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Cloning of a gene involved in cellulose biosynthesis in *Acetobacter xylinum*: Complementation of cellulose-negative mutants by the UDPG pyrophosphorylase structural gene

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Summary. Three cellulose-negative (Cel^-) mutants of *Acetobacter xylinum* strain ATCC 23768 were complemented by a cloned 2.8 kb DNA fragment from the wild type. Biochemical analysis of the mutants showed that they were deficient in the enzyme uridine 5′-diphosphoglucose (UDPG) pyrophosphorylase. The analysis also showed that the mutants could synthesize β(1-4)-glucan in vitro from UDPG, but not in vivo from glucose. This result was expected, since UDPG is known to be the precursor for cellulose synthesis in *A. xylinum*. In order to analyze the function of the cloned gene in more detail, its biological activity in *Escherichia coli* was studied. These experiments showed that the cloned fragment could be used to complement an *E. coli* mutant deficient in the structural gene for UDPG pyrophosphorylase. It is therefore clear that the cloned fragment must contain this gene from *A. xylinum*. This is to our knowledge the first example of the cloning of a gene with a known function in cellulose biosynthesis from any organism, and we suggest the gene be designated *cel*A.

Key words: Cellulose biosynthesis – *Acetobacter xylinum* – UDPG – Pyrophosphorylase – Gene

Introduction

Cellulose is the dominating biopolymer in the biosphere, with an annual production estimated to be about 10^{11} tons (Colvin 1980; Delmer 1983). Most of the cellulose is synthesized by the green land plants, and the abundance of this renewable resource makes cellulose one of the most attractive raw materials for large scale biotechnological utilization. In spite of these facts, the mechanism of cellulose biosynthesis is still not understood at the molecular level. A highly efficient system for in vitro cellulose synthesis in *Acetobacter xylinum* has been developed (Aloni et al. 1982). Synthesis of cellulose in this organism is carried out by a membrane-bound cellulose synthase, which is subject to a complex multi-component regulatory system (Ross et al. 1987). The synthase utilizes uridine 5′-diphosphoglucose (UDPG) as substrate, and is directly activated by cyclic diguanylic acid (c-di-GMP). Synthesis of UDPG is carried out by UDPG pyrophosphorylase from UTP and glucose-1-phosphate, which is a product of the phosphoglucomutase reaction. The c-di-GMP activator is formed from GTP by diguanylate cyclase, and is degraded to an inactive linear dimer (p5′G3′p5′G) by a membrane-bound phosphodiesterase (PDE-A), which is inhibited by Ca^{2+}. A second phosphodiesterase (PDE-B) further degrades the dimer, forming two molecules of 5′GMP.

None of the structural genes involved in cellulose biosynthesis in *A. xylinum* or any other organism (the *cel* genes, Valla and Kjosbakken 1982) has been isolated and characterized. Recently, however, a gene exerting a pleiotropic effect on polysaccharide biosynthesis (including cellulose) in *Agrobacterium tumefaciens* has been cloned by Tomashow et al. (1987). Our previous work on the *A. xylinum* strain ATCC 10245 also indicated that the biosynthesis of cellulose was affected by genes exerting pleiotropic effects which interfered with the synthesis of the polymer (Valla and Kjosbakken 1982).

We planned to isolate structural genes involved in the synthesis of cellulose by the potential ability of cloned wild-type genes to complement the deficiencies in Cel^- mutants. A major problem with this approach was to discriminate between mutants in the structural genes for cellulose synthesis, and mutants in genes exerting pleiotropic effects. We show in this report that by using another strain of *A. xylinum* (ATCC 23768) in the experiments, it was possible to isolate and complement mutants in at least one structural gene of cellulose synthesis. The gene was cloned in *E. coli*, and was found to be the structural gene for UDPG pyrophosphorylase.

Materials and methods

Bacterial strains and plasmids. For complementation studies, the Cel^- mutants were isolated from a spontaneous streptomycin-resistant derivative of the wild-type of *A. xylinum* strain ATCC 23768. Cel^- mutants isolated for comparison of mutagen-induced mutant frequencies in different strains, were isolated from the respective wild-type *A. xylinum* strains. *E. coli* strains and their relevant characteristics were: S17.1, *rec*A (RP4-2Tc::Mu Km::Tn*7* Tn*1*) (Simon et al. 1983), MC4100 (Casadaban 1976), and FF4001, MC4100 *gal*U95 (Giæver et al. 1988). Plasmids used were: pVK100 Km^r, Tc^r, Tra^- (Knauf and Nester 1982), and pUC19 Ap^r (Norrander et al. 1983).

Growth, harvesting of cells, and isolation and characterization of Cel^- mutants. Cellulose producing cells were grown statically while Cel^- mutants were grown on a gyratory shaker,

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in the medium described by Hestrin and Schramm (1954). Cel^+ cells were freed from cellulose, and Cel^- mutants were isolated after mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine (NG) as described previously for *A. xylinum* ATCC 10245 (Valla and Kjosbakken 1982), except that mutants used for complementation studies (from strain ATCC 23768) were isolated after mutagenesis with 5 µg/ml NG. This mutagen concentration gave rise to low cell killing (less than 50%). For comparison of mutant frequencies in different strains, 20 µg/ml of the mutagen was used. At this high mutagen concentration the frequencies of mutation were at their maximum and most reproducible levels, although cell killing was high for all strains (survival frequencies varied between 10^{-2}–10^{-4}). Mutant frequencies after mutagenesis were determined directly after treatment with the mutagen, as described previously (Valla and Kjosbakken 1982). Resting cells of *A. xylinum* were prepared from 24 h grown cells as described previously (Weinhouse and Benziman 1974). *E. coli* cells were grown in L broth (10 g tryptone, 5 g yeast extract, and 10 g NaCl per litre), or in M63 medium (Miller 1972) supplemented with 0.2% galactose as carbon source. Measurement of extracellular water-soluble polysaccharides, and induction of cellulose synthesis in Cel^- mutants by tetracycline, was performed as described previously (Valla and Kjosbakken 1982).

Isolation and purification of plasmids and total DNA. E. coli plasmids were isolated preparatively by a clear lysate technique (Timmis et al. 1978), while clonal analysis was performed essentially according to Birnboim and Doly (1979). *A. xylinum* plasmids and total DNA were isolated as described previously (Valla et al. 1983, 1987). We were originally unable to obtain *E. coli* transformants from *A. xylinum* plasmid preparations. Recently we found that these plasmid preparations could be used efficiently for transformation of *E. coli* if they were further purified by addition of 0.34 vol. 10 M ammonium acetate prior to the last ethanol precipitation step. The addition of ammonium acetate resulted in formation of a precipitate (after 20 min on ice) which could be removed by centrifugation.

Agarose gel electrophoresis of DNA, and hybridization analysis. Plasmids and linear DNA were separated on 0.7% agarose gels in the buffers described by Meyers et al. (1976) for plasmids, and by Depicker et al. (1980) for linear DNA. For further hybridization analysis, the DNA was transferred to Gene Screen filters (New England Nuclear, Boston, Mass), according to Bittner et al. (1980). Radioactive labelling of DNA was performed by nick translation (Rigby et al. 1977), while hybridization was performed according to Wahl et al. (1979).

Cloning and conjugative matings. For reasons described in the Introduction and Discussion, strain ATCC 23768 was used for the complementation experiments. We had previously made a partially representative gene library of this strain in the broad host range cloning vector pVK100. The library (in *E. coli* S17.1) consisted of about 680 clones with inserts (inactivating the kanamycin resistance marker), and was made by cloning completely *Hin*dIII-digested *A. xylinum* DNA into the *Hin*dIII site of pVK100. The *E. coli* strain S17.1 has a chromosomal insertion containing the functions necessary for mobilization of the pVK100 vector, and the library could therefore be used in conjugative matings with *A. xylinum* without the use of a helper plasmid. The matings were performed on membrane filters, as described previously (Valla et al. 1986). Transconjugants were selected on agar medium containing 1 mg/ml streptomycin and 100 µg/ml tetracycline.

Enzyme assays. Cell-free preparations were prepared according to Aloni et al. (1982). Washed membrane preparations derived from 1 mg cells (dry weight) typically contained 0.23–0.27 mg protein. The crude supernatant fraction derived from the membrane preparations contained 0.1–0.3 mg protein. Cellulose synthase activity was measured as the rate of incorporation of radiolabelled glucose from UDP-D-[U-^{14}C]glucose into an alkaline insoluble product, which has been characterized as a $\beta(1,4)$-glucan (Aloni et al. 1982). Diguanylate cyclase was determined by measuring the rate of formation of c-di-GMP from [α-^{32}P]GTP (Ross et al. 1987). PDE-A activity was assayed by following the rate of degradation of [α-^{32}P]c-di-GMP (Ross et al. 1986), and PDE-B activity was determined by following the rate of degradation of p5′G3′p5′G (Ross et al. 1987). The labelled guanyl dinucleotide substrates were prepared and purified according to Ross et al. (1987). Phosphoglucomutase activity was determined by assaying the rate of formation of glucose-6-phosphate from glucose-1-phosphate. UDPG pyrophosphorylase was assayed as the rate of formation of glucose-1-phosphate in the presence of UDPG and inorganic pyrophosphate. The coupled assay system for the two enzymes was employed as described by Joshi (1982), and Hansen et al. (1966). Cells were sonicated, and the supernatant (containing 1–2 mg/ml of protein) obtained after centrifugation (at 4° C) for 1 h at 100000 *g*, was used in the assay. For *A. xylinum* the sonication buffer consisted of 50 mM TRIS-HCl (pH 7.5), 10 mM $MgCl_2$, and 1 mM Na_2EDTA, while 100 mM TRIS-acetate (pH 7.8), 1 mM β-mercaptoethanol was used for *E. coli.* The assay mixture (1 ml) for phosphoglucomutase contained 100 mM TRIS-HCl (pH 7.8), 10 mM $MgCl_2$, 0.5 mM NaNADP, 5 mM glucose-1-phosphate, 66 µM glucose-1,6-diphosphate, and 1.3 units of glucose-6-phosphate dehydrogenase (Sigma, Bakers yeast). For UDPG pyrophosphorylase, the assay mixture (1 ml) for *A. xylinum* contained: 100 mM TRIS-HCl (pH 7.8), 5 mM $MgCl_2$, 0.5 mM NaNADP, 1.25 mM UDPG, 2.5 µM glucose-1,6-diphosphate, 5 units/ml phosphoglucomutase (Sigma, rabbit muscle), 5 units/ml glucose-1,6-phosphate dehydrogenase (Sigma, bakers yeast). For *E. coli* it contained: 100 mM TRIS-acetate (pH 7.8), 2 mM magnesium acetate, 0.4 mM NaNADP, 0.4 mM UDPG, 2.5 µM glucose-1,6-diphosphate, 1 unit/ml phosphoglucomutase (Sigma, rabbit muscle), 1 unit/ml glucose-1,6-phosphate dehydrogenase (Sigma, bakers yeast).

Results

Cloning of a DNA fragment which restores cellulose synthesis in Cel⁻ mutants

A gene library (in *E. coli*) representing parts of the genome of *A. xylinum* ATCC 23768 wild-type, was mated conjugatively with 14 Cel^- mutants of the same *A. xylinum* strain. Transconjugant colonies were selected on agar medium containing streptomycin and tetracycline. Since Cel^+ colonies can be distinguished from Cel^- colonies by direct visual

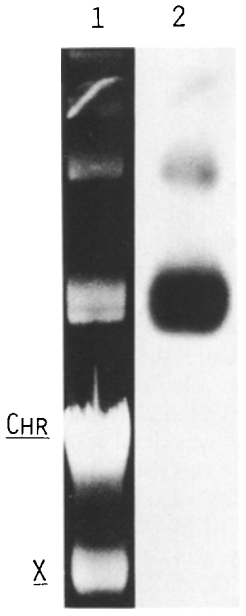

Fig. 1. Extrachromosomal localization of the complementing recombinant plasmid pVK100(246) in Cel28. Lane 1, plasmid profile of Cel28(pVK100(246)). Chr, chromosomal DNA fragments; X, naturally occurring plasmid of strain ATCC 23768. Lane 2, hybridization of DNA in lane 1 against ^{32}P-labelled pVK100(246) DNA. Prolonged exposure of the film showed that there is also weak hybridization to the chromosomal DNA fragments

inspection (Valla and Kjosbakken 1982), thousands of transconjugants could be screened rapidly for their cellulose synthesizing ability. In three of the crosses, using the Cel$^-$ mutants Cel11, Cel14, and Cel28 as recipients, a low frequency (10^{-3}–5×10^{-4}) of Cel$^+$ transconjugants per total transconjugants was detected, indicating that complementation might have taken place.

We were originally unable to obtain *E. coli* pVK100 transformants from *A. xylinum* transconjugants, but this problem has now been solved by a minor modification of the plasmid isolation procedure. Figure 1, lane 1, shows the plasmid profile of one of the Cel$^+$ transconjugants obtained after mating of the gene library with strain Cel28. This plasmid preparation was used to transform *E. coli* S17.1, selecting for tetracycline resistance. Analysis of the plasmids in the resulting transformants showed that they contained a pVK100 plasmid with a 2.8 kb DNA insert. The recombinant plasmid (pVK100(246)) was labelled with ^{32}P, and hybridized against the separated plasmids in Fig. 1, lane 1. The results of this experiment (lane 2), clearly identified the vector in an extrachromosomal location in the Cel$^+$ transconjugant, and it was also evident that there is at least one naturally occurring plasmid in this *A. xylinum* strain. Plasmid pVK100(246) was finally transferred conjugatively to Cel11, Cel14, and Cel28. All transconjugant colonies obtained in this mating had acquired the Cel$^+$ phenotype, thus confirming that the cloned fragment was complementing the cellulose synthesizing deficiency in the three Cel$^-$ mutants.

Biochemical characterization of the complemented Cel$^-$ mutants

Cellulose synthesis in vivo and β(1,4)-glucan synthesis in vitro. The cellulose synthesizing capacity of the various strains was tested in resting non-proliferating cells with D-[U-^{14}C]glucose as substrate (Table 1). In all cases tested, the rate of cellulose synthesis was linear up to 2 h. Although the three mutant strains grew readily on medium containing glucose, their ability to convert glucose into cellulose was greatly impaired. The complemented strains, on the other hand, had acquired a markedly restored cellulose synthesizing ability.

Table 1. Rates of cellulose synthesis in vivo, and cellulose synthase activity in vitro

Acetobacter xylinum strains	Glucose incorporation (pmol/min)	
	In vivo per mg cells	In vitro per mg protein (with/without c-di-GMP in assay)
23768 wild-type	1990	1120/10
Cel11	98	1010/ 2
Cel14	48	1120/ 1
Cel28	45	830/ 4
Cel11(pVK100(246))	613	892/ 7
Cel14(pVK100(246))	906	1010/ 4
Cel28(pVK100(246))	1270	865/12

Rates of cellulose synthesis in vivo were calculated from the amount of labelled glucose incorporated into alkaline insoluble product by resting cells (1 mg dry weight/ml) during 15, 30, and 120 min of incubation in the presence of 40 mM D-[U-^{14}C]glucose and 50 mM phosphate buffer, pH 5.0, at 30° C (Weinhouse and Benziman 1974). Cellulose synthase activity was assayed in the absence and presence of 10 μM cyclic diguanylic acid (c-di-GMP), in washed membrane preparations with 30 μM UDP-D-[U-^{14}C]glucose as substrate

The in vitro cellulose synthesizing capacity of the Cel$^-$ and wild-type strains was then measured. This was done by measuring the rate of transfer of glucose from UDP-D-[U-^{14}C]glucose into a cellulosic (β(1,4)-glucan), alkaline insoluble product under conditions standardized for other strains of *A. xylinum* (Aloni et al. 1982). As predicted, preparations from the Cel$^+$ strains exhibited high levels of cellulose synthase activity. Surprisingly, the Cel$^-$ mutants showed similar in vitro cellulose synthesizing capacity to the wild-type (Table 1). All mutant strains possessed a c-di-GMP sensitive, membrane-associated synthase which, when assayed at low concentrations of substrate in the presence of the activator, achieved a synthetic rate essentially equivalent to that of the cellulose producers. Significantly, these in vitro rates, when extrapolated to the K_m concentration (0.2 mM) of UDPG (Aloni et al. 1982), and expressed per milligram dry weight of cells, were comparable with the values obtained in vivo for Cel$^+$ strains utilizing glucose.

In vitro activity of diguanylate cyclase, PDE-A, PDE-B, phosphoglucomutase, and UDPG pyrophosphorylase. Diguanylate cyclase was assayed in a crude supernatant fraction of washed membranes. The phosphodiesterases PDE-A and PDE-B, degrading c-di-GMP and p5′G3′p5′G respectively, were assayed in membrane preparations. All three activities were detected in comparable levels in the strains (Table 2). Additionally, in all cases tested, PDE-A activity was inhibited more than 95% in the presence of 2.5 mM $CaCl_2$ (data not shown). Phosphoglucomutase and UDPG pyrophosphorylase were assayed in the crude supernatant

Table 2. Diguanylate cyclase (DC), phosphodiesterase PDE-A and PDE-B, phosphoglucomutase (PM), and UDPG pyrophosphorylase (UP) activity

Acetobacter xylinum strains	DC	PDE-A	PDE-B	PM	UP
	(nmol/min per mg protein)				
23768 wild-type	0.42	3.34	80×10^{-3}	1100	2200
Cel11	0.80	3.60	80×10^{-3}	610	135
Cel14	0.85	3.70	92×10^{-3}	1200	20
Cel28	0.55	2.23	85×10^{-3}	620	25
Cel11(pVK100(246))	0.63	1.92	65×10^{-3}	910	1250
Cel14(pVK100(246))	0.93	5.80	22×10^{-2}	600	780
Cel28(pVK100(246))	0.73	4.50	18×10^{-2}	610	807

Diguanylate cyclase activity is expressed as the amount of c-di-GMP formed from GTP, while PDE-A and PDE-B are expressed as the amount of substrate degraded. Phosphoglucomutase and UDPG pyrophosphorylase activities refer to the amount of substrate transformed

Table 3. Biological activity of the cloned DNA fragment in *Escherichia coli*

Escherichia coli strain	UDPG pyrophosphorylase activity (nmol/min per mg protein)
MC4100	42.8
FF4001(pUC19)	1.6
FF4001(pKF10)	539.0

Note that the activities are not directly comparable with the corresponding activities described in Table 2, since the assay conditions were not identical

fraction obtained after centrifugation of sonicated cells (Table 2). No discernable differences were observed among the strains in the level of phosphoglucomutase activity. The level of UDPG pyrophosphorylase, however, was significantly lower (less than 5% of that of the wild-type) in the Cel$^-$ mutants, and was restored (up to 50% of the wild-type level) after complementation. The level of this enzyme in extracts of Cel$^+$ strains was not affected by growth under shaking conditions (data not shown).

Analysis of the biological activity of the cloned DNA fragment in E. coli

The biochemical analysis of the Cel$^-$ mutants indicated that a deficiency in UDPG pyrophosphorylase was probably responsible for the Cel$^-$ phenotype of these mutants. The mutations would therefore either be located in the structural gene for this enzyme, or in some other gene which affected the activity of the enzyme. In order to distinguish between these possibilities, we first subcloned the 2.8 kb DNA fragment into the *Hin*dIII polylinker site of the high copy number *E. coli* vector pUC19. The recombinant plasmid (pKF10) was then transformed into an *E. coli* strain (FF4001) carrying a mutation in the structural gene (*galU*) for UDPG pyrophosphorylase. *E. coli galU* mutants are unable to grow on galactose as carbon source. Initial characterization of the pKF10 transformants of FF4001 showed that they were able to grow on minimal media containing galactose as carbon source (data not shown). The UDPG pyrophosphorylase activity in one of the transformants was therefore compared with the corresponding activity in FF4001 cells transformed with the cloning vector pUC19. As can be seen from Table 3, the UDPG pyrophosphorylase activity in FF4001(pKF10) was about 330 times higher than the activity in FF4001(pUC19). These data therefore clearly demonstrated that the cloned DNA fragment contained the *A. xylinum* structural gene for UDPG pyrophosphorylase, and we propose to call this *A. xylinum* gene *celA*. It was also interesting to note that the activity of UDPG pyrophosphorylase was more than ten times higher in FF4001(pKF10) than in the plasmid-free *galU*$^+$ parent strain of FF4001 (strain MC4100).

Discussion

Previous studies of *A. xylinum* strain ATCC 10245 have shown that the genetics of this organism is both complex and unusual. In order to avoid the difficulties involved in using this strain as a model organism, we studied the properties of Cel$^-$ mutants isolated from several different *A. xylinum* strains. These studies showed that the mutants isolated from strain ATCC 23768 differed from Cel$^-$ mutants of strain 10245 by three important criteria. The Cel$^-$ mutants were induced with NG at an about 30-fold lower frequency in strain ATCC 23768 than in strain ATCC 10245, indicating that cellulose synthesis might be affected by fewer genes in strain ATCC 23768. In contrast to the mutants from strain ATCC 10245 (Valla and Kjosbakken 1981, 1982), the Cel$^-$ mutants of strain ATCC 23768 did not produce detectable quantities of water-soluble extracellular polysaccharides, and could not be induced phenotypically by tetracycline to produce cellulose. We also found that it was difficult to use many of the Cel$^-$ mutants of strain ATCC 10245 in mating experiments, since the mating procedure itself seemed to induce high frequencies of spontaneous reversion to the Cel$^+$ phenotype (data not shown). Although the gene library of strain ATCC 23768 did not represent the entire genome of the organism, it was likely that it contained some of the genes of interest. Since we had access to a large number of Cel$^-$ mutants, the library would therefore probably be of satisfactory quality for testing the potential of strain ATCC 23768 for the complementation experiments.

The results of the experiments described above, showed that 3 of the 14 Cel$^-$ mutants tested could be complemented by a cloned 2.8 kb DNA fragment. Biochemical analysis of the 3 mutants indicated that all of them were deficient in the enzyme UDPG pyrophosphorylase, while the levels of all other known enzymes of cellulose synthesis (phosphoglucomutase, diguanylate cyclase, phosphodiesterases A and B, and cellulose synthase) seemed to be essentially unaffected. The lack of UDPG pyrophosphorylase activity in the Cel$^-$ mutants also explained the deficiency of cellulose synthesis in vivo from glucose, while the rates of in vitro synthesis of $\beta(1,4)$-glucan from UDPG were similar to the activity in the wild-type. The observation that the UDPG pyrophosphorylase activity, as well as the capacity to synthesize cellulose, were at least partly replenished in the complemented mutants, shows that the cloned gene is related to the activity of this enzyme. Since the cloned gene could also be used to complement an *E. coli* mutant known to be deficient in the structural gene for UDPG pyrophosphorylase, the experiments provide direct proof that the 2.8 kb DNA fragment contains the corresponding gene from *A.*

xylinum. It is not clear at present whether the expression of the gene in *E. coli* originates from an *E. coli* or *A. xylinum* promoter, but experiments designed to differentiate between these two possibilities are now in progress. If *A. xylinum* promoters are recognized by the *E. coli* RNA polymerase, it should be possible to clone many other *A. xylinum* genes by analogous complementation procedures.

The experiments described in this report demonstrate that the *A. xylinum* strain ATCC 23768 can serve as a model system for isolating the genes involved in cellulose biosynthesis. To develop this potential further, we are currently pursuing a biochemical analysis of the mutants not complemented by the UDPG pyrophosphorylase gene. By complementing these mutants with a representative gene library of strain ATCC 23768, we hope to clone more of the genes involved in cellulose biosynthesis in *A. xylinum*.

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References

Aloni Y, Delmer D, Benziman M (1982) Achievement of high rates of *in vitro* synthesis of 1,4-β-D-glucan: Activation by cooperative interaction of the *Acetobacter xylinum* enzyme system with GTP, polyethylene glycol, and a protein factor. Proc Natl Acad Sci USA 79:6448–6452

Birnboim HC, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7:1513–1523

Bittner M, Kupferer P, Morris CF (1980) Electrophoretic transfer of protein and nucleic acids from slab-gels to diazobenzyloxymethyl cellulose or nitrocellulose sheets. Anal Biochem 102:459–471

Casadaban MJ (1976) Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J Mol Biol 104:541–555

Colvin JR (1980) The biosynthesis of cellulose. In: Preiss J (ed) Biochemistry of plants, vol. 3. Carbohydrates: structure and function. Academic Press, New York, pp 543–570

Delmer DP (1983) Biosynthesis of cellulose. Adv Carbohydr Chem Biochem 41:105–153

Depicker H, De Wilde M, De Vos G, De Vos R, Van Montagu M, Schell J (1980) Molecular cloning of overlapping segments of the nopaline Ti plasmid pTiC58 as a means to restriction endonuclease mapping. Plasmid 3:193–211

Giæver HM, Styrvold OB, Kaasen I, Strøm AR (1988) Biochemical and genetic characterization of osmoregulatory trehalose synthesis in *Escherichia coli*. J Bacteriol 170:2841–2849

Hansen RG, Albrecht GJ, Bass ST, Seifert LL (1966) UDP-glucose pyrophosphorylase (crystalline) from liver. Methods Enzymol VIII:248–253

Hestrin S, Schramm M (1954) Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. Biochem J 58:345–352

Joshi JG (1982) Phosphoglucomutase from yeast. Methods Enzymol 89:599–605

Knauf VC, Nester EW (1982) Wide host range cloning vectors: a cosmid clone bank of an *Agrobacterium* Ti plasmid. Plasmid 8:45–54

Meyers JA, Sanchez D, Elwell LP, Falkow S (1976) Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J Bacteriol 127:1529–1537

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Norrander J, Kempe T, Messing J (1983) Improved M13 vectors using oligonucleotide-directed mutagenesis. Gene 26:101–106

Rigby PWJ, Dieckmann M, Rhodes C, Berg P (1977) Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J Bacteriol 113:237–251

Ross P, Aloni Y, Weinhouse H, Michaeli D, Weinberger-Ohana P, Mayer R, Benziman M (1986) Control of cellulose synthesis in *Acetobacter xylinum*. A unique guanyl oligonucleotide is the immediate activator of the cellulose synthase. Carbohydr Res 149:101–117

Ross P, Weinhouse H, Aloni Y, Michaeli D, Weinberger-Ohana P, Mayer R, Braun S, Benziman M (1987) Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid. Nature 325:279–281

Simon R, Priefer U, Puhler A (1983) A broad host range mobilization system for *in vivo* genetic engineering. Transposon mutagenesis in gram-negative bacteria. Bio/Technology 1:784–791

Thomashow MF, Karlinsey JE, Marks JR, Hurlbert RE (1987) Identification of a new virulence locus in *Agrobacterium tumefaciens* that affects polysaccharide composition and plant cell attachment. J Bacteriol 169:3209–3216

Timmis KN, Cabello F, Cohen SN (1978) Cloning and characterization of *Eco*R1 and *Hin*dIII restriction endonuclease-generated fragments of antibiotic resistance plasmids R6-5 and R6. Mol Gen Genet 162:121–137

Valla S, Kjosbakken J (1981) Isolation and characterization of a new extracellular polysaccharide from a cellulose-negative strain of *Acetobacter xylinum*. Can J Microbiol 27:599–603

Valla S, Kjosbakken J (1982) Cellulose-negative mutants of *Acetobacter xylinum*. J Gen Microbiol 128:1401–1408

Valla S, Coucheron DH, Kjosbakken J (1983) *Acetobacter xylinum* contains several plasmids: Evidence for their involvement in cellulose formation. Arch Microbiol 134:9–11

Valla S, Coucheron DH, Kjosbakken J (1986) Conjugative transfer of the naturally occurring plasmids of *Acetobacter xylinum* by incP-plasmid-mediated mobilization. J Bacteriol 165:336–339

Valla S, Coucheron DH, Kjosbakken J (1987) The plasmids of *Acetobacter xylinum* and their interaction with the host chromosome. Mol Gen Genet 208:76–83

Wahl GM, Stern M, Stark GR (1979) Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl paper and rapid hybridization by using dextran sulfate. Proc Natl Acad Sci USA 76:3683–3687

Weinhouse H, Benziman M (1974) Regulation of hexose phosphate metabolism in *Acetobacter xylinum*. Biochem J 138:537–542

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