG) and *Bam*HI (GGATCC)

DNA isolation, manipulation and transformation into *C. acetobutylicum*

All commercial enzymes used in this study (Vent DNA polymerase, restriction endonucleases, T4 DNA ligase and polynucleotide kinase [New England Biolabs, Inc., Beverly, MA]) were used according to manufacturers' recommendations.

Plasmids were purified from *E. coli* by using the Qiaprep miniprep protocols. PCR products and enzymatically manipulated DNA was purified from agarose gels using a Qiaquick gel extraction kit (Qiagen, Inc., Valencia, CA). Automated DNA sequencing was performed by LoneStar Laboratories Inc., Houston, TX)

Prior to transformation into *C. acetobutylicum*, plasmids were methylated by transformation into *E. coli* DH10*β* harboring plasmid pDHKM [17]. Electroporation of *C. acetobutylicum* was peformed according to the protocol developed by Mermelstein [18]. All strains are designated 824 for wild type or SK for SKO1 [3], followed by the plasmid name in parentheses.

mRNA collection and primer extensions

A controlled pH fermentation was performed in a 2 l Bio-Flo 110 Fermenter/Bioreactor (New Brunswick Scientific Co. Inc., Edison, NJ), according to a modified version of the protocol described by Green [19]. A total of 1.35 l of CGM was prepared and autoclaved in the fermenter according to manufacturer's instructions. A 150 ml CGM batch culture was inoculated with a single colony of *C. acetobutylicum* and grown to an OD_{600} of 0.2 in the anaerobic chamber

prior to injection into the fermenter. The pH was allowed to fall to 5.5, at which stage it was controlled via the addition of 3 M ammonium hydroxide solution. The agitation rate was set to 200 rpm, and the temperature was maintained at 37°C. Prior to inoculation, the fermenter was sparged with nitrogen gas to establish anaerobic conditions, and following inoculation, the nitrogen supply was turned off, with the gas production of the growing culture being sufficient to maintain a positive pressure within the fermenter and hence prevent entry of air and oxygen into the vessel.

Cell samples of 20 ml were harvested at OD_{600} points of approximately 0.2 (0.83 h), 0.4 (2.25 h), 0.8 (3.25 h), 1.2 (3.83 h), 1.6 (4.83 h) and 2.0 (6.25 h), and thereafter at 8, 12, 18 and 24 h post-inoculation. mRNA was prepared and primer extension reactions were performed using 5μg mRNA per reaction as previously described [8]. Primer extension products were separated via electrophoresis on a 12% acrylamide Readygel (BioRad laboratories, Hercules, CA). Gels were run at 100 V for 120 min prior to exposure to BAS-IIIS imaging plates for 15 h. Plates were imaged using a Fujix BAS1000 imager, and images were adjusted and extension product band intensity calculated using the Fujix MACBas Version 2.0 software (Fuji Photo Film USA Inc., Edison, NJ). Acid and solvent concentrations were assayed by injection of a 5 μl aliquot of sample supernatant into a Hewlett Packard 5890 Series II gas chromatograph. Beyond 24 h, it was not possible to purify mRNA in sufficient quantity for primer extension by this method.

Construction of pTL310-1 through -6

The *abrB310* promoter was amplified by PCR from *C. acetobutylicum* genomic DNA using primers 310DS and 310UPS, and cloned into plasmid pTrcHisTOPO-TA (Invitrogen, Carlsbad, CA) to yield vector pTrcHis310prom. Plasmid pThilac has a cloning site upstream of the *lacZ* open reading frame derived from *Clostridium thermosulfurogenes* coding for a thermostable β-galactosidase [20, 21]. pTrcHis310prom and pThilac were digested with *Bam*HI and *Xho*I, and the promoter fragment was ligated into pThilac to yield plasmid pTL310-1. Figure 1 shows the DNA sequence of the *abrB310* promoter in pTL310-1.

PCR amplifications were performed to construct linearized reporter vectors containing only the A1 or A2 transcription start sites, and variants of all three vectors without the spo0A binding site, as shown in Table 3. The linearized vectors were treated with polynucleotide kinase prior to circularization using T4 DNA ligase. The correct sequence of each vector was confirmed by DNA sequencing using primer pTLcheck, which hybridizes to all vectors 24 bases upstream of the *Xho*I restriction site.

Table 3 Construction and features of the pTL310 family of reporter vectors

Plasmid	Template	Primers	Features
pTL310-1	n/a	n/a	A1, A2, 0A box
pTL310-2	pTL310-1	0AL, 0AR	A1, A2
pTL310-3	pTL310-1	A1L, A1R	A2, 0A box
pTL310-4	pTL310-3	0AL, 0AR	A2
pTL310-5	pTL310-1	A2L, A2R	A1, 0A box
pTL310-6	pTL310-1	A2L, 0AR	A1

The template and primers used to construct each vector are shown, with the promoter elements of the resultant vector

A1, A2: A1 or A2 transcription start sites, 0A box: putative Spo0A binding site

Batch fermentations of *C. acetobutylicum*

Single colonies of transformed wild type and SKO1 *C. acetobutylicum* were grown in closed-cap batch fermentations of 100 ml CGM supplemented with thiamphenicol anaerobically at 37°C. To allow for differences in lag time following inoculation, zero hour (T_0) was determined when the culture had reached an OD_{600} of 0.1. Fermentations were allowed to proceed for 72 h. For each strain tested, four separate cultures were grown, with the exception of strain 824(pTL310-3), where three cultures were grown.

β-galactosidase activity assays

Samples of 5 ml were taken at the time points specified and the OD_{600} was measured prior to centrifugation at maximum speed for 20 min at 4°C in a Sorvall NT6000B centrifuge. Subsequent sample preparation and β-galactosidase activity assays were performed as previously described [21]. Enzyme specific activity was quantified as units of enzyme per milligram protein extracted.

Results

Primer extension analysis of *abrB310* expression

Figure 2 shows the primer extension transcripts for *abrB310* at sample points throughout the first 24 h of growth. Samples A through H were taken during the acidogenic phase of growth, whereas samples I and J were taken as the culture was transitioning from acidogenic to solventogenic growth, as indicated by the detection of acetone and butanol. The large *abrB310* transcript was detected in all samples, whereas the small transcript was first detected in sample C, corresponding to an OD_{600} of 0.8, 3.25 h following fermenter inoculation. In all samples after C, the large

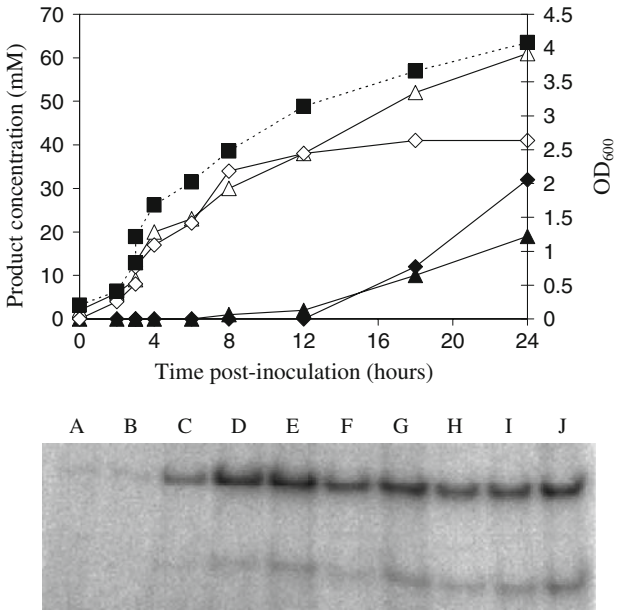

Fig. 2 Primer extension analysis of *abrB310* expression during 24 h growth. Graph shows OD_{600} (*filled square*), acetate (*open triangle*), acetone (*filled triangle*), butyrate (*open diamond*) and butanol (*filled diamond*). Sample points A through J can be seen unlabeled on the OD_{600} trace. Gel shows large (~200 bases) and small (~110 bases) primer extension transcripts of *abrB310* at the following sample points: *A* OD_{600} = 0.2; *B* OD_{600} = 0.4; *C* OD_{600} = 0.8; *D* OD_{600} = 1.2; *E* OD_{600} = 1.6; *F* OD_{600} = 2.0; *G* time = 8 h; *H* time = 12 h; *I* time = 18 h; *J* time = 24 h

transcript was five to ten times more abundant than the small transcript. The intensity of each transcript remained relatively constant in samples C through J.

OD_{600} normalization of reporter strains

To allow comparison of the *β*-galactosidase activity between the strains, it was necessary to determine the exponential and stationary growth phases in wild type and SKO1 strains by OD_{600} as shown in Fig. 3. All wild type cultures grew similarly, with the exponential phase lasting for the first 24 h, and stationary phase from 24 to 72 h. The

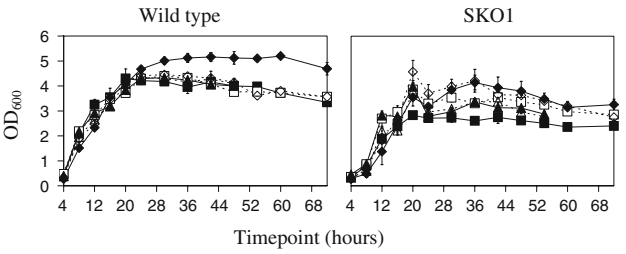

Fig. 3 Growth curves of strains transformed with pTL310 family of vectors. pTL310-1 (*filled square*), pTL310-2 (*open square*), pTL310-3 (*filled diamond*), pTL310-4 (*open diamond*), pTL310-5 (*filled triangle*), pTL310-6 (*open triangle*). Data are shown ±1 standard error

exponential growth phase of SKO1 strains lasted for the first 20 h, with the stationary phase extending from 20 to 72 h. Although the OD_{600} of SKO1 strains during stationary phase was more variable than that observed in wild type cultures, it appears to have had no significant impact in the activity of the reporter vectors.

β-galactosidase activity in wild type *C. acetobutylicum*

Figure 4a shows the *β*-galactosidase activity in strains 824(pTL310-1), 824(pTL310-3) and 824(pTL310-5). *β*-galactosidase activity in strain 824(pTL310-1) remained relatively constant through exponential growth at approximately 4,000 units per milligram protein, and declined as the cultures entered stationary phase. In strain 824(pTL310-5), where A1 is the sole transcription start site, *β*-galactosidase activity is reduced to approximately 30% of that in 824(pTL310-1). In strain 824(pTL310-3), where A2 is the sole transcription start site, *β*-galactosidase activity reached a maximum level of approximately threefold greater than that in 824(pTL310-1) after 20 h growth, then declined throughout the remainder of the fermentations.

The effect of spo0A and the putative 0A box on *β*-galactosidase activity

β-galactosidase activity in strains 824(pTL310-6), SK(pTL310-5) and SK(pTL310-6) were extremely low, similar to that in strain 824(pTL310-5) and have therefore been omitted. Figure 4b and c allows comparison of *β*-galactosidase activity between wild type and SKO1 strains transformed with plasmids pTL310-1 through pTL310-4.

In strains 824(pTL310-1) and 824(pTL310-2), *β*-galactosidase activity remained constant and similar during the first 24 h of growth, whereas in strains 824(pTL310-3) and 824(pTL310-4), *β*-galactosidase activity increased during the first 24 h of growth, reaching a maximum at 24 h. *β*-galactosidase activity in all four strains decreases thereafter for the remaining 48 h of growth.

β-galactosidase activity in SK(pTL310-1) and SK(pTl310-2) was slightly decreased compared to that in corresponding wild type strains, but remained constant throughout the first 30 h of growth before gradually declining. Strain SK(pTL310-3) exhibited the highest *β*-galactosidase activity of all strains tested, reaching a maximum peak after 8 h growth, then remaining relatively constant from 8 to 30 h prior to declining. *β*-galactosidase activity in SK(pTL310-4) reached a maximum after 16 h growth, remained constant between 16 and 30 h, then declined.

Only strains transformed with pTL310-3 and pTL310-4 exhibited significant differences in *β*-galactosidase activity as a result of the removal of the putative 0A box sequence.

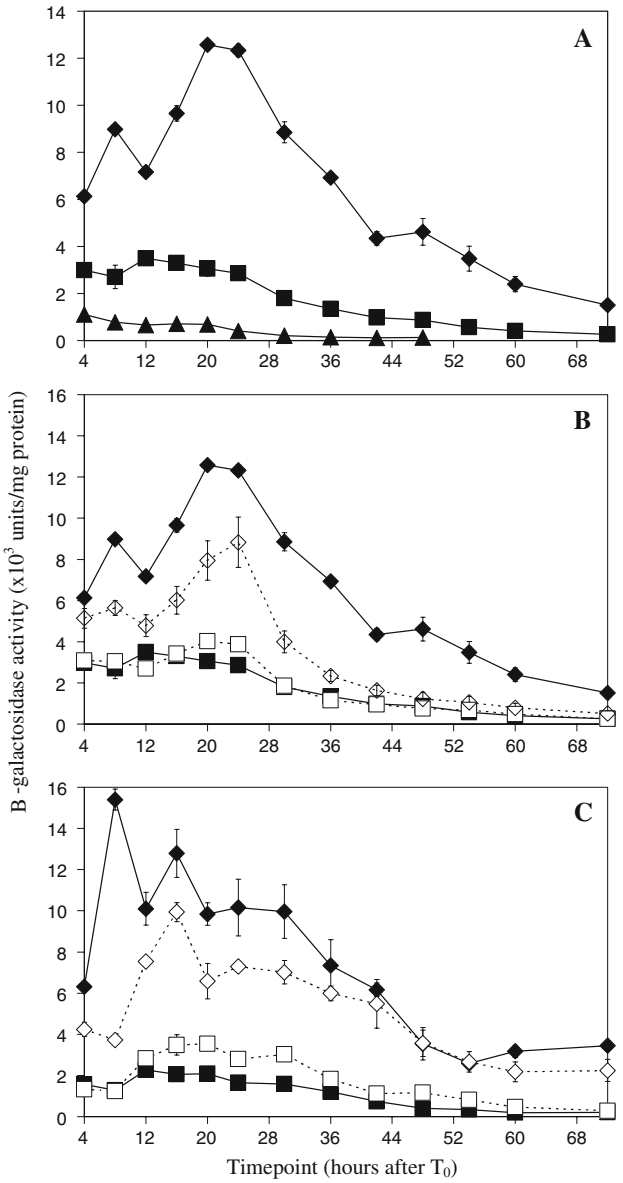

Fig. 4 β-galactosidase activity in strains transformed with pTL310 family of vectors. **a** Wild type *C. acetobutylicum* transformed with pTL310-1 (*filled square*), pTL310-3 (*filled diamond*), pTL310-5 (*filled triangle*); **b** Wild type *C. acetobutylicum* transformed with pTL310-1 (*filled square*), pTL310-2 (*open square*), pTL310-3 (*filled diamond*), pTL310-4 (*open diamond*); **c** Strain SKO1 transformed with pTL310-1 (*filled square*), pTL310-2 (*open square*), pTL310-3 (*filled diamond*), pTL310-4 (*open diamond*). Data are shown ± 1 standard error

However, in either wild type or strain SKO1, β-galactosidase activity from pTL310-4 transformants followed a similar pattern to that in the corresponding pTL310-3 transformants, but the magnitude was decreased. This shows that although the removal of the 0A box caused a decrease in β-galactosidase activity in these strains, this phenomenon is not dependent upon the presence of an intact copy of *spo0A*.

Discussion

The primer extension data show constant transcription of *abrB310* throughout the acidogenic and transition phases. As the culture entered the acidogenesis–solventogenesis transition, both the large and small transcripts of *abrB310* persisted, and it is likely that the mRNA samples did not extend sufficiently late in growth to detect any potential decreases in transcript. The large transcript remained approximately five to tenfold denser than the small transcript, at all stages assayed. Given that the large transcript is approximately twice as long as the small transcript, it can be estimated that there were two to five copies of the large transcript per copy of the small transcript.

A paradox emerges as the primer extension data is compared to the β-galactosidase activity in strains 824(pTL310-1), 824(pTL310-3) and 824(pTL310-5). Whilst the primer extension data suggests that transciption start site A1 was more active than A2, the β-galactosidase data indicate the reverse. In wild type strains, β-galactosidase activity derived from pTL310-3 (harboring A2 only) was significantly greater than β-galactosidase activity from either pTL310-1 (harboring the full-length *abrB310* promoter) or pTL310-5 (harboring A1 only).

All vectors in the pTL family are transcriptional fusions of the promoter or promoter fragments upstream of *lacZ*. The ribosome binding site and 67 bases located directly upstream of the start codon of *lacZ* are native to the *lacZ* gene. Therefore, differences in β-galactosidase activity are not attributable to translational signal variation between vectors, but to differences in transcriptional activity or mRNA stability.

Vector pTL310-3 contains only the A2 transcription start site and upstream sequence, yet strain 824(pTL310-3) exhibited two to four times higher β-galactosidase activity at all stages of growth than 824(pTL310-1). The removal of the A1 promoter and hence the large transcript resulted in elevated β-galactosidase activity, suggesting that the A1 promoter or large transcript may have a negative or inhibitory effect on the A2 promoter. In wild type cells, it is possible that RNA polymerase acting from the A1 site, as well as the growing mRNA strand, may physically block the A2 site, thus limiting the access of RNA polymerase. The removal of the A1 promoter and associated blockage may then allow RNA polymerase open access to the A2 site, thus causing an elevation in overall transcription, greater even than wild type levels.

Vector pTL310-5 contains the A1 transcription start site and promoter sequence upstream, yet strain 824(pTL310-5) exhibited approximately onethird of the β-galactosidase activity compared to strain 824(pTL310-1). The removal of the region between the A1 and A2 transcription start sites had a negative effect, resulting in decreased β-galactosidase activity. The β-galactosidase data indicate that transcription

from A1 alone is weak, but the mRNA derived from A1 in wild type cells may be stable, allowing its accumulation and persistence as observed in the primer extension data. Extensive mRNA secondary structure can be predicted for the region between the A1 and A2 (see Fig. 1), which may form stem-loop structures and subsequently may facilitate stabilization of the mRNA.

In *B. subtilis* AbrB has been shown to play a critical role in the transition between vegetative growth and sporulation [11, 14, 22]. It has previously been shown in *C. acetobutylicum* that decreased *abrB310* expression causes a delay in the transition from acidogenic to solventogenic growth, and it was suggested that overexpression of *abrB310* may be lethal to the cell [8]. The data presented here show that an intact *abrB310* promoter is required to allow the correct level of expression of AbrB310, which is critical to maintaining cell viability. In the absence of either the A1 or A2 promoter regions, and presumably their transcripts, correct control of expression is abolished. Whilst the detailed mechanism for this proposed model of *abrB310* expression regulation is unknown, we can speculate that the complex promoter arrangement allows a means of specifying the level and timing of expression in coordination with other early events of sporulation and thus might explain the relevance of the two transcription start sites of *abrB310* in *C. acetobutylicum*.

In all wild type strains transformed with a pTL310 vector, the point at which β-galactosidase activity began to decline (24 h) correlated exactly with the transition from the exponential to the stationary phase of growth, but this correlation was not affected by the presence or absence of the putative 0A box. Conversely, in all SKO1 strains transformed with a pTL310 vector, β-galactosidase activity began to decline after 30 h growth, at least 10 h after the transition from exponential to stationary phase.

The removal of the 0A box from the full length promoter in strain 824(pTL310-2) caused no changes in β-galactosidase activity compared to the wild type promoter in strain 824(pTL310-1), leading to the conclusion that it has no significant role in the regulation of *abrB310* expression. It is possible that the removal of the 0A box in vector pTL310-4 caused destabilization of the mRNA produced or an unexpected structural change resulting in a wholesale decrease in β-galactosidase activity relative to pTL310-3 unrelated to any transcription factor.

Taken together, these data suggest that an active copy of *spo0A* is required for the correct temporal regulation of *abrB310* expression, but these effects are not directly mediated via the putative 0A box within the *abrB310* promoter.

Experiments using a 242-base pair amplicon of *abrB3647* on a DNA microarray indicate that Spo0A downregulates *abrB* expression [4]. Although it has been shown that *abrB3647* is not transcribed [8], it is highly homologous to *abrB310* and cross-hybridisation of *abrB310* to *abrB3647* DNA on the microarray is likely. The persistence of β-galactosidase activity in SKO1 strains transformed with a pTL310 vector is consistent with the conclusion that Spo0A acts to downregulate *abrB310* expression. Recent array experiments [Cheng N, Jones SW, Tracy B, Paredes CJ, Sillers R, Papoutsakis ET (2008) The transcriptional program of clostridial sporulation, submitted] show elevated expression of *abrB310* in the period 10–18 h in their experimental conditions during the time when solvent production begins.

Strain SKO1 remained asolventogenic, despite the presumed presence of active AbrB310 indicating that the effects on the cell mediated by the absence of Spo0A, namely the abolition of the transition from acidogenesis to solventogenesis, with the subsequent defects in solvent production and sporulation, supercede any effects that AbrB310 may have. In conclusion, AbrB310 is necessary but not sufficient for the correct transition between acidogenic and solventogenic growth, and Spo0A remains the "master controller" of the onset of solventogenesis in *C. acetobutylicum*.

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