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Research in Microbiology 153 (2002) 205–212

Research in Microbiology

www.elsevier.com/locate/resmic

Mini-review

Molecular biology of cellulose production in bacteria

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Received 18 January 2002; accepted 8 March 2002

Abstract

Cellulose biosynthesis has recently been established for a variety of bacteria of diverse origin at the phenotypic and genetic levels. Novel regulatory pathways, which involve the second messenger bis-(3′,5′) cyclic diguanylic acid and several proteins with the GGDEF domain, participate in the regulation of cellulose biosynthesis. The biological significance of cellulose production in environmental, commensal and pathogenic bacteria is only punctually resolved. This review summarizes current knowledge on cellulose biosynthesis, its regulation and biological function.

Keywords: Cellulose biosynthesis; *Acetobacter xylinus*; *Salmonella typhimurium*; c-di-GMP

1. Introduction

Although cellulose production has been considered to be a domain of the plant kingdom, for a long time the model organism for the elucidation of basic features of cellulose biosynthesis has been the bacterium *Gluconacetobacter xylinus* (formerly called *Acetobacter xylinus(m)*). For example, efficient in vitro cellulose biosynthesis and the cloning of the cellulose biosynthesis operon in *G. xylinus* at the beginning of the nineties had been milestones in cellulose research [29,43]. To this end, based on the homology of conserved motives to the bacterial cellulose synthases, the first plant cellulose synthase genes were recently identified [24]. Although the role of a bacterium as a model organism for cellulose biosynthesis is now superfluous, the recent discovery of cellulose production (and the prediction of cellulose biosynthesis) in a wide variety of bacteria has opened up exciting perspectives for the elucidation of molecular mechanisms of cellulose biosynthesis and regulation, and for the role of cellulose in bacterial development and in the interaction of the bacterial cell with the environment and the host. In addition, cellulose production in bacteria is of potential economical interest.

2. Detection of cellulose production

Although only consisting of the monosaccharide glucose in (1 → 4) β-glycosidic bonds the polysaccharide cellulose is both a relatively simple and a complicated macromolecule. Several dozen (in some organisms up to 250) linear glucan chains are arranged in parallel and form highly regular intra- and interchain hydrogen bonds. In nature, the most frequently found crystalline structure is the metastable cellulose I allomorph. This particular tertiary structure actually defines the characteristics of cellulose that consist of water-insoluble crystalline microfibrils inert to the treatment even with strong alkaline and acidic solutions [40]. This peculiarity in the behavior of the macromolecule is responsible for the fact that production of cellulose by bacteria is not detected using conventional methodology for the analysis of exopolysaccharide production. However, convenient primary screens for cellulose-producing organisms are provided by the characteristic binding behavior of dyes to the cellulose molecule such as Congo red or calcofluor before detailed genetic and chemical analyses are established [2,6, 16,28]. Since cellulose production leads to the aggregation of bacteria, dissolution of cell clumps by the treatment with cellulase but not with other glucosidases such as amylase or proteinases is another hint that cellulose is produced [6,16, 44].

Cellulose production has been established for *G. xylinus*, *Agrobacterium tumefaciens*, *Rhizobium leguminosarum* bv. *trifolii*, *Sarcina ventriculi* and recently, for the enterobacteriaceae *Salmonella* spp., *Escherichia coli*, *Klebsiella pneu-*

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0923-2508/02/$ – see front matter
PII: S0923-2508(02)01316-5

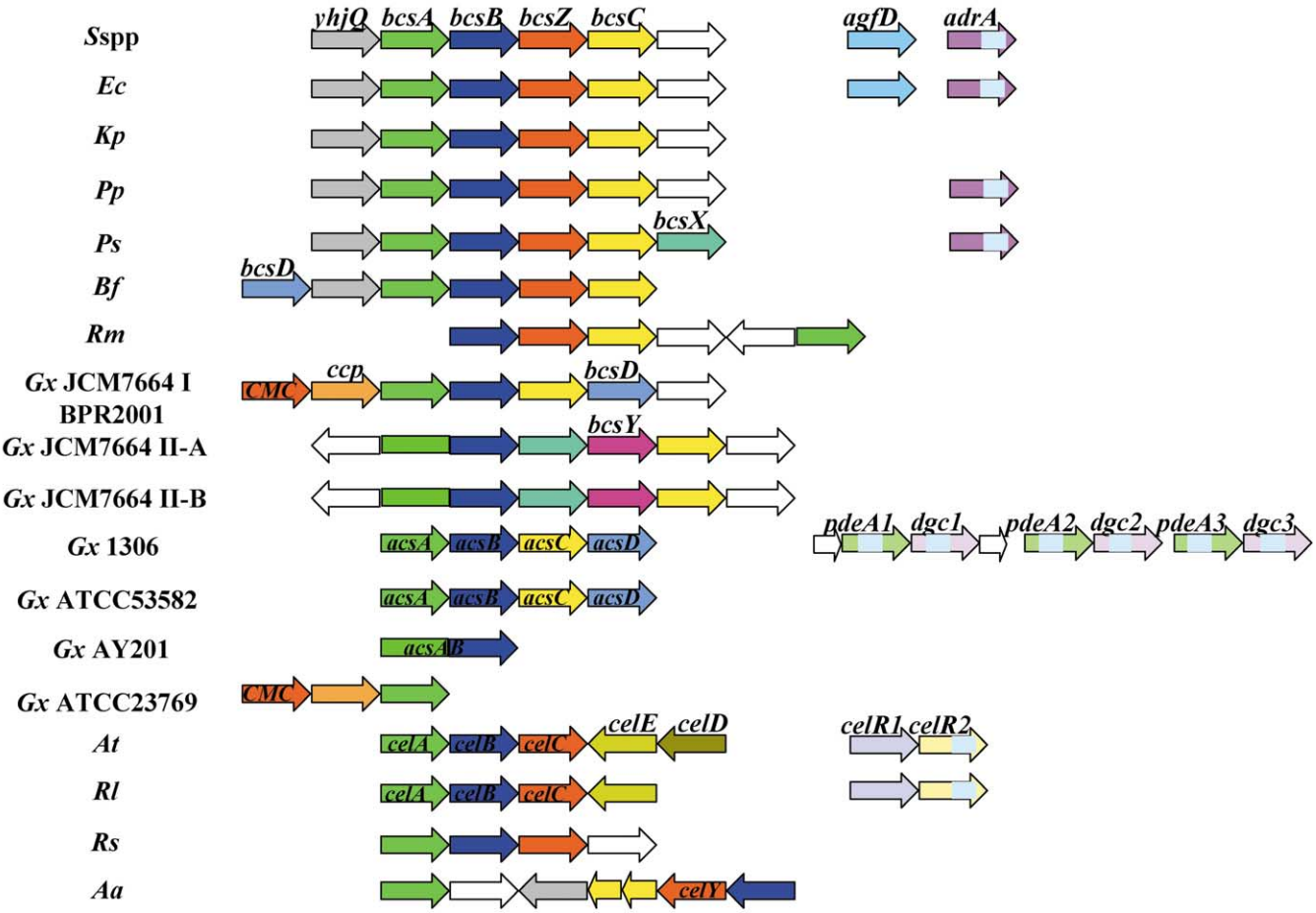


Fig. 1. Confirmed and predicted *bcs* operons and identified regulatory genes of cellulose biosynthesis. Open reading frames (ORFs; not drawn to scale) that encode homologous genes (as determined experimentally or by BLAST search) have the same color. Alternative designations for homologous genes as assigned in databases are indicated within the respective arrow. Grey and white arrows indicate genes not proven to be involved in cellulose biosynthesis. A light blue box stands for a GGDEF domain. *S*spp, *Salmonella* serotypes (serotype Typhimurium (NC_003197, AJ315770, AJ002301, AJ271071) ; serotype Typhi (NC_003198)); *Ec*, *E. coli* K12, O157:H7 EDL933, O157:H7 (NC_000913, AE005174, NC_002695); *Pp*, *P. putida* KT2440; *Ps*, *P. syringae* pv. *tomato*; *Bf*, *Burkholderia* sp. strain LB400; *Rm*, *R. metallidurans* CH34; *G. xylinus*, strain JCM7664 (I; AB015802); JCM7664 (II-A; AB015803); JCM7664 (II-B; AB015804); BPR2001 (AB010645); 1306 (M37202); ATCC53582 (X54676); AY201/ATCC23769 (U15957); ATCC23769 (M96060); *At*, *A. tumefaciens* C58 (NC_003063, L38609); *Rl*, *R. leguminosarum* bv. *trifolii* (AF121340, AF121341); *Rs, Rhodobacter sphaeroides* 2.4.1; *Aa*, *A. aeolicus* VF5 (NC_000918). Preliminary sequence data for *P. putida* and *P. syringae* were obtained from The Institute for Genomic Research website at http://www.tigr.org and for *Burkholderia* sp., *R. metallidurans* and *R. sphaeroides* at http://spider.jgi-psf.org/JGI_microbial/html.

moniae and several species of cyanobacteria [2,6,17,22,23, 30,44]. A cellulose synthase has been identified in all the above-mentioned organisms besides in the Gram-positive bacterium *S. ventriculi*. With the exception of the cyanobacteria, central structural genes required for cellulose biosynthesis, including the cellulose synthase, form an operon on the chromosome (Fig. 1). *G. xylinus* strains may have more than one cellulose biosynthesis operon, whereby cellulose biosynthesis is mediated only by one of the synthases in vivo under laboratory conditions [31].

Two genes are present in the cellulose biosynthesis operon throughout the species, the cellulose synthase and the bis-(3′,5′) cyclic diguanylic acid (c-di-GMP) binding protein. The cellulose synthase is the first gene in the operon encoded by *bcsA* (bacterial cellulose synthesis), which is also named *acsA* (*Acetobacter* cellulose synthesis) or *celA* (cellulose). Second comes the c-di-GMP binding protein encoded by *bcsB* (synonyms: *acsB*, *celB*). In *G. xylinus* the *bcsA* and *bcsB* genes are occasionally fused to a single open reading frame in type II cellulose synthases which shows the tight functional coupling of the two protein products for which spatial closeness has recently been demonstrated [12]. Otherwise, the present stage of knowledge suggests that there is variability in the location and possibly also in the requirement of additional genes for cellulose biosynthesis. *BcsZ* (synonyms CMC in *G. xylinus* and *celC* in *A. tumefaciens* and *R. leguminosarum* bv. *trifolii*) has been shown to encode a cellulase (family 8 glucosidase), which is required for cellulose synthesis. *BcsZ* present in all cellulose-producing species is encoded by the cellulose biosynthesis operons of enterobacterial species, *A. tumefaciens* and *R. leguminosarum* bv. *trifolii*, but located

outside yet adjacent to the cellulose biosynthesis operon in several *G. xylinus* strains [37]. *BcsC* required in vivo but not in vitro for cellulose biosynthesis is present in the enterobacterial species, the pseudomonads and *G. xylinus*. Whether other genes suspected of participating directly in cellulose biosynthesis such as *bcsD* (required in vivo, but not in vitro for cellulose biosynthesis [32,43]), *ccp* in *G. xylinus* [36] and *celDE* in *A. tumefaciens* and *R. leguminosarum* bv. *trifolii* [2,17] have functional homologues in the other cellulose-producing species remains to be shown.

Search of the databases of finished and unfinished bacterial genomes detected several bacterial species that contain homologues to the four genes of the enterobacterial *bcsABZC* operon on their chromosomes (Fig. 1). The genes of the *bcs* operons of two *Pseudomonas* species, *Pseudomonas putida* KT2440 and *Pseudomonas syringae* pv. *tomato* and of *Burkholderia* sp. strain LB400 are most closely related to the genes of the *bcs* operon of the enterobacteriaceae and show the same gene order. In *Ralstonia metallidurans* CH34 (formerly called *Alcaligines*/*Ralstonia eutrophus*) and *Aquifex aeolicus* VF5 a recombination event separated *bcsA* from *bcsBZC*, the rest of the operon. *Burkholderia pseudomallei* also contains highly conserved *bcsABZC* genes, but in the current state of the sequencing project it is not possible to determine whether they build up an operon. Based on the high homology of the genes it is expected that all those bacterial species mentioned above are capable of producing cellulose. For several *Pseudomonas* spp. strains production of cellulose has already been described [1]. Consequently, cellulose, which had once been considered to be produced only by a few soil bacteria of the α-branch of proteobacteria has become a common polysaccharide secreted by a variety of unrelated environmental, commensal and pathogenic bacteria.

3. The cellulose synthase BcsA

The cellulose synthase BcsA is between 723 to 888 amino acids (aa) long and is the most conserved gene of the *bcs* operon among the species. Although the N- and C-terminal part of the protein is less well conserved the homology is not restricted to the frequently analyzed D,D,D35Q(R,Q)XRW motif, which spans domains A and B (Fig. 2).

The D,D,D35Q(R,Q)XRW motif is actually characteristic of the whole group of processive β-glycosyltransferases which include, besides cellulose synthase, among others chitin synthase and curdlan synthase. In bacterial cellulose synthases, conserved aa suggest calling the D,D,D35Q(R,Q) XRW motif the D_3D_2D35QRXRWA motif (Fig. 2). When confirmed and predicted cellulose synthases were compared a region of high homology (~ 70% aa similarity) comprises approximately 350 aa. The region of high homology contains the D_3D_2D35QRXRWA motif and five additional

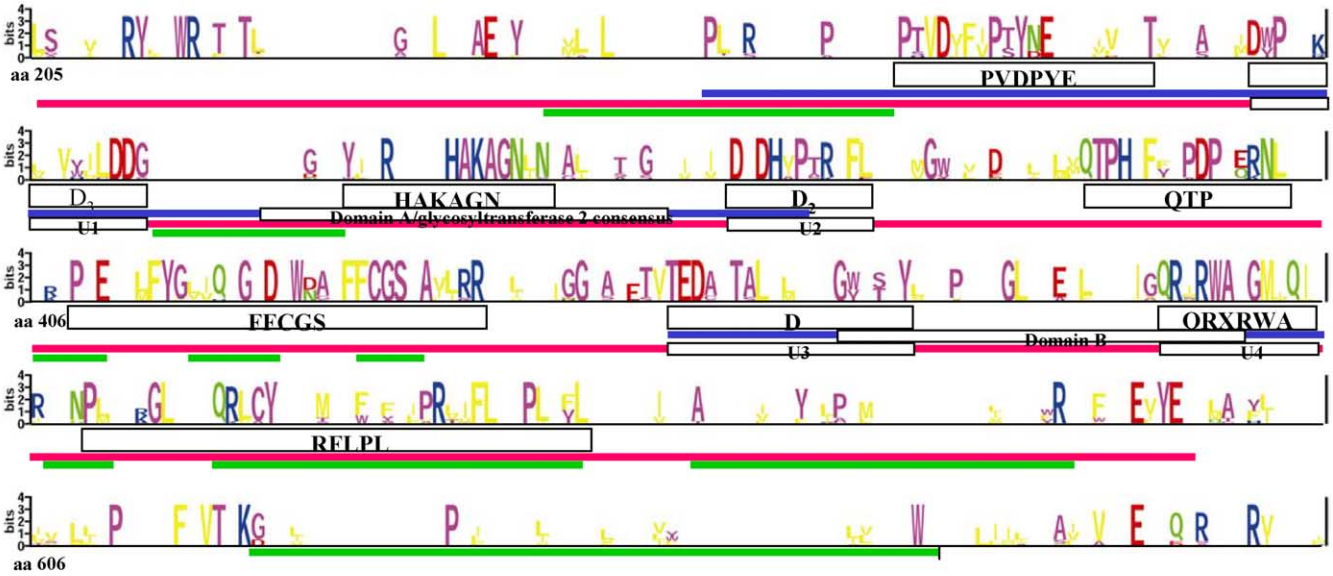


Fig. 2. Consensus sequence of bacterial cellulose synthase, catalytic subunit BcsA. Experimentally confirmed and selected predicted BcsA proteins were used. Multiple alignment of the amino acid sequences was performed by PileUp (GCG package version 9, University of Wisconsin) using standard parameters. The consensus sequence was drawn using the SeqLogo program [33] in the WWW-based implementation (http://www.bio.cam.ac.us/seqlogo). Only aa that are more than 80% conserved are shown with BcsA from *S. typhimurium* as frame. The height of each letter reflects the degree of conservation. The solid blue bar below the alignment indicates the regions of homology to processive glycosyltransferase domain A and domain B, respectively. Domain A overlaps with the glycosyltransferase 2 consensus sequences [4]. The solid red bar below the alignment indicates the region of homology to eukaryotic cellulose synthases with characteristic motives U1 to U4 [24]. The solid green bar below the alignment marks regions where the completely conserved amino acids of the cellulose synthases deviate from the sequence of the closest homologue, curdlan synthase from *Agrobacterium* spp., a (1 → 3) β-glucan synthase [38]. Protein sequences used: *G. xylinus:* BAA31463.1, P19449, BAA77585.1, P21877, BAA77593.1; *S. typhimurium*: CAC44015.1; *E. coli*: P37653; *A. tumefaciens*: NP_357298.1; *R. leguminosarum* bv. *trifolii*: AAC41436.1. *P. putida* and *P. syringae* pv. *tomato* BcsA proteins according to sequence data from http://www.tigr.org, *R. metallidurans*, *Burkholderia* sp. strain LB400 and *R. sphaeroides* 2.4.1 BcsA proteins from http://spider.jgi-psf.org/JGI_microbial/html.

highly conserved sequence stretches which cluster around positively or negatively charged aa and contain the majority of invariable aa, namely the PVDPYE, HAKAGN(L,I)N, QTP, FFCGS and RFLPL motives (Fig. 2). Most closely related to the bacterial cellulose synthases is the curdlan synthase, a (1 → 3) β-glucan synthase, from *Agrobacterium* spp. [38] which contains, besides the D,D,D35Q(R,Q)XRW motif, an identical HAKAGN(L,I)N motif, slightly altered PVDPYE and QTP motives, but almost completely lacks the FFCGS and RFLPL motives. This finding suggests that the FFCGS and RFLPL motives could be mainly responsible for the determination of the (1→ 4) specificity of the β-glucan bond. Divergence among the cellulose synthases is highest in an aa stretch between D_3 and the HAKAGN motif where the CelA and BcsA proteins contain a 34 and 15 aa long insertion, respectively.

The cellulose synthase is located in the cytoplasmic membrane with 8 to 10 predicted transmembrane domains. BcsA is considered to be the catalytic subunit for cellulose biosynthesis. Besides the high homology to other processive glycosyltransferases, BcsA has been experimentally shown to bind the substrate UDP-glucose [13].

4. The BcsB protein

The BcsB protein that was indirectly inferred to bind c-di-GMP [19] is less well conserved among the species. However, direct comparisons of the BcsB proteins with CelB from *A. tumefaciens* and *R. leguminosarum* bv. *trifolii* revealed significant homology (∼ 40% similarity) over the entire length of the proteins with several invariable residues (Fig. 3). An alanine/proline rich domain is located at the N-terminus of all proteins except *A. aeolicus*. One transmembrane domain located at the C-terminus has been predicted by various algorithms for all BcsB proteins.

5. Biosynthesis of cellulose

Although elucidation of the structure and function of the cellulose-synthesizing complex will be useful for the general understanding of membrane complexes that traverse both the inner and the outer membrane as well as for the directed manipulation of cellulose production, surprisingly little is known about the function and localization of the proteins participating in cellulose biosynthesis.

In *G. xylinus* the cellulose-synthesizing complex is a transmembrane complex over the cytoplasmic and outer membrane whereby the cellulose synthase (BcsA) and the c-di-GMP binding protein (BcsB) are considered to be localized in the cytoplasmic membrane [12]. In cells actively producing cellulose approximately 50 cellulose-synthesizing multienzyme complexes are organized in a single row along the longitudinal axis of the bacterial rod whereby each complex secretes approx. 12 to 25 glucan chains which assemble into larger microfibrils at the site of synthesis. This so-called linear terminal complex can be visualized by electron microscopy using freeze fracture as 35 Å pores in the outer membrane or as pits when the outer leaflet is fractured away [12].

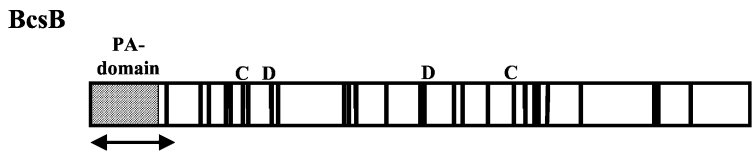
At the molecular level the mechanism of cellulose biosynthesis has not been resolved. In *A. tumefaciens* participation of two different lipid intermediates in cellulose biosynthesis has been postulated whereby the initial glucose-lipid derivative is formed by the *celDE* gene product [18]. There is no hint of lipid intermediates in cellulose biosynthesis nor in the occurrence of *celDE* homologues in *G. xylinus*.

6. c-di-GMP, activator of cellulose biosynthesis

In *G. xylinus* c-di-GMP has been identified as an activator of cellulose biosynthesis [29]. The free c-di-GMP in the cell is considered to allosterically activate the cellulose synthase BcsA. However, 90% of the cellular c-di-GMP is reversibly bound by the c-di-GMP binding protein BcsB, a membrane protein that is structurally associated with the cellulose synthase [12,19,43]. It is believed that the spatial proximity is necessary to direct c-di-GMP released from BcsB towards the cellulose synthase. The equilibrium between bound and free c-di-GMP is modulated by the intracellular potassium concentration [42].

The level of free c-di-GMP is regulated by the opposing action of two enzymes, diguanylate cyclase (DGC) that cycles two molecules of GTP under the release of two molecules of PP_i, and phosphodiesterase A (PDEA) that degrades c-di-GMP to the inactive pGpG. *G. xylinus* has three distinct operons each containing a PDEA/DGC pair (Fig. 1), which contribute at different levels to the c-di-GMP turnover [39] indicating that cellulose biosynthesis underlies various control mechanisms in *G. xylinus*.

At the N-terminus DGC as well as PDEA contain sensory domains for environmental signals [39, (Fig. 4)]. The



$GX_{43}NX_{14}GX_{10}PX_6NX_{13}\mathbf{C}X_8W X_{30}\mathbf{D}X_9PX_{74}PX_7LX_3GX_{41}PX_{38}\mathbf{PD}X_{25}SX_6NX_{37}PX_{27}\mathbf{C}X_{15}SX_{13}PX_1LX_8PX_{39}PX_{87}SPX_{35}G$

Fig. 3. Signature of completely conserved residues in BcsB/CelB proteins with BcsB from *S. typhimurium* (CAC44016.1) as an example. ↔; 100 aa.

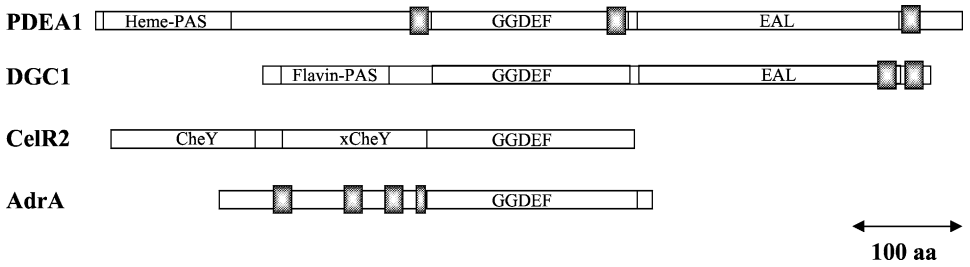


Fig. 4. Comparison of the domain structures of proteins involved in the regulation of cellulose biosynthesis. PDEA1 and DGC1 from *G. xylinus* (AF052517); CelR2 from *R. leguminosarum* bv. *trifolii* (AF121341): AdrA from *S. typhimurium* and *E. coli* (AJ271071). Heme-PAS is an oxygen-sensing domain similar to a domain in FixL from rhizobia, whereas flavin-PAS has homology to a redox-sensing domain in NifL from *K. pneumoniae* [39]; CheY indicates a domain homologous to the receiver domain of two-component systems; the GGDEF and EAL domains are described in the text. Shaded boxes indicate putative membrane domains.

activity of PDEA has been shown to be negatively regulated by oxygen sensed by a heme-containing PAS domain [5] DGC has been proposed to harbor a flavin-containing PAS domain, which senses the redox status of the cell.

DGC and PDEA are homologous multidomain proteins that have a GGDEF (also called DUF1) and EAL (synonym: DUF2) domain in common (Fig. 4). Both domains are widely represented in bacteria and may occur alone, together or with well-established domains for signal transduction [8]. Recently, the detection of sequence similarities between the GGDEF domain and eukaryotic adenylate cyclases predicted a nucleotide cyclase activity as a possible function for the GGDEF domain [25]. In that line, unrelated proteins of different bacteria that only had the GGDEF domain in common were able to induce cellulose biosynthesis in a cellulose-deficient *R. leguminosarum* bv. *trifolii* wild-type strain when plasmid-expressed [3]. However, there exists no truly convincing explanation as to why certain bacterial species like *E. coli* and *S. typhimurium* have about 20 proteins with the GGDEF-based nucleotide cyclase activity all producing the soluble activator molecule c-di-GMP. The function of the EAL domain still remains unknown.

Although there is evidence, it is not yet clear whether c-di-GMP acts in general as an activator of cellulose biosynthesis in bacteria. Besides *G. xylinus*, the occurrence of c-di-GMP and activation of cellulose biosynthesis by c-di-GMP has only been shown for *A. tumefaciens* [1]. The discovery of DOS, a protein encoded by *E. coli* and *S. typhimurium* that is highly homologous to PDEA from *G. xylinus* [7], is a hint of the occurrence of c-di-GMP in those organisms and at least partially overlapping signal transduction pathways leading to cellulose biosynthesis.

Strikingly, by genetic analysis, individual proteins with GGDEF domains have been identified as participating in the positive regulation of cellulose biosynthesis in *R. leguminosarum* bv. *trifolii* and *S. typhimurium* [2,27]. The GGDEF domain is the only homologous domain among the proteins (Fig. 4). Otherwise, CelR2, which controls cellulose biosynthesis in *R. leguminosarum* bv. *trifolii*, is homologous to PleD, a response regulator of cell differentiation in *Caulobacter crescentus* [10]. On the other hand, AgfD-regulated protein (AdrA), a regulator of cellulose biosynthesis in *S. typhimurium*, has four transmembrane N-terminal domains of the GGDEF domain. Yet too little is known about the regulation of cellulose biosynthesis to predict distinct roles for these proteins at the molecular level. Based on the few phenotypes that are known for genes encoding GGDEF-domain-containing proteins it can be concluded that GGDEF domains play a role in bacterial morphogenesis.

7. Regulation of cellulose biosynthesis in *S. typhimurium*

In *S. typhimurium* a regulatory cascade that leads to cellulose biosynthesis has been elucidated using a strain that shows semi-constitutive cellulose expression [27,44]. AgfD, a response regulator of the LuxR-family that was initially detected as regulating the biosynthesis of thin aggregative fimbriae [26], was shown to transcriptionally regulate *adrA* under a variety of environmental conditions [27]. The expression of AgfD itself is mainly restricted to the stationary phase of growth and subject to regulation by global regulatory proteins and a variety of environmental conditions [26]. Expression of AdrA in turn activates cellulose biosynthesis even when *agfD* is deleted, while *adrA* is plasmid-expressed [44]. Since it has been shown that transcription of *bcsA* and *bcsC* is not dependent on AgfD and therefore AdrA, activation of cellulose biosynthesis by AdrA must occur at a posttranscriptional level [44]. Stabilization or activation of the Bcs proteins by protein-protein interactions with AdrA or production of c-di-GMP by the GGDEF domain of AdrA could trigger cellulose biosynthesis. Since the above described genes involved in cellulose biosynthesis are highly conserved among *S. typhimurium*, other *Salmonella* serotypes and *E. coli*, it is expected that a similar regulatory cascade leading to cellulose biosynthesis works in the respective organisms.

8. Biological significance of cellulose production in bacteria

Most knowledge of the biological role of cellulose biosynthesis has been gathered on the interaction of soil

bacteria of the family Rhizobiaceae, *Rhizobium* spp. and *A. tumefaciens,* respectively, with plants. *Rhizobium* spp. are plant symbionists that fix nitrogen when living inside root nodule cells of leguminous host plants. After the initial attachment of the individual bacterial cells to plant root hairs in culture, cellulose biosynthesis is required for the second step in adherence, firm adherence plus aggregation of bacteria at the root hair tip (cap formation) [36]. However, cap formation is not a prerequisite for nodulation, the next step in the interaction of the bacteria with the plant. The physiological role of cap formation, although performed by various *Rhizobium* spp. [35], is not resolved. A contribution of cellulose to more effective nodulation has been proposed [22], but never convincingly proven. A similar role for cellulose in sequential attachment to carrot tissue culture cells has been shown for *A. tumefaciens* the causative agent of crown gall tumours on dicotyledonous plants [14].

Mimicking environmental conditions colonization of roots in soil was significantly reduced by cellulose mutants of *A. tumefaciens* [15]; however, the survival rate of the wild-type and the cellulose mutants in soil has not been determined. Anchoring of the bacterial cells to the plant tissue, which leads to a survival advantage under natural conditions, might be a function of cellulose biosynthesis in Rhizobiaceae. A similar explanation concerning the function of cellulose might hold for *G. xylinus*, considering that the natural environment of *G. xylinus* is the surface of fruits, vegetables and decaying material [34]. Although the most popular argument for cellulose biosynthesis in *G. xylinus* is the maintenance of the organism in an aerobic environment in liquids [11], this view might be biased by observations from a laboratory perspective.

The effect of cellulose on the virulence of *A. tumefaciens* in a leaf infection model was not consistent, since some of the cellulose-minus mutants were not affected in virulence, whereas others showed reduced virulence [20]. This finding suggests that some mutations occurred in genes that regulate cellulose biosynthesis but also affect other cellular functions. Another phenotype that has been observed with cellulose pellicles is the protection of cells from the hazardous effect of UV radiation [34].

Although cellulose biosynthesis is common among several species of the Enterobacteriaceae the biological function of cellulose in these organisms is not clear. Cellulose is produced by the majority of *S. typhimurium* and *S. enteritidis* isolates from disease origin, but not by *S. typhimurium* isolates from doves and other *Salmonella* spp. with a narrow host range which cause invasive disease (unpublished observations). This fact indicates that there is a strong selective pressure against cellulose production under certain conditions in the host. I hypothesize that cellulose biosynthesis in Enterobacteriaceae once played a role in the bacterial life cycle outside the animal host in biofilm formation, cell-cell interaction and/or persistence of the organisms. In that environment species of Enterobacteriaceae might have acquired the cellulose biosynthesis operon *bcsABZC* and *adrA*, the activator of cellulose biosynthesis, from *Pseudomonas* spp. or related soil organisms, as concluded from the high homology of the respective operons on the nucleotide and amino acid level [44]. Whether a critical (positive) role for cellulose biosynthesis in bacterial-host interaction emerged later in evolution remains to be determined.

Cellulose biosynthesis in *Salmonella* spp. and *E. coli* occurs concomitantly with the production of thin aggregative fimbriae (AGF), the second component of the extracellular matrix of a multicellular morphotype [27]. Each of these substances mediates a specific type of cell-cell as well as cell-surface interaction [27]. While thin aggregative fimbriae form rigid, but fragile interconnections between cells, cellulose connects the cells through elastic, but stable bonds. One developmental characteristic of the multicellular morphotype is biofilm formation on abiotic surfaces where cells producing cellulose and thin aggregative fimbriae form distinct adherence patterns. In a steady state model, cells which express AGF fimbriae form a hard-to-remove biofilm that starts below the air-liquid interface, while cellulose-expressing cells form a loosely adherent biofilm at the air-liquid interface [27,44]. When expressed together in a colony on plates the two substances form a highly inert, hydrophobic extracellular matrix around the cells which enables the bacteria to act like a multicellular organism and not as individual cells as detected by various types of phenotypic assays [27]. This developmental behavior of *Salmonella* and *E. coli* strains has been named the rdar (red, dry and rough) morphotype inspired by the characteristic phenotype of the colony exhibited on Congo red containing agar plates [26].

9. Impact of bacterial cellulose production in medical settings and industrial applications

Besides its role in the natural environment cellulose biosynthesis of bacterial organisms has its impact in medical settings. Enterobacteriaceae, in particular *E. coli*, frequently cause nosocomial infections such as sepsis, biliary tract infections and catheter-related cystitis that are caused by biofilm-forming isolates. Since the major components of the extracellular matrix of biofilm forming *E. coli* have been identified, more rational approaches to prevent adhesion to catheter material can be designed.

Plant-derived cellulose is used in high quantities as a starting material in various industrial branches. Since bacterial cellulose is of high purity and displays special physico-chemical characteristics, it has found numerous applications in the paper and food industry (as acoustic membrane and food texture) and in the medical field (as an artificial skin and blood vessel substitute) [9,41]. However, the industrial production of bacterial cellulose is yet fairly inefficient, although strain selection, genetic manipulation and process optimisation have been used to enhance productivity of cellulose [21,41].

10. Perspectives

For some time cellulose biosynthesis has been receiving attention in basic as well as applied research. Until recently, basic questions about cellulose biosynthesis had been answered in the model organism *G. xylinus*. With the discovery of cellulose biosynthesis in well-characterized organisms such as *E. coli* and *S. typhimurium* cellulose biosynthesis can be elucidated using all the well-established molecular tools and sequence information available for these organisms. A better understanding of the molecular mechanisms of cellulose biosynthesis and regulation will also help to develop strategies for the eradication of biofilm-forming bacteria and the optimization of cellulose production for industrial applications.

Acknowledgements

I would like to thank the members of my group and collaborators who contributed to the fundamentals of this review; in particular, X. Zogaj and M. Nimtz. W. Rabsch and H. Tschäpe are gratefully acknowledged for collaboration. This work was supported in part by the Bundesministerium für Forschung und Technologie (BMFT) program "Infektionsbiologie" and by the Deutsche Forschungsgemeinschaft (Ro2023/3-1). Release of sequence data prior to publication from the Sanger Institute, TIGR, JGI and the University of Washington is gratefully acknowledged.

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