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Molecular basis of cellulose biosynthesis disappearance in submerged culture of *Acetobacter xylinum*⋆

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Acetobacter xylinum **strains are known as very efficient producers of bacterial cellulose which, due to its unique properties, has great application potential. One of the most important problems faced during cellulose synthesis by these bacteria is generation of cellulose non-producing cells, which can appear under submerged culture conditions. The reasons of this remain unknow. These studies have been undertaken to compare at the molecular level wild-type, cellulose producing (Cel$^+$)** ***A. xylinum*** **strains with Cel$^-$ forms of cellulose-negative phenotype. Comparison of protein profiles of both forms of** ***A. xylinum*** **by 2D electrophoresis allowed for the isolation of proteins which were produced exclusively by either Cel$^+$ or Cel$^-$ cells. Sequences of peptides derived from these proteins were aligned with those of proteins deposited in databases. This analysis revealed that Cel$^-$ cells lacked two enzymes: phosphoglucomutase and glucose-1-phosphate uridylyltransferase, which generates UDP-glucose being the substrate for cellulose synthase. DNA was analyzed by ligation-mediated PCR carried out at low denaturation temperature (PCR-MP). Two DNA fragments of different thermal stability (218 and 217 bp) were obtained from the DNA of Cel$^+$ and Cel$^-$ forms, respectively. The only difference between these Cel$^-$ and Cel$^+$ DNA fragments is deletion of one T residue. Alignment of those two sequences with those deposited in the GenBank database revealed that similar fragments are present in the genomes of some bacterial cellulose producers and are located downstream from open reading frames (ORF) encoding phosphoglucomutase. The meaning of this observation is discussed.**

Keywords: bacterial cellulose, *Acetobacter xylinum*, 2-D electrophoresis, PCR-MP, phosphoglucomutase, UDP-glucose pyrophosphorylase

Bacterial cellulose has found multiple applications in various fields owing to its unique physicochemical and mechanical properties (Ring *et al*., 1986; Ross *et al*., 1991). However, the scale of its production, processing and use is relatively small because of problems with selection of sufficiently efficient producers and costs of culture media.

The most efficient producers of bacterial cellulose are the Gram-negative *Acetobacter xylinum* rods (reclassified as *Gluconacetobacter xylinus*) (Yamada, 2000). They occur singly, in pairs or in chains, reproduce by binary fission, are motile by flagella, and do not form endospores. Under limiting conditions, *A. xylinum* strains form involution forms, i.e. swollen or elongated filaments. Under conditions appropriate for bacterial growth these forms atrophy or fragment to shorter pieces which enables recovery of normal cells. The optimum temperature for *A. xylinum* growth is 25–30°C, and optimum pH ranges from 5.4 to 6.2. *A. xylinum* produces cellu-

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Abbreviations: Cel$^+$ cells, wild-type cellulose-producing cells of *Acetobacter xylinum*; Cel$^-$ cells, non-reverting and cellulose-nonproducing forms of *A. xylinum*; c-di-GMP, cyclic di-guanosine monophosphate; CS, cellulose synthase; CTAB, cetyltrimethylammonium bromide; DTT, dithiothreitol; PCR-MP, ligation-mediated PCR performed at low denaturation temperatures; TAE, Tris/acetate/EDTA buffer.

lose on the surface of liquid and solid culture media. Gelatinuous, leather-like mats formed on the surface of liquid culture media under stationary culture conditions contain bacterial cells entrapped in a network of cellulose fibers. Under agitated culture conditions, deposition of pellicle is disrupted and cellulose forms irregular granules stellate and fibrous strands (Bielecki *et al*., 2001). On agar media *A. xylinum* forms colonies with equal or undulate edges, transparent or white, smooth or rough, flat or convex.

A. xylinum strains are prone to spontaneous mutations yielding cellulose non-producing cells, which is one of the major problems facing commercial exploitation of bacterial cellulose biosynthesis. The appearance of Cel^- forms in agitated cultures was first described by Schramm and Hestrin (1954) who isolated three different types of *A. xylinum* cells distinguished by morphology of colonies and efficiency of cellulose biosynthesis :

— Type I: wild-type, cellulose-producing (Cel^+) cells;
— Type II: cellulose-nonproducing forms (Cel^-) capable of reverting through passages;
— Type III: non-reverting cellulose-nonproducing forms (Cel^-).

The morphology of Cel^+ and Cel^- colonies is different. Colonies of Cel^- cells are rough, flat, slimy with undulate edges, in contrast to those of the Cel^+ type, which are spherical with smooth edges, gelatinous and convex.

The frequency of Cel^+ to Cel^- conversion depends on culture conditions and their changes. Cellulose-producing cells dominate in stationary cultures and produce on the surface of culture medium a thick cellulose mat, called a pellicle, in which the embedded bacterial cells have contact with the oxygen-rich liquid/air interface. Sufficient and uniform aeration of liquid culture media under agitated culture conditions favors spontaneous appearance of Cel^- cells, which become dominating in the population. Thus aeration of culture media is believed to be a factor against discriminating Cel^+ *A. xylinum* cells (Leisinger *et al*., 1966).

Studies on the effect of culture medium composition on the efficiency of cellulose synthesis under agitated culture conditions revealed that the highest yields of this biopolymer were achieved in media supplemented with ethanol (2%, v/v). According to Son *et al*. (2001) this phenomenon resulted not from a change in metabolism but from the lack of conversion of Cel^+ cells to Cel^- ones. Hence, the spontaneous change of *A. xylinum* Cel^+ cells to the cellulose-negative phenotype in agitated cultures can be decreased by addition of ethanol to culture media (Son *et al*., 2001; Krystynowicz *et al*., 2002).

Cellulose synthesis by *A. xylinum* cells is catalyzed by four key enzymes:

- glucokinase (EC 2.7.1.2), responsible for phosphorylation on C-6 of glucose,
- phosphoglucomutase (EC 5.4.2.2), which catalyzes isomerization of glucose-6-phosphate to glucose-1-phosphate,
- glucose-1-phosphate uridylyltransferase (EC 2.7.7.9), which synthesizes UDP-glucose (UDPG),
- cellulose synthase (EC 2.4.1.12), which produces cellulose from UDP-glucose.

Glucose-1-phosphate uridylyltransferase (also known as UDPG pyrophosphorylase) is thought to play an important role in cellulose synthesis because the Cel^- forms of *A. xylinum* lack this enzyme (Valla *et al*., 1989). However, the genetics and regulation of transcription of this enzyme in bacteria producing cellulose or related polysaccharides have not been explained.

According to Valla *et al*. (1987), also plasmids can be involved in cellulose biosynthesis. Based on electrophoretic separations of plasmid DNA isolated from *A. xylinum* it was found that nine of thirteen isolated mutants displayed changes in plasmid DNA profile as compared to the wild-type strain.

It was also reported that insertion elements could be involved in polysaccharide synthesis in *Pseudomonas atlantica* (Bartlett & Silverman, 1989) and *Xanthomonas campestris* (Hotte *et al*., 1990). An unstable polysaccharide production by *Zoogloea ramigera* resulted from rearrangement of its DNA (Easson *et al*., 1987). The presence of insertion element IS1031 (950 bp) was detected in *A. xylinum* ATCC 23769. Cel^- mutants of this strain possessed two or more IS1031 elements, and furthermore, their DNA was rearranged within the IS elements, as compared to cellulose-producing wild-type strain (Coucheron, 1991). Therefore, the loss of capability of some *A. xylinum* strains to produce cellulose is supposed to result from dislocation of insertion elements and inactivation of gene(s) responsible for cellulose synthesis.

The presented studies have been undertaken to compare wild *A. xylinum* E_{25} strain and Cel^- forms possessing cellulose-negative phenotype at the molecular level. Genome DNA was isolated from Cel^+ and Cel^- *A. xylinum* E_{25} cells, and compared by PCR-MP (PCR-melting profiles) analysis. Independently, protein profiles of both types of cells were compared by means of two-dimensional electrophoresis. Spots corresponding to the proteins inherent for only one type of the cells were excised from gels, digested and sequenced. The peptide sequences determined in this way were subsequently aligned with protein sequences deposited in databases. Two enzymes involved in the synthesis of various glucose-containing polysaccharides, namely phosphoglucomutase and glucose-1-phosphate uridylyltransferase, were identified as lacking in the Cel^- phenotype. Possible reasons of this phenomenon are discussed.

MATERIALS AND METHODS

Microorganism. *A. xylinum* E_{25} strain from the pure culture collection of the Institute of Technical Biochemistry of the Technical University of Lodz was used for the studies. It was maintained on agar slants at 4°C.

Culture medium. The Schramm and Hestrin (1954) medium (SH medium), containing (g/l): glucose 20.0, yeast extract 5.0, bactopeptone 5.0, Na_2HPO_4 2.7, citric acid 1.15, and $MgSO_4 \times 7H_2O$ 5.7, with initial pH adjusted to 5.7 was used throughout the cultures.

Culture conditions. Inoculum was prepared by transferring a single *A. xylinum* Cel^+ or Cel^- colony from SH agar medium into a 50-ml Erlenmeyer flask containing 10 ml of liquid SH medium. This cell suspension (10 ml) was added into a 500 ml Erlenmeyer flask containing 100 ml of a fresh SH medium. Agitated cultures were incubated at 30°C on a rotary shaker at 90 r.p.m.

Selection of non-reverting mutant. A non-reverting form of *A. xylinum* E_{25} was obtained by reiterated passages under agitated culture conditions. The cultures were carried out for 48 h at 30°C on a rotary shaker at 90 r.p.m. This process was repeated three times (3 passages). Cell suspension from each passage was spread on SH agar medium and incubated for 6 days at 30°C. Colonies with morphology characteristic for Cel^- forms were suspended in 5 ml of liquid SH medium and incubated for 3 days at 30°C to test if the revertants were able to form the cellulose pellicle. Non-reverting forms were isolated from cellulose-free culture broths and used in further studies.

Sample preparation for 2D electrophoresis. A single colony of Cel^+ or Cel^- *A. xylinum* E_{25} was suspended in 5 ml of SH medium and incubated for 48 h. From this culture, a 0.5 ml inoculum was added do 10 ml of SH medium (in a 50 ml Erlenmayer flask) and incubated for 48 h at 30°C. Bacterial cells were harvested by centrifugation (9000 r.p.m., 20 min, 4°C), washed with 40 mM Tris/HCl and disrupted by sonication (3 × 2 min). Proteins were separated using 2-D Clean-Up Kit (Amersham Biosciences) and suspended in a solution containing: 7 M urea, 2 M thiourea, 4% Chaps, 1% DTT and 0.8% ampholites pH 3–10, according to Westemeier *et al*. (2002).

Two-dimensional gel electrophoresis. Two-dimensional electrophoresis was carried out in an Ettan apparatus (Amersham Biosciences). In the first dimension the separation of proteins was based on their isoelectric points (Immobiline DryStrip gel pH 3–10), and in the second dimension the proteins were separated by SDS/PAGE gel (12.5%, 200 × 260 mm) according to the protocol of an Amersham Pharmacia Biotech Technical Manual (1999). The analyzed samples contained 75 μg of proteins which were visualized by Silver Staining Kit (Sigma).

DNA isolation. DNA was isolated from Cel^+ and Cel^- cells of *A. xylinum* E_{25} according to Maniatis *et al*. (1989). Bacterial cells were pelleted by centrifugation (1300 × *g*, 15 min), washed with 10 ml TGE (25 mM Tris/HCl, 50 mM glucose, 10 mM EDTA, pH 8) and again centrifuged (1300 × *g*, 15 min). The pellet was suspended in 10 ml TGE supplemented with lysozyme (10 mg/ml) and incubated at room temperature for 10 min. Next, proteinase K (100 μg/ml) and 1% SDS were added and the mixture was kept at 37°C for 30 min. Then, 2 ml of 5 M aqueous NaCl solution and 1.5 ml of 10% CTAB in 0.7 M NaCl were added to the mixture, which was incubated at 65°C for 20 min. After mixing with equal volume of phenol/chloroform, the mixture was centrifuged (3600 × *g*, 10 min), extracted with a double volume of chloroform/isoamyl alcohol (24:1, v/v), carefully mixed with 2.5 volumes of cold (–20°C) ethanol (96%), and left for 15 min. DNA was collected by centrifugation, the pellet washed 2 times with 70% ethanol, centrifuged at 3600 × *g* for 10 min and dried.

PCR-MP (melting profiles). The PCR-MP procedure was carried out as described by Masny and Płucienniczak (2003). Bacterial DNA prepared as described above was digested with restriction endonuclease *Hin*dIII, extracted with phenol/chloroform, precipitated, the precipitate was dissolved in ligation mixture containing two oligonucleotide adapters: 5′-CTCACTCTCACCAACGTCGAA-3′ (POWIH) and 5′-AGCTTTCGACGTTGG-3′ (HIL) (20 pmol each), in a total volume of 20 μl ligation buffer (66 mM Tris/HCl, pH 8.5, 6.6 mM $MgCl_2$, 10 mM DTT, 66 mM ATP; Amersham Pharmacia Biotech). The mixture was heated in a water bath for 2 min at 56°C and cooled for 10 min at room temperature. Subsequently, 1 μl of T4 DNA ligase (1U/μl) was added and the samples were incubated overnight at 16°C.

Then, PCR reaction was carried out in an MJ Research PTC200 thermocycler at denaturation temperatures (T_d) 80, 81 and 82°C. The reaction mixture contained 50 pmol of POWBAGCT primer 5′-CTCACTCTCACCAACGTCGAAAGCTT-3′, 100 μmol each dNTPs and 1 μl of ligation mixture in 50 μl of PCR buffer. Samples (8 μl out of 50 μl) were loaded on 6% polyacrylamide gel (bisacrylamide/acrylamide = 1:60) with TAE buffer. The gel was stained with ethidine bromide and visualized in UV at 302 nm, according to the protocol of Masny and Płucienniczak (2003). DNA fragments of different thermal stability were isolated from the gel (Dybczyński & Płucienniczak, 1988), digested with *Hin*dIII restriction endonuclease, ligated with the pBluScriptSK$^-$ plasmid (Stratagene) and cloned in *Escherichia coli*, strain NM522. The nucleotide sequences of the inserts were

determined in the Institute of Biochemistry and Biophysics (PAS, Warsaw, Poland).

Protein identification. Proteins were identified by peptide mass fingerprinting using MALDI-TOF mass spectrometry and by partial peptide sequencing with ion trap electrospray mass spectrometry. Trypsin autodigestion peaks were exluded from the database searching. Molecular mass data were obtained from EXPASY (http://www.matrixscience.com/). Monoisotopic peptide masses were used to search protein databases to match and subsequently identify individual protein spots.

RESULTS

PCR-MP

Figure 1 presents a profile of DNA fragments liberated by *Hin*dIII restrictase from the genomes under investigation and amplified at low T_d's according to the procedure described by Masny and Płucienniczak (2003). It appeared that in the case of the cellulose producer strain (Cel^+ form) one of the DNA fragments migrating just above 242 bp was less stable than the corresponding fragment from Cel^-. The DNA fragment from Cel^+ cells was amplified at the denaturation temperature of 81°C, while its counterpart from Cel^- became visible when the T_d was 1°C higher. The less stable fragment (from Cel^+ cells) has a stretch of four T-residues (positions 76–79, Fig. 2), while its counterpart from Cel^- contains at the same positions three T residues. One can suggest that the deletion of a single T residue in the Cel^- fragment is responsible for its increased thermal stability as compared to that of the corresponding Cel^+ DNA fragment.

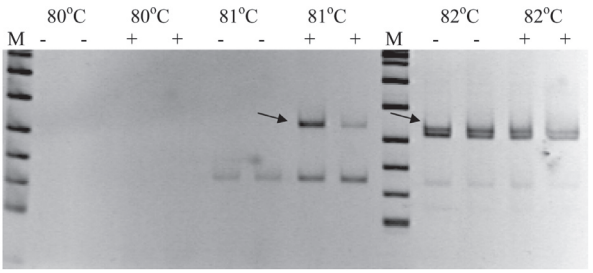

Figure 1. Electrophoretic pattern after PCR-MP of DNA preparations from Cel^- and Cel^+ bacteria (lanes – and +, respectively).
Experiments for each temperature were repeated two times. T_d's of the PCR are marked above electrophoretic lines. DNA bands taken for cloning and nucleotide sequencing are marked with arrows. Lanes marked M, DNA markers: 110+111, 147, 190, 242, 331, 404, 476 and 489 base pairs.

Two-dimensional electrophoresis

Figures 3A and 3B present intracellular proteins (75 μg per gel) isolated from the wild *A. xylinum* E_{25} (Cel^+) strain and its Cel^- counterpart. Electrophoretic separation was carried out in the pH range from 4 to 7 in 12% polyacrylamide gel. Proteins produced only by Cel^+ cells and not synthesized by the Cel^- mutant, as well as proteins found only in Cel^- mutant and lacking in wild-type strain are marked on the gels.

Protein identification

Proteins unique to Cel^+ or Cel^- cells were excised from the gel and sequenced. The results of the analysis of peptides derived from intracellular *A. xylinum* E_{25} (Cel^+) proteins supposedly involved in cellulose biosynthesis and missing in cells of the cellulose-nonproducing mutant are collected in Table 1.

These peptides are similar to amino-acid sequences of the following enzymes:

— phosphoglucomutase from: *Gluconacetobacter xylinus*, *Mycobacterium tuberculosis* (CDC1551), *Bifidobacterium longum* (NCC2705 and DJO10A), *Streptomyces avermitilis* (MA-4680), *Pseudomonas fluorescens* (PfO-1), *Desulfovibrio vulgaris subsp. vulgaris str.*, and *Leifsonia xyli subsp. xyli str.* (CTCB07),

— glucose-1-phosphate uridylyltransferase from: *Gluconacetobacter xylinus* and *Acetobacter pasteurianus.*

DISCUSSION

Cellulose non-producing forms of *A. xylinum* were for the first time described in 1954 (Schramm & Hestrin, 1954). An influence of culture conditions on the frequency of Cel^- phenotypes (Leisinger *et al.*, 1966; Son *et al.*, 2001; Krystynowicz *et al.*, 2002) and activities of enzymes involved in the synthesis of this polysaccharide (Valla *et al.*, 1989) have been reported by several groups. An influence of plasmid DNA and insertion elements in the *A. xylinum* genome on the efficiency of cellulose synthesis was also described (Valla *et al.*, 1987; Coucheron, 1991).

However, the reasons of the appearance of cellulose non-producing *A. xylinum* forms in agitated cultures remain obscure. One can suppose that this effect results from different expression of genes encoding enzymes responsible for glucose metabolism and/or cellulose synthesis. Wong *et al.* (1990) identified in *A. xylinum* the *bcs* operon encoding four proteins essential for bacterial cellulose synthesis. The first gene in this operon, *bcsA,* encodes the regulatory subunit of cellulose synthase (CS), which binds c-di-GMP acting as bacterial second messanger and activator in the cellulose synthesis process (Wong *et al.*, 1990). The second gene, *bcsB,* encodes the catalytic subunit of CS, which binds the substrate, UDP-

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AAGCTTCGAAAGAACGCCACCTTTTTGAAAAAAGGCGGCACCCAAAAACTTTTATTGTTT  60
|||||||| |||  | || ||||||||||||||||||||| |||||||||||| || |||
aagcttcggaag-gcaccgccttttttgaaaaaaggcggcatccaaaaactttttcttctttt

TCTATCAATAAGTTATTTTTCAAACAGTCTCTTGTTGAATTCGGACCGTTCTGGATGATGT 120
| ||||||| |  | |||||||| ||
t-tatcaatgatctgttttcaaatag [AB071166]

ATTGCGCAATAAGGTATTGTTAAAAAAAATAAATAATAAAA-TTTCTGGGTGTCGCCTTTTT 180
                            ||||| ||||||| ||| |||| ||||||||||
                           ataaaaaataaaagtttttgggggtcgccttttt

T---AAAGGCGACGTTCCGGGACATTTTTTGAAAAAAGCTT 218
|    |||||| | || |||||   ||  || |||||||||
Tcaggaaggcggcatttcgggaagcttcctg-aaaaagctt [Y08696.1]
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Figure 2. The nucleotide sequence of the 218 nt element from Cel⁺ *A. xylinum* E_{25} strain isolated after PCR-MP (shown in capital letters) and its comparison with the corresponding *Gluconacetobacter xylinus* and *Acetobacter europaeus* nucleotide sequences.
The array of T residues where deletion of one T occurs in Cel⁻ is shown in bold. Inverted repeat sequences are shown by gray background. The GenBank accession numbers of the sequences under comparison are shown in square brackets.

glucose, and presumably catalyzes the polymerization of 1,4-β-D-glucan. Two other genes belonging to this operon, *dgc* and *pdeA*, encode diguanylate cyclase and phosphodiesterase A, which are responsible for the synthesis and degradation of c-di-GMP, respectively (Ross *et al.*, 1991). Coucheron (1991) reported insertion of the IS1031 element upstream from the transcription start of this operon, which resulted in the deficiency of cellulose synthesis.

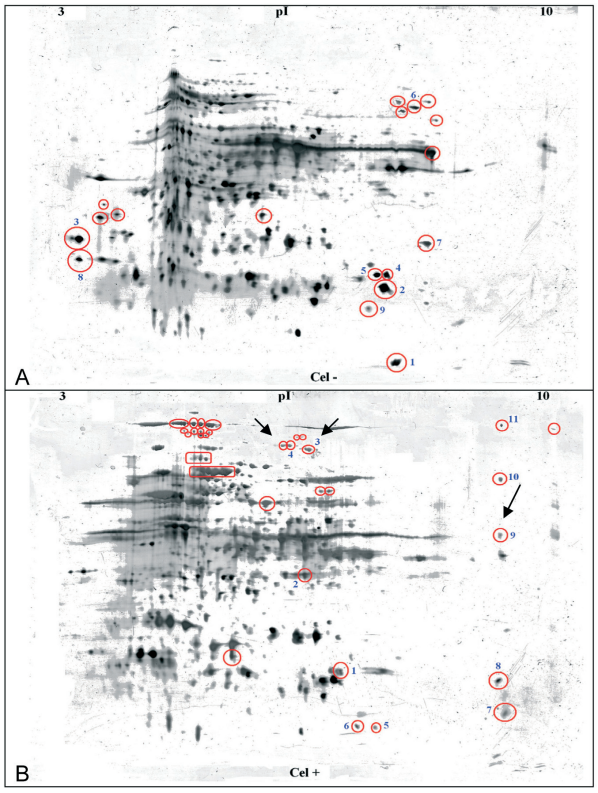

Figure 3. 2-D separation of a lysate of *Acetobacter xylinum* E_{25} cells.
Proteins from (Cel⁻) (A) and wild-type (Cel⁺) (B) were separated by 2-D electrophoresis and visualized by silver staining. The pH gradient (horizontal direction) runs from pH 3–10 and the SDS/PAGE separation (perpendicular direction) was performed in 12.5% gel. The arrows indicate the proteins identified as phosphoglucomutase (spots 3 and 4) and UDP-glucose pyrophosphorylase or glucose-1-phosphate uridylyltransferase (spot 9).

Our studies carried out with the use of the PCR-MP approach (Masny & Płucienniczak, 2003) indicate that genomic libraries from Cel⁺ and Cel⁻ strains contain DNA fragments of different thermal stability. In the case of the cellulose producer (Cel⁺ form) a DNA fragment migrating just above 242 bp is less stable than the corresponding fragment from Cel⁻ cells. A comparison of their sequences has revealed that the thermally less stable fragment (Cel⁺) has array of four T-residues (positions 76–79, Fig. 2), while its counterpart from Cel⁻ cells contains at the same positions only three T residues.

Alignment of the 218 nt sequence identified in our studies with those deposited in the GenBank database (National Center for Biotechnology Information) reveals that several genes of *Acetobacter xylinum* and *Gluconacetobacter oxydans* contain in their 3′-terminal parts (downstream from their ORFs) nucleotide sequences similar to the fragment sequenced in our studies. The 2,702,173 nt-long *Gluconacetobacter oxydans* genome contains five regions showing significant homology to this fragment (Table 4). However, it should be noticed that all these sequences are shorter than 218 nt (see Fig. 2). Because the 218 nt fragment contains inverted repeats (see Fig. 2), one can speculate that the corresponding RNA arising during transcription can form stem-loop or hairpin secondary structures (see Fig. 4). It is known that these types of RNA structures can regulate gene expression by their involvement in the transcriptional terminator/antiterminator mechanism (Turner *et al.*, 1994; Yanofsky, 2000). It cannot be excluded that the loss of the single T residue results in a change of the RNA secondary structure which may influence transcription of the genes involved in cellulose synthesis.

Table 1. Results of analysis of peptide fragments derived from gel slices numbered 3, 4 and 9 (according to Fig. 3).

Gel slice number 3	Gel slice number 4	Gel slice number 9
phosphoglucomutase (PGM)	phosphoglucomutase (PGM)	glucose-1-phosphate uridylyltransferase (UDPGP)
amino acids: 555; M_r: 59654	amino acids: 555; M_r: 59654	amino acids: 284 M_r: 31792
protein ID: P38569 (swissprot); gi|585669 (according to NCBI)	protein ID: P38569 (swissprot); gi|585669 (according to NCBI)	protein ID: P27897 (swissprot); gi|7381245 (according to NCBI)
score: 1394	score: 1735	score: 207
queries matched: 36	queries matched: 44	queries matched: 5
sequence coverage: 68%	sequence coverage: 76%	sequence coverage: 19%

Table 2. Localization of DNA fragments homologous to the 218 nt element

Bacterial strain	GenBank Accession Number (literature reference)	Region of homology within the 218 nt DNA Element-coordinates	Region of homology in bacterial DNA, data from GenBank-coordinates	Genes in the closest proximity to the fragment homologous to the 218 nt element; (gene coordinates; name of the protein; protein id)
Gluconacetobacter xylinus b	AB071166 (Tajima *et al.*, 2001)	1–86	716–799	1137..3356; β-glucosidase
Gluconacetobacter xylinus b	AB091059 (unpublished)	1–86	378–461	808..3036; β-galactosidase
Acetobacter xylinum	L24077.1 (Brautaset *et al.*, 1994)	1–75	1942–2012	135..1812; phosphoglucomutase
Gluconobacter oxydans 621H	CP000009.1 (Prust *et al.*, 2005)	56–1	1137409–1137463	1136433..1137404; putative oxidoreductase; protein id: AAW60805.1 and 1137522..1137968; hypothetical protein; protein id: AAW60806.1
Gluconobacter oxydans 621H	CP000009.1 (Prust *et al.*, 2005)	154–208	1137409–1137467	1136433..1137404; putative oxidoreductase; protein id: AAW60805.1 and 1137522..1137968; hypothetical protein; protein id: AAW60806.1
Gluconobacter oxydans 621H	CP000009.1 (Prust *et al.*, 2005)	1–56	797775–797829	795730..797730; oligopeptidase; protein id: AAW60504.1 and 797839..798789; putative multidrug efflux pump; protein id: AAW60505.1
Gluconobacter oxydans 621H	CP000009.1 (Prust *et al.*, 2005)	1–55	916535–916588	915033..916508; NADPH-dependent L-sorbose reductase; protein id: AAW60623.1 and 916634..917890 (c); UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase; protein id: AAW60624.1
Gluconobacter oxydans 621H	CP000009.1 (Prust *et al.*, 2005)	218–149	916521–916594	915033..916508; NADPH-dependent L-sorbose reductase; protein id: AAW60623.1 and 916634..917890 (c); UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase; protein id: AAW60624.1
Acetobacter xylinus	AB010645 (Nakai *et al.*, 1998)	84–13	16568–16638	12448..14655; β-glucosidase; protein id: BAA31467.1
Acetobacter xylinus	AB010645 (Nakai *et al.*, 1998)	1–60	15344–15402	12448..14655; β-glucosidase; protein id: BAA31467.1
Acetobacter europaens	Y08696.1 (Thurner *et al.*, 1997)	60–1	5991–6049	3604..5925; aldehyde dehydrogenase; protein id: BAA00408.1
Acetobacter europaens	Y08696.1 (Thurner *et al.*, 1997)	148–218	5989–6062	3604..5925; aldehyde dehydrogenase; protein id: BAA00408.1
Acetobacter polyoxogenes	D00521.1 (Tamaki *et al.*, 1989)	62–1	2623–2683	236..2557; aldehyde dehydrogenase precursor; protein id: BAA00408.1
Acetobacter xylinum	Y18467.1 (Edwards *et al.*, 1999)	1–86	1041–1123	32..997; glucosyl transferase; protein id: CAB44443.1

A comparison of the protein profiles of wild-type (Cel^+) *A. xylinum* strains and their Cel^- mutants revealed some differences. Cel^- cells do not synthesize two key enzymes involved in cellulose biosynthesis, namely phosphoglucomutase and glucose-1-phosphate uridylyltransferase. The lack of glucose-1-phosphate uridylyltransferase in cellulose non-producing cells was reported earlier (Valla *et al*., 1989). Our studies proved that *A. xylinum* cells of Cel^- phenotype are also deprived of phosphoglucomutase. Although it is known that phosphoglucomutase and glucose-1-phosphate uridylyltransferase (or UDP-glucose pyrophosphorylase) play a critical role in cellulose synthesis, their genetics and regulation have not been studied in detail.

Taking into account the results of the PCR-MP analysis and 2D electrophoresis as well as the alignment of the nucleotide and peptide sequences, one can suggest that the cellulose deficiency in *A. xylinum* Cel^- cells can result from the lack of expression of the genes encoding phosphoglucomutase and glucose-1-phosphate uridylyltransferase. Our finding that in the Cel^- form the ORF encoding phosphoglucomutase may be flanked by a DNA fragment of a sequence different than that in Cel^+ cells can indicate a possible transcriptional repression of the gene expression.

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