Differential gene expression in *Thermotoga neapolitana* in response to growth substrate

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Abstract

We have previously shown that \( \beta \)-galactosidase activity expressed in *Thermotoga neapolitana* cells grown on lactose is subject to repression by glucose when they are grown on both substrates whereas \( \beta \)-galactosidase and \( \beta \)-glucosidase activities observed in cells grown on cellobiose are not repressed by growth on both glucose and cellobiose. To examine the differential expression of \( bgalA \), \( bgalB \), \( bglA \) and \( bglB \) in *T. neapolitana*, total RNA was isolated from cells growing on either glucose, lactose or cellobiose as the sole source of carbon and transcripts encoding these genes were quantitated by Northern blot analyses. \( BglA \) expression was induced by cellobiose while \( bglB \) was expressed under all three conditions at a lower level. Expression of the \( \beta \)-galactosidase genes, \( bgalA \) and \( bgalB \), was detected only in lactose-grown cells. \( \beta \)-Glucosidase enzyme activity was only found in cell extracts of cellobiose-grown cells while \( \beta \)-galactosidase activity was found in both lactose- and cellobiose-grown cell extracts. Our results show that in cellobiose-grown cells, the high \( \beta \)-glucosidase activity is likely due to expression of \( bglA \) and that neither \( bgalA \) nor \( bgalB \) is responsible for the \( \beta \)-galactosidase activity. ß 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

*Thermotoga neapolitana* is an obligately anaerobic, Gram-negative bacterium that grows optimally at 77°C by fermentation of a number of sugars. By 16S rRNA sequence analysis, *T. neapolitana* belongs to the deepest branch of bacterial heterotrophs [1] so early members of this lineage may have been among the first organisms to develop the means to control expression of genes allowing catabolism of different substrates. Early, and perhaps simpler, versions of the complex mechanisms of catabolite repression observed throughout the *Bacteria* may have arisen in this lineage. Elucidating the circuits governing the regulation of expression of catabolic genes in *Thermotoga* spp. will help understand the evolution of catabolic pathways and their regulation in bacteria.

Previous studies in our laboratory demonstrated that *T. neapolitana* exhibits a typical diauxic pattern of growth and repressible \( \beta \)-galactosidase activity in medium containing both glucose and lactose, indicating that it possesses a \( \beta \)-galactosidase activity subject to catabolite repression [2]. When grown on cellobiose, *T. neapolitana* cells express both \( \beta \)-galactosidase and \( \beta \)-glucosidase activities. However, these two activities are not subject to catabolite repression by glucose when cells are given both glucose and cellobiose and these activities are catalyzed by two different enzymes. To identify the genes responsible for the \( \beta \)-galactosidase and \( \beta \)-glucosidase activities previously observed in lactose- and cellobiose-grown cells, we present here an examination of the differential expression of genes encoding the known *T. neapolitana* \( \beta \)-galactosidases (\( bgalA \) and \( bgalB \)) and \( \beta \)-glucosidases (\( bglA \) and \( bglB \)) in cells grown on glucose, lactose or cellobiose.

2. Materials and methods

2.1. Organism and growth conditions

*T. neapolitana* NS-E was grown under anaerobic conditions as previously described [3]. The defined TB medium was slightly modified as follows: PIPES buffer was re-
placed with HEPES buffer to a final concentration of 20 mM, and 10 mM Na₂S₂O₃ was added. Resazurin was omitted to avoid interference with the aerobic optical density measurements. Sugar stock solutions of 20% (w/v) were filter-sterilized under anaerobic conditions and added to the medium prior to inoculation.

2.2. RNA preparation

Cultures were harvested in the early exponential phase as determined by optical density and direct cell counts. Typically, 5 ml of an overnight culture was transferred to 500 ml of pre-warmed medium and grown at 77°C to the desired optical density at which time the cultures were rapidly cooled to 4°C. Cells were harvested by centrifugation at 4225×g for 30 min at 4°C and washed once in fresh medium lacking a carbon source and resazurin. Total RNA was isolated using the QIAGEN RNeasy mini-kit with a modified lysing method. For each column, 4×10⁸ cells resuspended in 100 μl 1× SSC were lysed with SDS (0.5% final concentration). Subsequent steps were performed following the QIAGEN RNeasy mini-kit protocol. Contaminating DNA was removed from the RNA solution by treatment with DNase I (Pharmacia FLPCpure) in 40 mM Tris-HCl, pH 7.5, 6 mM MgCl₂ at 37°C for 10 min. Approximately 1 μg of RNA was obtained from 3–5×10⁸ cells. RNA was stored at −80°C until use.

2.3. Northern hybridization

Total RNA extracted from cells grown under each growth condition was resolved in duplicate lanes of FMC Reliant pre-cast RNA gels. The blot probed with bgalB contained 5 μg of RNA in each lane; those probed with bgalA, bgA and bgB contained 7.5 μg. Denaturation of RNA, electrophoresis and ethidium bromide staining were as recommended by the manufacturer (FMC). The molecular mass marker used was Boehringer Mannheim RNA DIG-labeled molecular mass marker II. RNA was transferred by capillary transfer to MSI Magna nylon membranes in 10× SSC and Northern hybridizations were done using digoxigenin-labeled probes following procedures of the Boehringer Mannheim Genius system. DIG Easy Hyb solution was used with a probe concentration of 10–12 ng ml⁻¹. Hybridization conditions, post-hybridization washes and detection of the blots by CSPD were as described [4]. The substrates were o-nitrophenyl-β-D-galactopyranoside and o-nitrophenyl-β-D-glucopyranoside. To determine each specific activity, the linear correlation between activity and amount of extract was determined using 3–12 μg of total protein of extracts of lactose-grown cells for β-galactosidase and of extracts of cellobiose-grown cells for β-glucosidase. The specific enzyme activities in each cell extract were then calculated from those amounts that fell within this linear range.

3. Results and discussion

3.1. Substrate-dependent gene expression

Fig. 1 shows blots of total RNA of T. neapolitana cells grown on either glucose, lactose or cellobiose.
with probes derived from the genes bgalA, bgalB, bglA, bglB and gap. While transcripts of bgalA and bgalB were only detected in cells grown on lactose (Fig. 1A,B), bgA mRNA was only present in celllobiose-grown cells (Fig. 1C) and bglB was found in cells grown under all three conditions (Fig. 1D). As a housekeeping gene, gap was expected to be expressed under all conditions and its signal intensity was used as a measure of the actual amount of RNA present in each lane on the blot and to normalize the signals of the other genes. On the blot shown in Fig. 1E, the amount of gap RNA appears to decrease in the order glucose, lactose and celllobiose. This order of abundances was not observed in other blots probed with gap (not shown), so this pattern is likely due to differences in transfer efficiency during the blotting process.

The estimated sizes of each transcript in kb were 1.45 (bgalA), 1.15 (bgalB), 1.60 (bglA), 2.40 (bglB) and 1.45 (gap). The respective genes encoding these enzymes are 1947 bp (bgalA), 3258 bp (bgalB), 1275 bp (bglA) and 2163 bp (bglB). The gene for gap in T. neapolitana has not been sequenced but the corresponding gene in T. maritima is 1002 bp [5]. The observed sizes of the two β-galactosidase transcripts are smaller than their respective genes while the other three mRNAs are all larger than their coding sequences by a few hundred base pairs. In addition, bgalA, bgalB and bglB mRNAs appeared as smears instead of sharp, distinct bands like the bglA and gap transcripts. Smaller than expected transcripts and diffuse bands could indicate general degradation of RNA, but this possibility does not account for the sharper bgalA and gap transcripts observed in the same RNA samples. Moreover, these samples also showed sharp 16S and 23S rRNA bands on agarose gels stained with ethidium bromide (not shown). A more likely explanation for the smaller than expected bgalA and bgalB transcripts was that they were less stable than the other transcripts and were selectively degraded by Thermotoga ribonucleases.

Gene sequences from both T. neapolitana and T. maritima can account for some of the observed transcripts. The genes bgalA and bgalB in T. neapolitana are separated from one another by an open reading frame (ORF) with little intergenic space (GenBank, accession number AF055482) and bgalB overlaps agalA (α-galactosidase) by 4 bp, suggesting the transcripts we observed likely resulted from transcript-specific loss. The T. maritima bglA gene has been sequenced and a putative promotor and transcriptional terminator flank it ([6] GenBank accession number X74163), but there is no obvious promotor upstream of bglA from T. neapolitana although a potential terminator can be seen downstream ([7] GenBank accession number Z97212). In T. neapolitana, bglB is located 16 bp upstream from lamA, encoding laminarinase [8]. There is a potential terminator downstream of lamA and a gap between an upstream ORF and bglB which contains no obvious promotor, but appears to encode a ribosome binding site. BglB is thus likely encoded on a larger tran-

Fig. 1. Northern blots showing T. neapolitana RNA hybridized with probes derived from (A) bgalA, (B) bgalB, (C) bglA, (D) bglB and (E) gap. Each blot contains duplicate samples of total RNA of T. neapolitana cells grown on glucose (G), lactose (L) and celllobiose (C). The three bars next to each blot indicate the migration of the molecular mass marker fragments of 2.8, 1.9 and 1.6 kb. The membrane used for the blot shown in (E) was the same as that hybridized with bgalA and bglA (A and C, respectively).
Therefore, if the relative L4 identified.

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References


