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Identification and molecular characterization of an endoglucanase gene, *celS*, from the extremely thermophilic archaeon *Sulfolobus solfataricus*

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Abstract A genomic region upstream of the alcohol dehydrogenase (*Ssadh*) gene was cloned and sequenced from a library of *Sulfolobus solfataricus* MT4 strain. The isolated 4,040-bp DNA fragment revealed an open reading frame (*celS*), lying in the opposite direction to *Ssadh*, which showed significant similarity to *endo*-β-1,4-glucanases from *Pyrococcus furiosus*, *Thermotoga maritima*, and *Thermotoga neapolitana*. *celS* was shown to be a functional gene in vivo: a specific *celS* mRNA was detected by primer extension analysis showing a unique initiation transcription site coinciding with the ATG translation initiation codon. The specific gene product was detected as an extracellular cellulase after enzyme staining by carboxymethyl cellulose (CMC) SDS-PAGE, showing a molecular weight in agreement with that deduced from the open reading frame. Depending on growth conditions, different levels of cellulase activity and specific *celS* transcript were detected, revealing an inductive effect of CMC and suggesting a repressive role of glucose.

Key words Cellulase · *Sulfolobus solfataricus* · Glycosyl hydrolase · *endo*-b-1,4-Glucanase · Archaeon

Introduction

Cellulose is one of the most abundant biopolymers on earth (Krassing 1993) and represents an important source of renewable energy. It is composed of D-glucose units linked together to form linear chains via 1,4-β-glucosidic bonds (Salomon and Hudsun 1997). Cellulosic compounds are structurally heterogeneous and have both amorphous and highly ordered crystalline regions that are resistant to enzymatic hydrolysis. The microbial conversion of cellulose to soluble compounds requires several types of enzymes: *endo*-β-glucanases (1,4-β-D-glucan 4-glucohydrolase [EC 3.2.1.4]), exoglucanases (1,4-β-D-glucan cellobiohydrolase [EC 3.2.1.91]), glucan glucohydrolases (1,4-β-D-glucan glucohydrolases [EC 3.2.1.74]), and β-glucosidases (β-D-glucoside glucohydrolase [EC 3.2.1.21]). Endoglucanases decrease the polymer length and increase the concentration of reducing sugars by randomly hydrolyzing internal glycosidic bonds (Wood and Bhat 1988); exoglucanases hydrolyze cellulose chains by removing cellobiose from either the reducing or the nonreducing ends (Teeri 1997); glucan glucohydrolases and β-glucosidases, acting on cello-oligomers and on cellobiose, respectively, produce glucose (Goyal and Eveleigh 1996; Rixon et al. 1992).

Enzyme systems that degrade cellulose have been widely studied in various fungal species (Kitamoto et al. 1996; Ooi et al. 1990), as well as in mesophilic (Spiridonov and Wilson 1999; Wittman et al. 1994; Park et al. 1997) and hyperthermophilic bacteria (Bok et al. 1998; Liebl et al. 1996; Hreggvidsson et al. 1996), mainly because of their huge economic potential in the conversion of plant biomass into fuel and chemicals, as well as in the application of these enzymes in the food and detergent industries (Niehaus et al. 1999).

Cellulolytic organisms also frequently produce other polysaccharases, such as xylanases, mannanases, galactosidases, and β-1,3–1,4-glycanases, which facilitate cellulase access to the substrate. Thermostable poly- and oligosaccharases from hyperthermophilic Archaea have also received considerable attention; among these, the α-amylase (Haseltine et al. 1999) and the β-glycosidase (Cubellis et al. 1990; Pisani et al. 1990) from *Sulfolobus solfataricus*, able to hydrolyze cellobiose and short cello-oligomers (Nucci et al. 1993), and the pullulanase from *Desulfurococcus mucosus* (Duffner et al. 2000) have been identified and extensively characterized. Recently, an endoglucanase, EglA, has been

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characterized from the hyperthermophilic archaeon *Pyrococcus furiosus* (Bauer et al. 1999), and two different glycosyl hydrolase genes, namely *celB* and *lamA*, were found in a locus containing two alcohol dehydrogenase (ADH) open reading frames (ORFs).

The aim of the present work was to search for the presence of glycosyl hydrolase genes on the genomic region preceding the *Ssadh* gene of the hyperthermophilic archaeon *S. solfataricus*. Sequencing and analysis of a cloned DNA region upstream of the *Ssadh* gene showed an ORF (*celS*) that encodes a polypeptide with significant identity degree to *P. furiosus*, *Thermotoga maritima*, and *T. neapolitana* endoglucanases. This gene was demonstrated to be transcribed in vivo, and the transcription start site was identified by primer extension analysis. The corresponding enzyme activity capable of degrading the β-1,4 bonds of carboxymethyl cellulose (CMC) was identified, and different conditions of growth were also investigated to analyze *celS* expression at both mRNA and protein levels.

Materials and methods

Bacterial strains, media, and growth conditions

S. solfataricus MT4 strain was cultivated on gelrite (Phytagel; Sigma, St. Louis, MO, USA) plates at 80°C according to Cannio et al. (1998). *Escherichia coli* BO3310 (Cannio et al. 1994) was used as host cells for library propagation.

S. solfataricus MT4 strain was grown at 80°C with rotary shaking in different media. Brock's salt (Brock et al. 1972) was supplemented alternately with 0.2% glucose, 0.2% lichenan, 0.2% CMC, or 0.2% Avicel as the carbon sources for minimal medium and supplemented with 0.1% yeast extract and/or 0.1% casamino acids for rich media. *E. coli* BO3310 was grown at 37°C with rotary shaking in Luria-Bertani (LB) medium (Miller 1972) containing 100 μg/ml ampicillin.

Construction of *S. solfataricus* MT4 strain gene bank

A gene bank (2×10^7 independent clones per microgram of plasmid DNA) of *S. solfataricus* MT4 strain was constructed as described previously (Cannio et al. 1994). Chromosomal DNA was extracted and partially digested with *Sau*3AI; purified fragments of 2–4 kb were cloned into pGEM7Zf(+) (Promega Italia, Milano, Italy). The screening of the genomic bank of *S. solfataricus* MT4 strain was performed by colony hybridization using as a probe a DNA fragment containing 315 bp upstream of the *Ssadh* gene (Cannio et al. 1999) and labeled by the incorporation of [α^{32}]dATP with a random priming kit (Roche, Monza, Italy). Hybridization was carried out for 16 h at 65°C in 10 ml of hybridization mixture (6 × SSC [sodium saline citrate], 5 × Denhardt's, 0.5% SDS [sodium dodecyl sulfate]); the filters were washed twice at 65°C in 2 × SSC, 0.5% SDS, once in 1 × SSC, 0.5% SDS, and at 68°C in 1 × SSC, 0.5% SDS. Clones that proved positive at the first selection were purified after a second colony hybridization screening before being digested with several restriction enzymes and analyzed by Southern blot in the stringency conditions described.

DNA sequencing was carried out using Sanger's dideoxynucleotide termination method (Sanger et al. 1977) with a Sequenase version 2.0 kit (Amersham, Milano, Italy) on double-stranded DNA using universal forward and reverse primers and primers constructed ad hoc. DNA sequence analysis, including assembling and editing of the data, analysis of the putative ORFs, and restriction map, was performed using the Strider program. Homology comparison and multiple sequence alignment were performed using Blast and Clustal W programs respectively, available on the Internet.

Isolation of total RNA and primer extension analysis

Total RNA was extracted in the late exponential growth phase by the guanidinium isothiocyanate method (Sambrook et al. 1989). The integrity and concentration of total RNA were verified by electrophoretic analysis by fractionating the total RNA on 1% agarose gels containing formaldehyde. Primer extension was performed according to Limauro et al. (1992) using the synthetic oligonucleotide 5′-CACTGAAAGGACGATTATGAGC-3′. The sequencing reaction of the corresponding DNA fragment cloned, which had been primed with the same synthetic oligonucleotide, was used as a marker to locate the products on 6% urea polyacrylamide gel.

Detection of the extracellular cellulase

Cellulase activity was detected in *S. solfataricus* MT4 strain both from cells cultivated on solid media and from culture supernatants. To reveal cellulase activity on plates, cells were grown for 4 days at 80°C on plates on rich medium (yeast extract and casamino acids) containing 0.2% CMC. The plates were stained with 0.1% (w/v) Congo red solution for 30 min and washed with 1 M NaCl for 10 min. Cellulase activity (CMCase) was visualized as a clear halo zone around colonized areas against the red background.

Cellulase activity was then assayed on the culture supernatants of cells grown to early stationary phase. Cells were harvested by centrifugation at 5,000 *g* for 10 min at 4°C. The culture supernatant was concentrated 1,000-fold by ultrafiltration through a YM10 membrane (cut-off, 10 kDa) in an Amicon cell and dialyzed against 50 mM phosphate buffer, pH 7.4.

The CMCase assay was performed in triplicate in a mixture containing 50 mM K_2HPO_4, 12.5 mM citric acid, 0.25% CMC, at pH 5.8 and 65°C. The enzymatic activity was measured by monitoring the release of reducing sugars (Nelson 1944); boiled samples were used as a control to cut off the reducing sugars already present in the supernatant. One unit of enzyme activity is defined as the amount of enzyme required to release 1 μmol of glucose-equivalent reducing groups per minute at 65°C under described conditions. Specific activity was expressed as units per milligram of protein.

The culture supernatants were also analyzed on CMC-SDS-PAGE (polyacrylamide gel electrophoresis) to detect CMCase activity. After separation of protein samples in SDS polyacrylamide gel containing 0.1% (w/v) CMC, the proteins were renatured by washing the gel at room temperature for 30 min in a mixture (4 : 1 v/v) of 100 mM succinate buffer, pH 5.8, and 2-propanol, and for 30 min in 100 mM succinate buffer, pH 5.8. Last, the gel was submerged in 100 mM succinate buffer, pH 5.8, at 65°C for 120 min, renewing the buffer every 30 min. The gel was stained with 0.1% (w/v) Congo red solution for 30 min and destained with 1 M NaCl (Beguin 1983).

Results

Cloning and sequence of *S. solfataricus* MT4 *celS* gene

The *adh* locus in *S. solfataricus* MT4 strain was studied in the search for sequences related to glycosyl hydrolase by characterizing the more extended *Ssadh* flanking regions cloned in a highly representative genomic library. This study began by selecting and analyzing a recombinant plasmid, pGEM1.1, which contained the longest 5′-flanking region of the *Ssadh* gene with an insert of 4,040 base pairs (bp).

Figure 1 shows the physical map of this region and the relative position of the two different ORFs (*orf1* and *celS*) identified by nucleotide sequence analysis. The protein translated from *celS* was found to be significantly similar to prokaryotic thermophilic glycosyl hydrolases. This ORF was separated from ORF1 by a 376-bp spacer and encoded a protein of 322 amino acids whose calculated molecular mass was 36,680 kDa. The CelS putative protein showed the highest identity (Fig. 2) with two thermostable *endo*-β-1,4-glucanases from *T. maritima* (CelB) and from *T. neapolitana* (CelB) (Bok et al. 1998; Liebl et al. 1996) and with a hyperthermophilic archaeal endoglucanase from *P. furiosus* (EglA) (Bauer et al. 1999). CelS, as the similar thermophilic enzymes mentioned, showed significant homology with the glycosyl hydrolases classified in family 12 (Henrissat 1998), albeit restricted to the catalytic region, with two residues, glutamate 197 and glutamate 299; these were recognizable as the possible active site nucleophile and proton donor, respectively. Unlike some of the family 12 endoglucanases and similar to EglA from *P. furiosus*, CelS seems to contain no cellulose-binding domain.

Cellulase activity expressed by *S. solfataricus* in different media

The functional role of the *celS* gene in vivo was first studied at the enzyme expression level under different medium compositions supporting cell growth. The utilization of 1,4-β-glucans by *S. solfataricus* MT4 was investigated by testing the ability of the cells to grow on Avicel, CMC, or lichenan as the sole carbon source in batch cultures. No growth was observed in these complex carbohydrates, even after very prolonged incubation times; in minimal media, this strain was able to grow on simpler cellulose-related mono- and disaccharides, such as glucose and cellobiose.

To demonstrate the presence of cellulase activity, *S. solfataricus* MT4 was cultivated under the optimal conditions supporting growth, i.e., in rich medium containing yeast extract and casamino acids, and aliquots were spotted onto plates containing the same medium supplemented with 0.2% CMC. Figure 3B shows a clear halo zone of CMC hydrolysis around the growth area of the cells after staining with Congo red, indicating cellulase activity in the MT4 strain; the negative control plate (Fig. 3A) shows that *S. solfataricus* MT4 strain and the medium used do not interfere per se with the staining procedure.

Depending on growth conditions, different expression of cellulase activity was revealed by enzymatic assays in the supernatant of the MT4 cultures. In all media tested, the highest activity levels were detected in the supernatants of cultures grown to the early stationary phase in the presence of CMC as indicated in Table 1; however, the presence of casamino acids in the medium interfered with the inducing effect of CMC. In addition, CMCase activity was completely undetectable in minimal medium containing only glucose as carbon source, suggesting a possible repressive role of the monosaccharide on enzyme expression.

The rich medium (yeast extract and casamino acids) without CMC was chosen to produce the cellulase and facilitate further analysis because of the lower viscosity of the concentrated supernatants when compared to those containing the inducer. The *S. solfataricus* cellulase enzyme was clearly identified in CMC-SDS-PAGE analysis; after

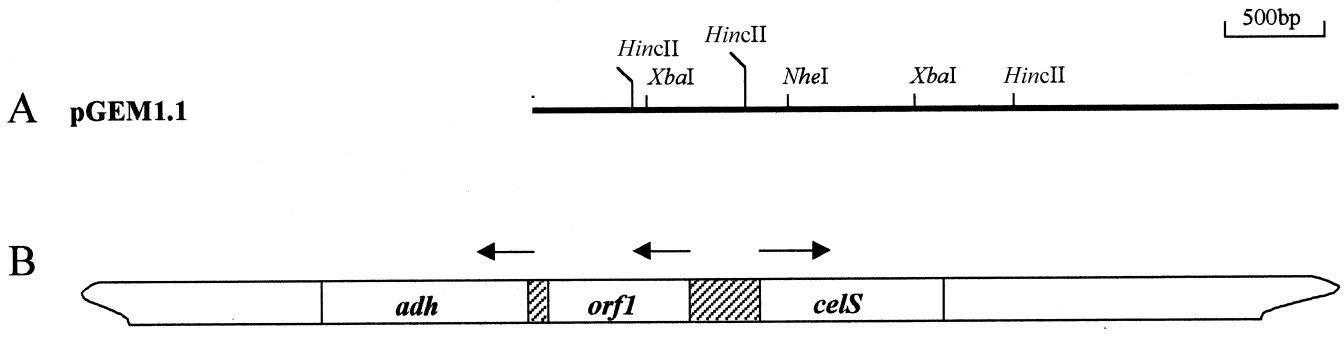

Fig. 1. Physical and genetic maps of *Ssadh-orf1-celS* genes. **A** Restriction map of the recombinant plasmid pGEM1.1. **B** Genetic organization of the *Ssadh* region

Fig. 2. Multiple sequence alignment of thermophilic endoglucanases: *Ss CelS*, *Sulfolobus solfataricus* cellulase S; *Pf EglA*, *Pyrococcus furiosus* endoglucanase; *Tm CelB*, *Thermotoga maritima* cellulase B; *Tn CelB*, *Thermotoga neapolitana* cellulase B. The percentages shown to the *right* of the sequences indicate the amino acid sequence identities between the *S. solfataricus* MT4 cellulase and the thermophilic enzymes reported here. *Numbering* of *S. solfataricus* CelS is indicated; the nucleophile and proton donor residues of the CelS putative active site are in *bold*. *Asterisks* and *dots* indicate the identical residues and the conservative substitutions, respectively

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                1                                                    53
Ss CelS         --MNKLIPIFVVVIIVLGIIVSIEFG-----KFHQNASLTRSTERFTLFPAHNRPFSVLG
Pf EglA         MSKKKFVIVSILTILLVQAIYFVEKYHTSEDKSTSNTSSTPPQTTLSTTKVLKIRYPDDG
Tm CelB         ------MRWAVLLMVVFSALLFSS-----------------EVVLTSVGATDISFN---
Tn CelB         ------MRLVVSFLLVVSAFLFSA-----------------EVVLTDIGATDITFK---
                      :   :  :::.  :                       ::   . .  :

                54                                                  111
Ss CelS         NYSSNSADALAILNSSTNATLMVSPFLWNIGYALG-NVNMTININYLHVAINLSQ-ISKI
Pf EglA         EWPGAPIDKDGDG----NPEFYIEINLWNILNATGFAEMTYNLTSGVLHYVQQLDNIVLR
Tm CelB         ---GFPVT-------------MELNFWNVKSYEG--ETWLKFDGEKVEFYADLYNIVLQ
Tn CelB         ---GFPVT-------------MELNFWNVKSYEG--ETWLKFDGEKVQFYADIYNIVLQ
                   . .               :.  :**:    *  :   .:    :.   :: :*

                112                                                 170
Ss CelS         S-SNVVDGYPGLMYGQELWWPFMYRTTQLQFLSLPMIVLRLPNFYSILNYSVYLINGSID
Pf EglA         DRSNWVHGYPEIFYGNKPWNANYATD---GPIPLPSKVSNLTDFYLTISYKLEPKNGLPI
Tm CelB         NPDSWVHGYPEIYYGYKPWAGHNS-----GVEFLPVKVKDLPDFYVTLDYSIWYENNLPI
Tn CelB         NPDSWVHGYPEIYYGYKPWAAHNS-----GTEILPVKVKDLPDFYVTLDYSIWYENDLPI
                . .. *.*** : ** : *             **  *  *.:**  :.*.:   *.

                171                                                 229
Ss CelS         DFSYDIWLSQNPNITS-LQYGDFEIMIWMYWNENLSHTPYFIYVGNMSIPTLINGKIENL
Pf EglA         NFAIESWLTREAWRTTGINSDEQEVMIWIYYDG---LQPAGSKVKEIVVPIIVNGTPVNA
Tm CelB         NLAMETWITRSPDQTS-VSSGDAEIMVWFYNNV---LMPGGQKVDEFTTTVEINGVKQET
Tn CelB         NLAMETWITRKPDQTS-VSSGDVEIMVWFYNNI---LMPGGQKVDEFTTTIEINGSPVET
                ::: : *::::..  *: :. .: *:*:*:* :      *    * ::  .  :**   :

                230                                                 289
Ss CelS         SWEVYVLPRTGSANGWTGVYFLSPLKEPKAEFGVPIGYILKNMGSYIEKAGVNIYNVNTY
Pf EglA         TFEVWKAN-----IGWEYVAFRIKTPIKEGTVTIPYGAFISVAAN-ISS----LPNYTEL
Tm CelB         KWDVYFAP-----WGWDYLAFRLTTPMKEGKVKINVKDFVQKAAEVVKKHSTRIDNFEEL
Tn CelB         KWDVYFAP-----WGWDYLAFRLTTPMKDGRVKFNVKDFVEKAAEVIKKHSTRVENFDEM
                .::*:         **  : *       .. . .    ::.  .. :..    : *

                290                             322
Ss CelS         YLDAIQVGMEFSD-NQGTAIMGYYLYSWQIWLLS-----
Pf EglA         YLEDVEIGTEFGTPSTTSAHLEWWITNITLTPLDRPLIS            30.0%
Tm CelB         YFCVWEIGTEFGDPNTTAAKFGWTFRDFSVEVVK-----            29.8%
Tn CelB         YFCVWEIGTEFGDPNTTAAKFGWTFKDFSVEIGE-----            29.0%
                *:   ::* **.  .  :* : : : .  :   .
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Fig. 3. Detection of cellulase activity. **A** *Sulfolobus solfataricus* MT4 strain grown on yeast extract and casamino acids. **B** *S. solfataricus* MT4 strain grown on yeast extract, casamino acids, and 0.2% carboxymethyl cellulose (CMC). Clear halo zones of CMC hydrolysis are detected around the growth area of the MT4 (**B**)

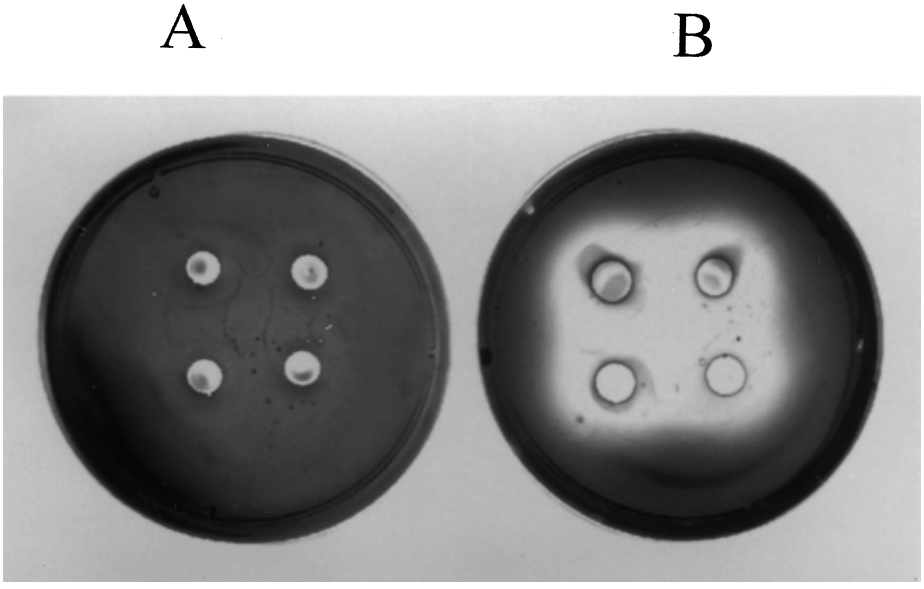

Table 1. Specific cellulase activity in response to different growth conditions

Medium	Specific activity (U/mg)
Brock's salt, yeast extract + casamino acids	0.014 ± 0.002
Brock's salt, yeast extract + casamino acids + CMC	0.170 ± 0.04
Brock's salt, yeast extract + CMC	0.490 ± 0.05
Brock's salt + glucose	NA

The enzymatic activity was measured as indicated in Materials and methods
One unit (U) of enzyme activity is defined as the amount of enzyme required to release 1 µmol of glucose-equivalent reducing groups/min at 65°C under described conditions
Specific activity is expressed as units/mg protein
CMC, carboxymethyl cellulose; NA, no activity detectable

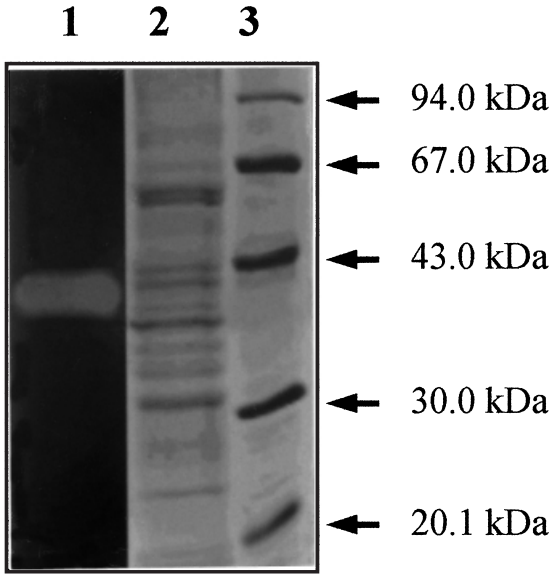

Fig. 4. Detection of cellulase activity by CMC SDS-PAGE. Proteins in the culture supernatant of *S. solfataricus* grown in yeast and casamino acids were loaded on SDS-PAGE containing 0.1% CMC. *Lanes 2 and 3*, staining with Coomassie blue of proteins from culture supernatant and molecular weight marker (indicated on *right*), respectively; *lane 1*, cellulase activity staining with Congo red after protein renaturation

electrophoresis, the gel was treated for protein renaturation and for specific enzyme staining with Congo red. As shown in Fig. 4, a halo was produced by one clear protein band with an apparent molecular weight of about 38.4 ± 2.0 kDa, which is in agreement with that deduced from the translation of the *celS* gene.

Transcriptional activity and identification of the transcriptional start site of *celS*

The expression of *celS* at the transcriptional level was evaluated in the same cultured cells used to determine cellulase activity. RNA was harvested from MT4 grown to the early stationary phase in the different media previously described. Northern blot analysis was not sensitive enough to reveal any hybridization signals in all growth conditions tested, indicating that the amount of specific mRNA was low; consequently, a primer extension analysis was performed. The specific 5′-end of *celS* mRNA was successfully revealed and showed that the transcriptional start site was coincident with the first nucleotide of ATG translation initiation codon (Fig. 5). The TATA box (TTTAAA) and BRE element were centered 27 and 33 nucleotides upstream of the initiation site, respectively, and these closely matched the consensus sequences found in archaeal promoters (Dalgaard and Garrett 1993; Qureshi and Jackson 1998).

As shown in Fig. 5, the levels of *celS* transcript changed in response to the growth conditions, with the absence of specific mRNA signals when the strain was grown in glucose minimal medium and an increase in specific mRNA signals at the highest levels in the presence of CMC, i.e., a general trend matching the enzyme production profile.

Discussion

Sugar fermentative metabolism often requires ADHs as the last step of the catabolic pathway both for energy production and for cell defense against toxic oxidizing by-products. In fact, ADHs can concur with glycosyl hydrolases in the metabolism of carbohydrate polymers and, in a few cases, genes coding for these two classes of enzymes can even be found to be physically associated. In the euryarchaeote *P. furiosus*, a cluster of three genes *adhA–adhB–lamA* (*lamA* operon) composed of two tandem *adhs* followed by an *endo*-β-1,3 glucanase (*lamA*) and a β-glycosidase gene (*celB*) are closely linked, and the two transcriptional units are divergent but coregulated by cellobiose and laminarin (Gueguen et al. 1997; Voorhorst et al. 1999).

Similarly, we have isolated a gene close to *adh* from *S. solfataricus* MT4 that shows high similarity with thermophilic endoglucanases belonging to family 12 of glycosyl hydrolases. Nevertheless, physical analysis performed on this *celS-adh* region showed substantial differences with the organization of the *celB-lamA* region of *P. furiosus*. The *celS-adh* gene arrangement seems to be general for the *Sulfolobus* genus, as suggested by the presence of an identical region on the recently sequenced genome of the P2 strain (http://niji.imb.nrc.ca/sulfolobus/), with 100% of identity between the two *celS* nucleotide sequences. A cellulase-specific zymostaining on SDS-PAGE performed on proteins extracted from the culture supernatant of *S. solfataricus* highlighted the presence of a protein with an apparent molecular mass matching the size of the protein deduced from the *celS* sequence.

The results obtained from the transcriptional analysis of *celS* by primer extension showed a unique transcription start site and also revealed infrequent features. In fact, the 5′-end of the transcript coincides with the first nucleotide of the translation initiation codon and is not preceded by any Shine–Dalgarno (SD)-like motif. The presence of mRNAs with very short or entirely absent 5′-untranslated sequences

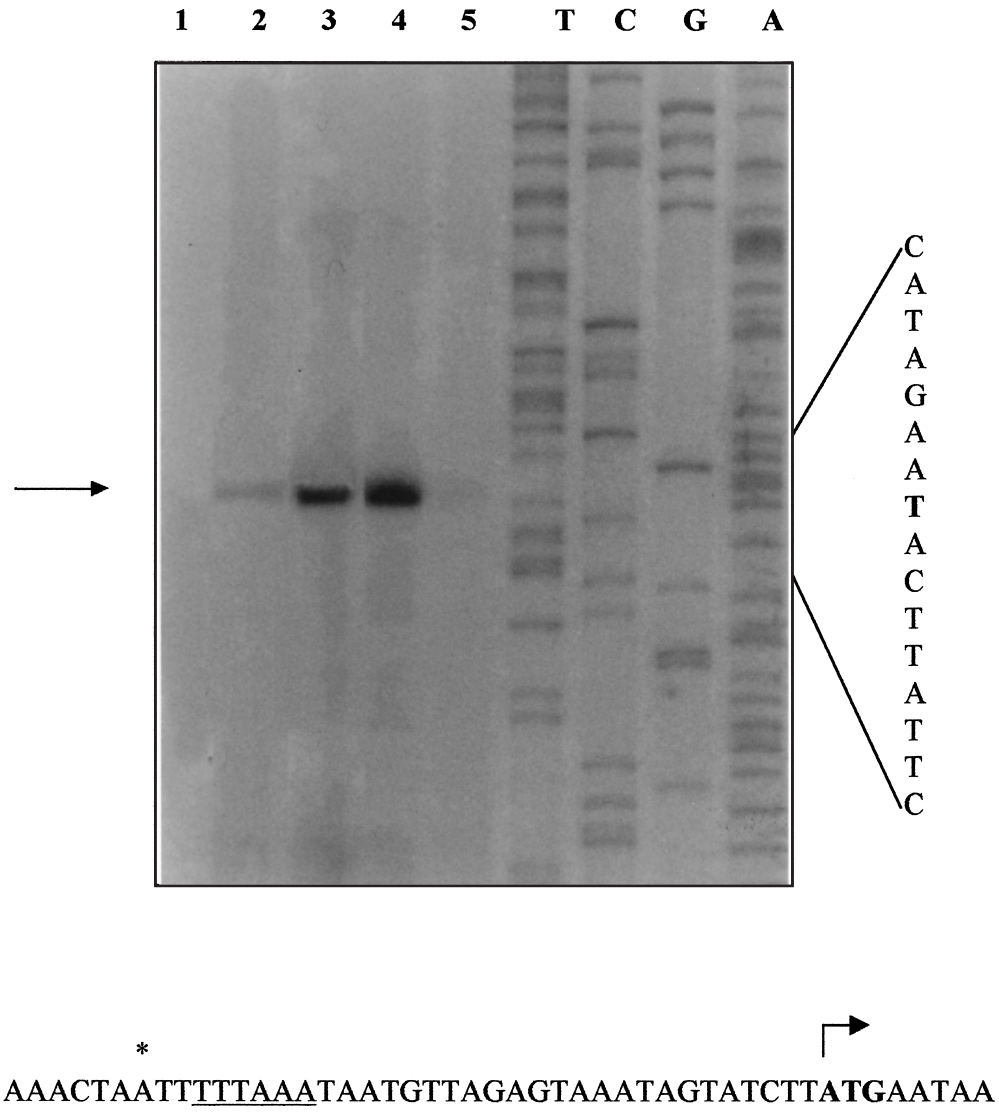

Fig. 5. Primer extension analysis of *celS* mRNA from bovine intestine was used in the primer extension as negative control (*lane 1*). Primer extension products obtained from mRNA of cells grown in different culture media: *S. solfataricus* MT4 grown in Brock's salt supplemented with yeast extract and casamino acids (*lane 2*); yeast extract, casamino acids, and 0.2% CMC (*lane 3*); yeast extract and 0.2% CMC (*lane 4*); or 0.2% glucose (*lane 5*). The sequence ladder derived from pGEM1.1 plasmid annealed to the same primer on the noncoding strand is indicated by *lanes T, C, G,* and *A*. *Below: celS* 5′ region: the transcription initiation site is indicated by the *right-angle arrow*; the initiation codon is in *bold*; the TATA box is *underlined*; the BRE element is indicated by an *asterisk*

was first described in the halophiles (Betlach et al. 1984; Das Sarma et al. 1984) and has also been recently found in *S. solfataricus* (Bell and Jackson 2000). These data were in agreement with the sequence analysis of different archaeal genomes, which indicated that this features is especially represented in the Crenarchaeota domain (Condò et al. 1999); furthermore, leaderless mRNAs have been discovered in all three domains of life.

The levels of the CMCase in the crude culture supernatants varied in response to the carbon sources used for the growth of *S. solfataricus* and followed the same general trend of the transcriptional regulation. In fact, the role of CMC as inducer was demonstrated by both enzymatic and transcriptional analysis; our results showed an increase of about tenfold in CMCase-specific activity when the cells were grown in rich medium containing yeast extract, casamino acids, and CMC with respect to the cells grown without the substrate; in addition, primer extension analysis performed in these conditions followed a similar trend. However, as shown by transcriptional and enzymatic analysis, the inductive effect of CMC was higher when the cells were grown in the absence of casamino acids, indicating their possible repressive role. These results will be further investigated to better understand if specific amino acids can repress cellulase expression, as already described for other glycosyl hydrolases in *S. solfataricus* (Haseltine et al. 1999).

Finally, when MT4 strain was grown in minimal medium in the presence of glucose as the sole carbon source, both the specific *celS* transcript and enzymatic activity were undetectable, suggesting that *celS* could be subjected to a possible catabolite repression-like system (Saier et al. 1996) as observed for other glycosyl hydrolases in *S. solfataricus* (Haseltine et al. 1999; Leichtling et al. 1986).

The DNA sequence data reported here have been submitted to the GenBank data base and have been assigned accession number AJ296029

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