Starvation stress modulates the expression of the *Aspergillus nidulans* brlA regulatory gene

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Expression of the *Aspergillus nidulans* brlA gene plays a fundamental role in the switch from vegetative growth to asexual reproduction. Using a media-shifting protocol to induce submerged sporulation and brlA-lacZ as an expression marker, it was shown that carbon and nitrogen starvation stress induced brlA transcription to different degrees. Glucose starvation induced brlA rapidly to high levels and resulted in spore formation on reduced conidiophores, whereas nitrogen starvation induced brlA gradually to lower levels and sporulation occurred to a lesser extent but from more complex conidiophores. β-Galactosidase activity paralleled brlAa and brlAb mRNA. No clear qualitative differences between the two brlA transcripts were found in these starvation conditions, suggesting that the different patterns of sporulation could be explained by quantitative expression differences. Since brlA mRNA did not accumulate in the presence of a high glucose concentration, we investigated the role of other carbon sources on brlA expression. Non-repressing carbon sources such as glycerol, acetate and arabinose were as effective as glucose in preventing brlA mRNA accumulation, suggesting that the glucose effects on brlA expression could be explained as a response to nutrient starvation, rather than by carbon catabolite repression. Despite similar low levels of brlA transcripts being detected during growth in glucose or non-repressing carbon sources, conidiophores were formed only in medium containing glycerol, acetate or arabinose. When mycelia were not shifted to starvation conditions, sporulation was not observed in standard minimal medium even after glucose was exhausted, unless the medium was buffered. This and other results suggest that strong deviation from external neutral pH partially prevented full induction and/or function of brlA.

Keywords: *Aspergillus nidulans*, brlA, starvation stress, glucose regulation, submerged sporulation

INTRODUCTION

Development in many micro-organisms has often been shown to be induced by nutrient limitation and other conditions which have a detrimental effect on growth, but very little is known about how these environmental signals lead to development (for a discussion see Hansberg & Aguirre, 1990; Strauch & Hoch, 1992). Asexual reproduction (conidiation) of the Ascomycete fungus *Aspergillus nidulans* is an excellent experimental system to study these signals, since the different conditions able to induce sporulation will converge upon expression of the brlA (Bristle) gene. This is an early regulatory gene involved in the formation of the conidiophores of *A. nidulans*. These multicellular, spore-producing structures are composed of a basal foot cell, an aerial stalk ending in a multi-nucleate globose vesicle, a layer of uninucleate primary sterigmata or metulae, and a layer of uninucleate secondary sterigmata or phialides, from which spores are produced by repeated division (Clutterbuck, 1969; Oliver, 1972; Mims et al., 1988). Mutants in brlA form aerial stalks that fail to differentiate vesicles, metulae, phialides or spores (Clutterbuck, 1969). They also fail to express many genes associated with later stages of conidiophore development (Zimmermann et al., 1980; Boylan et al., 1987; Adams et al., 1988; Mirabito et al., 1989; Clutterbuck, 1990; Stringer et al., 1991; Timberlake, 1991). The brlA gene encodes two mRNAs, designated brlAa and brlAb. The brlAb mRNA encodes a polypeptide...
which is a putative Zinc-finger transcription factor identical to brlAa, except that 23 additional amino acids are present at the amino terminus (Adams et al., 1988; Prade & Timberlake, 1993). brlAa/P transcripts are present at barely detectable levels in hyphae, and begin to accumulate about 5 h after induction of development by exposure to air (Boylan et al., 1987; Prade & Timberlake, 1993), primarily in conidiophore vesicles, metulae and phialides (Aguirre et al., 1990). Under conditions where sporulation does not normally occur, expression of either brlAa or brlAβ from the strongly inducible alcA promoter results in spor production from hyphal tips (Adams et al., 1988; Han et al., 1993).

Since expression of brlA leads to sporulation, it is important to understand how this gene is regulated. In A. nidulans the ability to conidiate, and therefore to express brlA, is not reached until a certain amount of mycelial growth is completed, a phenomenon described as competence (Axelrod et al., 1973). Competent mycelia, however, will not differentiate until sporulation is induced by exposure to air (Axelrod et al., 1973; Champe & Simon, 1992), or as shown in this paper, by nutrient limitation.

It has been reported that other A. nidulans developmental regulatory genes (such as staA, metA, and abaA; Mirabito et al., 1989) control the pattern of expression of the brlA gene, including brlA itself (Aguirre, 1993). However, half of the total induction of brlA transcription is brlA-independent (Aguirre, 1993), and nothing is known about the signals that initiate brlA expression. The study of such signals has been limited by the fact that sporulation in A. nidulans is typically induced by exposure to air in solid media, a condition where environmental factors such as the nutritional status of the cells are difficult to evaluate and manipulate.

In this paper, we show that the brlA gene can be induced in submerged liquid culture, either by carbon or nitrogen starvation, or both carbon and nitrogen starvation and that the rate and amount of brlA mRNA correlates inversely with the morphological complexity of the conidiophore. We propose that brlA is induced as a response to a general starvation stress condition.

METHODS

Aspergillus strain and growth conditions. A. nidulans strain TJA22 [brlA1; brlA(-2913 p/)/lacZ(argB+/argB::CAT); metG7] (Aguirre, 1993) was grown in appropriately supplemented minimal medium (C, glucose 1%; N, NaNO₃ or NH₄Cl; Käfer, 1977) at 37°C for 18 h and 300 r.p.m. (culture volume was kept to one-fifth of the flask volume).

Freshly collected 5-d-old spores were filtered through Miracloth (Calbiochem), washed four times with 10 ml sterile water, and used immediately to inoculate a single culture at a density of 5 x 10⁸ conidia ml⁻¹. The inoculated medium was then divided into 50 ml samples per 250 ml Erlenmeyer flask and incubated at 37°C for 18 h and 300 r.p.m. Fresh spores were used because variability in results was observed when using spore suspensions stored at 4°C for different times.

For starvation experiments, 50 ml cultures incubated for 18 h were filtered through Miracloth. Mycelia were briefly rinsed with minimal glucose/nitrate-free medium, and resuspended in 50 ml of either glucose-free or nitrogen-free medium (250 ml flask) and incubation was continued under the same conditions. For shifts to media with different glucose concentrations or with different carbon sources, filtered cultures were washed with 50 ml pre-warmed sterile water, then with 50 ml supplemented minimal medium with no glucose, and finally resuspended in 50 ml of the appropriate medium. Care was taken to control the amount of biomass transferred to the starvation media, since this can affect both the kinetics and the absolute levels of brlA/lacZ induction.

β-Galactosidase activity, glucose determination and microscopy. β-Galactosidase activity was determined as previously reported (Miller, 1972; Aguirre et al., 1990).

Glucose was measured directly from fresh or frozen culture filtrates using a quantitative, enzymic (glucose oxidase) determination kit from Sigma.

Mycelia from different culture conditions were examined under a microscope for conidiophore formation and spore counting, and were photographed using a green filter and Kodak Tri-X Pan 400 film.

RNA preparation and analysis. Total mRNA was extracted as described by Timberlake (1980), fractionated by electrophoresis in formaldehyde-agarose gels (10–15 µg per lane), transferred to Hybond-N membranes (Amersham), and hybridized to 32P-labelled random-primed specific probes (BRL) according to the membrane manufacturer’s instructions. A brlA BamHI–EcoRI fragment from pBS2-5 (Boylan et al., 1987) was used as the brlAβ-specific probe, and an argB HindIII fragment from pDGH25 (Gems et al., 1991) was used as a probe to confirm equal loading of mRNA samples. For dot-blot analysis, 15 µg total mRNA was applied to a Hybond-N membrane as recommended by the manufacturer, and hybridized to 32P-labelled random-primed specific probes for wA (SalI–XbaI fragment from pNK15; Mayorga & Timberlake, 1990), yA (XbaI–EcoRI from pRA6; R. Aramayo, unpublished) and CAT (Boylan et al., 1987).

RESULTS AND DISCUSSION

brlA can be induced in submerged cultures by carbon or nitrogen starvation, resulting in different patterns of sporulation

Martinelli (1976) reported submerged sporulation by A. nidulans in liquid cultures using different carbon sources. Since the high surface area/volume ratio (20 ml medium in 250 ml flasks) used in her experiments could have induced air interface sporulation, we produced submerged sporulation using lower surface area/volume ratios (50 ml medium in 250 ml flasks), as well as by media shifting, to test the ability of carbon and nitrogen starvation to induce the brlA gene.

A. nidulans strain TJA22, which contains a single copy of a brlAα/β-lacZ gene fusion integrated at the argB locus (Aguirre, 1993) was grown in liquid culture for 18 h at 37°C and then the mycelia were shifted to glucose-free medium, nitrate-free medium, or standard medium (Käfer, 1977). Samples were taken at different times and processed for β-galactosidase assay, mRNA extraction, and microscopic examination. The β-galactosidase assays showed that both glucose- and nitrate-starvation induced
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(a) Accumulation of β-galactosidase activity in A. nidulans grown for 18 h as described in Methods and shifted to media with no glucose (●) or no nitrate (○), or to standard medium (□) as a control. Incubation was continued under the same conditions and mycelia from entire cultures were harvested at the indicated times, frozen, and lyophilized. Part of the samples were processed for β-galactosidase determination.

(b) brlAβ/brlAα accumulation induced by glucose or nitrate starvation. Total mRNA was isolated from samples of the experiment shown in (a), Northern blotted and probed with a brlAα/β-specific probe. The lanes correspond to different times after shifting to the indicated media: 0 h after shifting corresponds to 18 h growth in standard minimal medium. Equal loading of mRNA was confirmed by stripping the membrane and reprobing with an argB-specific probe. (c) Conidiophore morphology 20 h after shifting to media with no glucose (top) or no nitrate (middle), or to standard medium (bottom). The long arrow in the top panel indicates a typical reduced conidiophore; arrowheads indicate spores. Short arrows in the middle panel point to complex conidiophores. The bottom panel shows non-differentiated hyphae. Bars, 50 μm.

The brlAβ/β-lacZ gene; glucose starvation induced brlA expression more rapidly and to a higher level than nitrate starvation (Fig. 1a). Shifting to standard glucose-nitrate medium caused a slight induction of brlA at 20 h. Endogenous β-galactosidase activity was barely detectable in all conditions tested (not shown).

Distinctive morphological changes were observed at 20 h following shifting to the different media. Glucose starvation induced sporulation from highly reduced conidiophores, bypassing the vesicle and metulae stages (Fig. 1c, top). This morphology was similar to that produced when either brlAβ or brlAα are induced rapidly to high levels from the inducible alcA promoter (Adams et al., 1988; Han et al., 1993). By contrast, nitrate starvation gave rise to more elaborate conidiophores, similar to those produced at an air interface (Fig. 1c, middle). No obvious signs of sporulation were observed when shifting was to standard medium (Fig. 1c, bottom).

These two different morphological patterns of sporulation may have resulted from differential induction of either brlAβ or brlAα transcripts. To address this question, the presence of both transcripts was assayed by Northern blotting. The results presented in Fig. 1(b) show that both brlAα and brlAβ mRNAs had accumulated by 10 and 20 h after shifting to glucose- or nitrate-free medium. Accumulation paralleled β-galactosidase activity and no clear differences, other than quantitative ones, were observed. In fact, densitometric analysis of Fig. 1(b) showed that the brlAβ/brlAα ratio was around 1:4 in all cases (brlAβ is usually more abundant than brlAα; Prade & Timberlake, 1993). Longer autoradiographic exposure of this Northern blot showed a slight accumulation of brlA mRNA when mycelia were transferred to standard 1% (w/v) glucose medium, but no obvious morphological changes were detected under those conditions (Fig. 1c, bottom). Other starvation experiments, shifting from glucose-NH₄Cl to glucose-free or NH₄Cl-free media, produced similar results to those shown in Fig. 1(a) and b, i.e. brlA/lacZ was expressed less when mycelia were nitrogen-starved than when they were glucose-starved, and complex and reduced conidiophores were formed, respectively. Cultures starved of both glucose and nitrate gave results similar to cultures starved of glucose only (some of

specific activity corresponds to U (mg protein)⁻¹ (Miller, 1972). About 300 U is reached 35 h after induction of sporulation in an air interface (Aguirre, 1993). A representative experiment is shown. (b) brlAβ mRNA accumulation induced by glucose or nitrate starvation. Total mRNA was isolated from samples of the experiment shown in (a), Northern blotted and probed with a brlAβ/β-specific probe. The lanes correspond to different times after shifting to the indicated media: 0 h after shifting corresponds to 18 h growth in standard minimal medium. Equal loading of mRNA was confirmed by stripping the membrane and reprobing with an argB-specific probe. (c) Conidiophore morphology 20 h after shifting to media with no glucose (top) or no nitrate (middle), or to standard medium (bottom). The long arrow in the top panel indicates a typical reduced conidiophore; arrowheads indicate spores. Short arrows in the middle panel point to complex conidiophores. The bottom panel shows non-differentiated hyphae. Bars, 50 μm.
The results represent mean values from two independent the times indicated and /?-galactosidase was assayed as in Fig. 1.

**Fig. 2.** brlA expression in creA-repressing and non-repressing carbon sources. (a) A. nidulans was grown for 18 h in glucose medium and shifted to media containing either no glucose (■), 0.1% glucose (▲), 1% glucose (■), 1% arabinose (□), 1% glycerol (△) or 100 mM acetate (□). Samples were isolated at the times indicated and /?-galactosidase was assayed as in Fig. 1. The results represent mean values from two independent experiments with a maximum variation of 16% about the mean. (b) Total mRNA was isolated from 24 h samples, Northern blotted and probed with a brlAa//?-specific probe. Lanes: 1, no glucose; 2, 0.1% glucose; 3, 1% glucose; 4, 1% arabinose; 5, 1% glycerol; 6, 100 mM acetate. Equal loading of mRNA was confirmed by stripping the membrane and reprobing with an argB-specific probe.

The observed correlation between the rate and level of brlA induction and the different conidiophore morphologies, suggests that conidiophore cell-types could result from gradual increases in brlA activity, the lowest dosage corresponding to vesicle formation and the highest to phialide- and spore-development. This proposal is elaborated upon below.

**Non-repressing carbon sources can also prevent brlA accumulation**

To investigate the effect of glucose on brlA expression, A. nidulans TJA22 was grown as before and shifted to media containing glucose (0, 0.1 or 1.0%, w/v), or the creA-mediated non-repressing carbon sources arabinose (1%, w/v), glycerol (1%, w/v) (Arst & Bailey, 1977) or acetate (100 mM). Cultures were sampled at different times to determine /?-galactosidase activity and brlA mRNA accumulation (Fig. 2a, b), morphology (Fig. 3) and spore numbers (Table 1). /?-Galactosidase activity was higher when cultures were shifted to 0.1% glucose than when shifted to glucose-free medium, whereas acetate, arabinose or glycerol were as good as 1% glucose in preventing brlA promoter activity (Fig. 2a), allowing a low level of brlA expression. The brlA promoter/β-lacZ activity also correlated with the actual brlA mRNA accumulation (Fig. 2b) and, within the limitations of the technique (low brlA transcription is not easily detectable in Northern blots, probably because the brlA mRNA is unstable; Prade & Timberlake, 1993), no obvious difference in the relative abundance of brlA or brlAβ transcripts was observed between 1% glucose and the non-repressing carbon sources (Fig. 2b). The high levels of brlA expression with 0.1% glucose could be related to the fact that this glucose concentration is low enough not to repress brlA, but high enough to fuel the sporulation process. However, glucose was exhausted from the medium by 18 h after shifting to 1% glucose (not shown), and the low brlA induction observed under this condition was probably due to partial carbon starvation.

The results obtained with arabinose, acetate and glycerol make it unlikely that brlA glucose repression could be directly mediated by a creA-dependent carbon catabolite
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**Fig. 3.** Conidiophore morphologies 24 h after shifting of glucose-nitrate grown mycelia to different carbon sources: (a), 0.1% glucose; (b), 1.0% arabinose; (c), 1.0% glycerol; and (d), 100 mM acetate. The long arrow in (a) indicates a reduced conidiophore and arrowheads indicate spores. The thick arrows in (b)-(d) indicate complex conidiophores. Bars, 20 \mu m.

repression mechanism (Arst \& Bailey, 1977; Dowzer \& Kelly, 1991). Instead, we propose that \textit{briA} is regulated by nutrient limitation and that starvation for carbon, nitrogen, carbon and nitrogen, or other nutrients can lead to a similar kind of metabolic stress (see Hansberg \& Aguirre, 1990).

**Rate and level of \textit{briA} expression correlates with the complexity of conidiophore development**

Figure 3 shows the morphology observed in some of the 24 h samples exposed to different carbon sources from the experiment presented in Fig. 2. Again, rapidly increasing levels of \( \beta \)-galactosidase activity and \textit{briA} mRNA corresponded with high spore numbers and reduced conidiophore morphology (Fig. 1c, top, Fig. 3a, and Table 1), whereas low but gradually increasing levels corresponded with either the absence of sporulating structures (as in Fig. 1c, bottom), or the presence of few conidiophores with more complex morphology and low spore production (Fig. 3b–d; Table 1). As in the experiments shown in Fig. 1, these different conidiophore morphologies could not be correlated with differential expression of either \textit{briA} or \textit{briAp} transcripts, as judged from Northern blots (Fig. 2b). These results support a quantitative model for \textit{briA} regulation of conidiophore development, which would be consistent with the following findings. Firstly, high induction of either of the two \textit{briA} transcripts (\( \alpha \) or \( \beta \)) from the strongly inducible \textit{alcA} promoter causes spore production directly from hyphal tips (Adams \textit{et al.}, 1988; Han \textit{et al.}, 1993), a morphology virtually identical to that observed in the glucose starvation experiments (Figs 1c and 3a). Secondly, \textit{briA} and \textit{briAp} seem to perform similar functions, since multiple copies of either \textit{briA} or \textit{briAp} can overcome the need for the other transcript during normal development (Prade \& Timberlake, 1993). Thirdly, developmental mutants such as \textit{medA} and \textit{stuA} (Clutterbuck, 1969; Miller \textit{et al.}, 1992) present aberrant conidiophore morphologies that are consistent with their abnormal temporal or spatial transcription of \textit{briA} (Aguirre, 1993).

Several \textit{cis}-acting regulatory elements could regulate \textit{briA} transcription rates through multiple inputs such as carbon and nitrogen starvation. It is important to note that \textit{briA} regulatory sequences, up to \(-2913\) bp, are required for full \textit{briA} expression, and progressive deletion of these sequences in a \textit{briAx}/\( \beta \)-lactamase fusion gradually decreases
maximum β-galactosidase levels but does not alter the developmental timing or cellular specificity of expression (J. Aguirre & W. E. Timberlake, unpublished). Miller (1993) has pointed out that several brlA-dependent genes contain more than one copy of brlA-responsive elements (Chang & Timberlake, 1992), providing a mechanism for a brlA dosage control of development. In microorganisms, incremental regulation of gene expression has been shown for the Myxococcus xanthus csgA gene, which encodes an extracellular protein whose steady increases are regulated at the transcriptional level and determine the proper ordering of developmental steps (Li et al., 1992). We cannot explain why some conidiophore development occurs in non-repressing carbon sources (Fig. 3b–d), but it does not occur in 1% glucose (Fig. 1c), despite similar moderate levels of brlA expression being detected in all these conditions. It is possible that although brlA itself does not seem to be repressed by glucose through a creA-dependent mechanism, other genes necessary for sporulation could be repressed by the glucose-creA system. The different carbon sources could also have diverse effects on brlA at the translational level (i.e. by stabilizing the brlA messages), since there is evidence showing that brlAβ, but not brlAα, is regulated by translational repression (Han et al., 1993). Further work is necessary to answer these questions.

**Table 1. Spore production and external pH after shifting to different carbon sources**

Mycelia grown in minimal nitrate-glucose (1%) media for 18 h were shifted to the indicated carbon sources and sampled for spore production after 24 h and for external pH after 6 and 24 h. Spore numbers shown are mean values from duplicates with a maximum variation of 13% about the mean.

<table>
<thead>
<tr>
<th>Minimal medium plus:</th>
<th>10^3 × Spores per culture</th>
<th>External pH</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>No glucose</td>
<td>50000</td>
<td>6.8</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>0.1% glucose</td>
<td>130000</td>
<td>7.1</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>1% glucose</td>
<td>8</td>
<td>7.6</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>1% arabinose</td>
<td>20</td>
<td>7.5</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>1% glycerol</td>
<td>14</td>
<td>6.9</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>100 mM acetate</td>
<td>14</td>
<td>8.5</td>
<td>8.8</td>
<td></td>
</tr>
</tbody>
</table>

Maximum pH changes during starvation-induced brlA expression

Espeso et al. (1993) have shown that the ipnA gene, which encodes isopenicillin N-synthase, is carbon repressed by a creA-independent mechanism that can be overcome by external alkaline pH. We monitored pH changes during growth and liquid sporulation in different carbon sources. The pH values in Table 1 show that incubation in the different carbon sources resulted in different degrees of medium alkalization. The largest differences occurred during the first 6 h of incubation, and acetate and 1% glucose resulted in the strongest alkalinization after 24 h. When mycelia were shifted to 1% glucose containing NH₄Cl rather than NaNO₃, brlA was fully repressed and, in this case, external pH became acidic instead of alkaline (not shown), indicating that glucose prevented brlA expression in both alkaline and acidic conditions.

These results argue against external pH changes as the sole explanation for the different morphologies observed in repressing and non-repressing carbon sources. However, we observed that submerged sporulation induced either by starvation of glucose, nitrate, ammonium, or by simultaneous glucose and nitrate starvation, always resulted in external pH values close to neutrality (Table 2); in fact, sporulation was reduced, but not prevented, when mycelia were starved of both glucose and nitrate in medium previously adjusted to pH 4 or 8.

**Table 2. Spore production, conidiophore morphology and external pH changes induced by different types of starvation**

Experimental conditions were as indicated for Table 1, except that shifting was to media lacking either glucose, nitrate or ammonium, or both glucose and nitrate. When shifting was to media with or without ammonium, the 18 h pre-growth was done in glucose-ammonium medium. Spore production and conidiophore morphology were determined on 24 h samples. pH was determined in medium samples from 6 and 24 h. Representative results are shown.

<table>
<thead>
<tr>
<th>Type of starvation</th>
<th>10^3 × Spores per culture</th>
<th>Conidiophore morphology</th>
<th>External pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 h</td>
</tr>
<tr>
<td>Glucose</td>
<td>50000</td>
<td>Reduced</td>
<td>6.8</td>
</tr>
<tr>
<td>+ Nitrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Glucose</td>
<td>3100</td>
<td>Reduced</td>
<td>6.0</td>
</tr>
<tr>
<td>+ Ammonium</td>
<td>64900</td>
<td>Complex</td>
<td>6.2</td>
</tr>
<tr>
<td>+ Nitrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Glucose</td>
<td>52800</td>
<td>Complex</td>
<td>6.3</td>
</tr>
<tr>
<td>+ Ammonium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>107000</td>
<td>Reduced</td>
<td>6.5</td>
</tr>
<tr>
<td>Nitrate</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**Without media shifting, submerged sporulation does not occur, even after glucose exhaustion**

All the experiments reported above were done by growing mycelia for 18 h in standard minimal nitrate-glucose medium (Käfer, 1977), and then transferring to different media. The same kind of experiment was performed without media shifting and the resulting culture was sampled at different times. It was found that external pH became strongly alkaline and brlA/lacZ was induced to moderate levels after glucose was exhausted (Fig. 4a). However, no signs of sporulation were observed even after prolonged incubation (morphology was similar to that shown in Fig. 1c; bottom). Most of the reluctance to
maintained around 6.5 and that sodium phosphate buffer. Minimal nitrate-glucose (1 %) samples were harvested and assayed for P-galactosidase.

Fig. 4. brlAllacZ induction (•), glucose consumption (■) and external pH (▲) during growth of A. nidulans in minimal nitrate-glucose liquid medium without (a) or with (b) 0.1 M sodium phosphate buffer. Minimal nitrate-glucose (1 %) medium with or without buffer was inoculated with A. nidulans and grown for 42 h. At the indicated times, mycelial samples were harvested and assayed for β-galactosidase. Medium samples were used for glucose and pH determinations (Methods). A representative experiment is shown.

consider submerged sporulation as a common occurrence, and starvation as a triggering factor, could be related to this finding, since Käfer’s medium is used extensively. Considering that the buffering capacity of Käfer’s medium is very limited (11 mM KH₂PO₄), and that our previous results suggested that strong deviation from external neutral pH could partially prevent full induction and/or function of brlA, we decided to include 0.1 M sodium phosphate buffer in Käfer’s medium and follow external pH, brlA/lacZ induction and morphological changes during growth. Fig. 4(b) shows that external pH was maintained around 6.5 and that brlA/lacZ was induced to higher levels (∼200 U) than those shown in Fig. 4(a) (∼70 U). Abundant sporulation (complex conidiphores) was observed under these conditions by 42 h, showing that reproducible submerged sporulation can be obtained in standard buffered media without media shifting. Even in this case, starvation (glucose exhaustion) seems to be a necessary step. It should be noted that previously reported submerged sporulation was observed in media that included 0.1 M phosphate buffer (Martinelli, 1976).

Concluding remarks

The results presented in this paper show that the brlA gene and sporulation can be induced under defined liquid culture conditions by different environmental stimuli. This could be an important tool to study the signals that initiate development in A. nidulans. Further studies are needed to elucidate the molecular mechanisms by which nutrient starvation induces brlA and how this is affected by external pH. Particularly relevant to this understanding would be the study of starvation effects on genes known to affect brlA expression, such as stnA and modA (Clutterbuck, 1969; Miller, 1993; Aguirre, 1993), as well as the roles that the carbon and nitrogen catabolite regulator genes creA and areA (Arst & Bailey, 1977; Kudla et al., 1990) could have in this process. Developmental competence (Axelrod et al., 1973) is necessary for air-interface-induced conidiation. Thus, it is relevant to address the question of whether or not competence is required for nutrient stress induced sporulation, and we are currently working on this.

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