

High-Producing Strains, Cellulose Synthase and Regulation of Bacterial Cellulose

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Bacterial cellulose which is produced by several bacteria has played an important role in industry and medicine area based on its unique structural, functional, physical and chemical properties. However, both the scale and cost of production impede a wide range of applications of bacteria cellulose. The paper concludes the current state of knowledge of high-producing strains and discusses the mechanism of cellulose biosynthesis. Cellulose synthase, as the key enzyme, is identified from the structure, the organization of gene and the regulation. The cyclic diguanylic acid regulatory system is important in the progress of bacterial cellulose biosynthesis.

Key words: Bacterial Cellulose, Metabolic Network, High-producing Strains, Cellulose Synthase, Regulation.

Cellulose is the most abundant polysaccharide on earth, which is the main structural element of plant cell wall. In 1886, Brown¹ first reported an extracellular gelatinous mat, named bacterial cellulose (BC), synthesized by *Bacterium aceti* when fermenting acetic acid. He verified this substance and determined that the chemical component of this material was same as plant-derived cellulose. Subsequently, scientists found that bacterial cellulose (BC) is a biological nanomaterial synthesized by several microorganisms belonging to the genera *Acetobacter*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Azotobacter*, *Pseudomonas*,

Salmonella, *Escherichia*, *Rhizobium* and *Sarcina ventriculi* with intensive studies on bacterial cellulose synthesis^{2,3}. The exceptional structure of higher crystallinity and higher polymerization endows BC with superior properties, such as high purity, high water retention, high tensile strength, ultrafine reticulated structure and excellent biodegradability⁴. Resulting from these unique properties, BC has found a wider applications in paper, textile, food industries, and is an ideal biomaterial in cosmetics and medicine industries as a renewable natural resource⁵. With the increase of demand for BC, it is urgent to improve BC production at the lowest cost possible. The strategies mainly include screening of high-producing strains, optimization of metabolic process, regulatory of synthesis, and so on. Therefore, a major goal of BC research has been used to enhance the microbial conversion of glucose into cellulose. The process analysis of bacterial cellulose production provides a platform for understanding the synthesis of BC.

In the present review, we have summarized various high-producing strains and the recent

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progress and regulation made to enhanced production of BC. Accordingly, this review will provide ideas for the development of novel strategies for BC production.

Screening of high-producing strains

Cellulose is the most abundant organic polymer produced by plants, some animals, algae, fungi, flagellates and bacteria [6]. In 1998, Jonas *et al.*⁷ gave an overview about the bacteria which can secrete bacterial cellulose. It was reported that so many strains are in conditions to produce cellulose, such as the strains from the genera *Gluconacetobacter* (formerly *Acetobacter*), *Agrobacterium*, *Pseudomonas*, *Rhizobium* and *Sarcina*. Among these, *Gluconacetobacter* is the most efficient bacterial cellulose producing strain and used as model organism in research and commercial production, whose high BC yield reaching to around 7-8 g/L in industrial scale (Fig.1)⁸.

The choice of production strains is critical for industrial production. Two strategies are proposed for the isolation of high producing strain: (1) Isolation high yield strain from environment; (2) Mutation breeding in the laboratory. The first strain producing bacterial cellulose was isolated from home made vinegar¹. Yang *et al.*⁹ has isolated a strain identified as *Gluconacetobacter intermedius* Cls26 from rotten mandarin fruit with a yield of 7.2 g/L. And this strain appeared to have potential in BC manufacture on an industrial scale. Tanskul¹⁰ has isolated one genus namely *Rhodococcus* sp. MI 2 from ripe fruits and vegetables. Castro¹¹ isolated a new acid-resistant strain of *Gluconacetobacter medellensis* from the fermentation of Colombian bomemade vinegar. The optimum yield of this strain was 4.5 g/L at pH 3.5, which was highly tolerant to low pH conditions. Due to bacterial cellulose producing strains can utilize glucose effectively to form cellulose, most of the strains are separated from materials with high sugar content. *Gluconacetobacter* sp. was found in the production of grape wine. The BC produced by this strain has indicated a high oxygen barrier but low water barrier¹². Besides, scientists screen efficient strains from mutants induced by physical and chemical methods. Wu¹³ has got a *Gluconoacetobacter xylinus* strain through mutagenesis induced by high hydrostatic pressure treatment. And the strain showed the highest BC

yield increased from 106.03 g/L to 158.56 g/L (in the wet state).

Analysis of BC Synthesis

Bacterial cellulose is the end product of carbon metabolism. The first step of BC producing is the polymerization of several ²-1,4-glucan chains through the cellulose precursor, UDPGlc (uridine diphosphoglucose), at an extrusion site on the cell surface. After that, the nascent individual glucan chains are forced to form characteristic ribbons, which later secreted into the surrounding medium (Fig.2)¹⁴. This process is closely related to hexose monophosphate pathway (HMP), the Embden-Meyerhof-Parnas Pathway (EMP) and the Tricarboxylic Acid Cycle (TCA)[15], which involves a large number of complexes of catalytic and regulatory proteins. It is still a difficult task to find out that how the nutrients metabolism in cellulose-producing bacterium, referring to carbon metabolism, nitrogenous metabolism and respiration of the microorganism. Due to the lack of the key enzyme in the 6-phosphofructokinase (PFK) or lower activity of PFK in EMP, carbohydrate catabolism of *Acetobacter xylinum* may be dominated by HMP, and provide energy through TCA pathway. In 1996, Tonouchi *et al.*¹⁶ characterized the biosynthetic pathway of cellulose from glucose and fructose in *A. xylinum* BPR2001 and investigated several enzyme activities in the medium containing different carbon sources.

Simultaneously, strain BPR2001 was found to have a fructose-specific phosphoenolpyruvate-dependent phosphotransferase system (PTS). The research indicated that the carbohydrate metabolism pathway is independent with the formation of protein synthesis. De Wulf *et al.*¹⁷ constructed the metabolic network of *Gluconacetobacter xylinus*, but the major product was keto-gluconate, which influenced pH, cell breed and BC production. In this process, they considered a parallel byproduct of acetan, a viscous water-soluble polysaccharide. In order to increase the BC production, they generated a non-keto-gluconate-producing mutant strain through UV mutagenesis. The production of BC had increased obviously. Hugo-Velasco-Bedrán and Felipe-López-Isunz¹⁸ also have made a simple analysis about the *A. xylinum* metabolism, but the bacterial synthesis effect on metabolic flux does not take into account. Makoto and Yasushi¹⁹

got a simplified scheme of bacterial cellulose production, which used glucose or fructose as the carbon source and considered the metabolism of gluconic acid (Fig.3).

Bacterial cellulose production can be improved by reducing the related byproducts, which suggests an important approach of metabolic engineering²⁰. Metabolic engineering is a subject trying to get a better insight into the metabolism of organisms²¹. Vallino *et al*²² assumed that intracellular intermediate metabolites were in the quasi-steady state with the base of quasi-steady state assumption. They estimated the metabolic flux distribution in cells combined with biochemical reaction pathway. The steady-state of metabolic networks can be described in terms of mass balance equations. The basic metabolites within metabolic network is proportional to the energy synthesized²³. In the fermentation prophase, bacteria consumes glucose to synthesis bacterial cellulose and cells rapidly mainly through the HMP pathway. In the second stage, the metabolic flux is redistributed and a mass of carbon source flows to byproducts. It concluded that the metabolic flux of main node G6P and acetate in different stage of the biosynthesis of bacterial cellulose. Li²⁴ gave a further research. Their results indicated that ethanol functions as energy source for ATP generation at the early stage, which can inhibit the activity of G6PDH by the increased levels of ATP. Furthermore, by adding sodium citrate, the main byproduct citric acid in the TCA cycle is also reduced significantly. The metabolic flux in the process of cellulose production is affected by the culture conditions. Pyruvate is another key metabolite of respiration and fermentation. Through the use of metabolic networks and metabolic flux balance model, quantitative analysis of metabolic network can provide theoretical guidance for fermentation control optimization and genetic operation to generate bacteria bacterial cellulose.

Genetics and enzyme regulation

Cellulose biosynthesis both in plants and in prokaryotes is catalyzed by the uridine diphosphate (UDP)-forming cellulose synthase complexes (CESAs), which are membrane-embedded glycosyltransferases (GT) (Fig.3) [3]. CESAs involved the transfer of consecutive glucopyranose residues from UDP-activated

glucose (UDP-Glc) to the newly formed polysaccharide chain at the anomeric carbon of the newly added sugar from $\pm$ to ^{2} , translocation of glucan across the plasma membrane, and coalescence of multiple glucan chains to form paracrystalline microfibrils^{25,26}. Research indicated that prokaryotic and eukaryotic CESAs shared a similar predicted topology including eight transmembrane helices and at least one extended intracellular glycosyltransferase loop (Fig.3) [3]. In 1958, Roelofsen first suggested that cellulose fibrils could be assembled by means of enzyme complexes located at their tips via a vitro assay and a purification protocol²⁷. Then in 1990, Lin and Brown²⁸ used Specific-labeling technique to support the opinion that cellulose synthase consisted of three polypeptide chains. The calculated molecular masses of these polypeptide chains are 90, 67 and 54 kDa, respectively. Saxona²⁹ and his companions purified the cellulose synthase, and provided evidence that the CESAs is comprised two polypeptide chains with the molecular size of 83kDa and 93kDa, simultaneously corroborated contain UDPG-binding site via azine group-UDPG affinity labeling method. Inder and his colleagues³⁰ gave the same point, they purified the cellulose synthase from *A. xylinum* ATCC 53582 by production entrapment, and the purified preparation showed two major polypeptides of 93 and 83 kDa on lithium dodecyl sulfate(LDS)-polyacrylamide gel following electrophoresis. Further analysis corroborated that the 83kDa polypeptide is a glycoprotein which contains substrate-binding sites and active sites by lectin affinity chromatography²⁸. For Gram-negative bacteria, the protein complex used to product and secrete cellulose was consisted of at least three subunits (BcsA, BcsB and BcsC)³¹. Detailed information about the atomic structure and biochemical mechanisms of the A and B subunits of CESAs were presented³. Cellulose synthesis occurs within the cytosolic domain of inner membrane protein BcsA, whereas BcsB resides in the periplasm which may help to guide the chain in the extrusion process. They are fused as a single polypeptide in some species. And BcsC just required for cellulose synthesis *in vivo* but not *in vitro*^{32,33}.

Back to genetic basic, the cellulose synthase genes from the bacterium

Gluconoacetobacter hansenii were described in 1990. The cellulose synthase gene of *Acetobacter xylinum* and other cellulose producing strains was all located on chromosome³⁴, rather than considered the plasmid in the past. But the functional cellulose synthase is located in the cytoplasmic membrane with several predicted transmembrane domains³⁵. Cellulose synthase gene and several other genes are included in the same operon in *G. Xylinus*³³. In most species, the different subunits of the CS are encoded by different genes. As mentioned earlier, the two polypeptides of the CS are encoded by *acsAB* of *acs* operon in *G. Hansenii* ATCC 23769 and *G. xylinus* ATCC 53582³⁶, while the two sigmasubunits of the CS are determined by *bcsA* and *bcsB* in *G. xylinus* 1306-3³⁷. *AcsA* considered to be the catalytic subunit has been experimentally shown to bind the precursors UDP-glucose for the synthesis of BC. The length of the cellulose synthase *acsA* is between 723 to 888 amino acids. *AcsB* contains a regulatory domain, and binds cyclic diGMP, which is shown to be the allosteric activator of this enzyme³⁸. *AcsC* was thought to

form pores in cell walls through which synthesized cellulose can pass. Recently, Naoki Sunagawa³⁹ verified that the cellulose synthase complex in *Acetobacter xylinum* contains a new member named cellulose complementing factor (Ccp). They successfully identified the coding region of the *ccpAx* gene, it is involved in the production and crystallization of cellulose I microfibrils. And most of CcpAx existed in the membrane fraction and contained rich structure of $\pm$ -helices. These data indicate that this protein has a direct interaction with AxCeSD through pulldown assays and ITC analysis. This work lays a foundation for further studying the mechanisms of the regulation for the cellulose synthesis. Very recently, a cellulose synthase has been identified the most conserved gene of the *bcs* operon among the species⁴⁰. But the C- and N- terminal part of the protein is less well conserved. The FFCGS and RFLPL motives included in the CS could be mainly responsible for the determination of the (1'!4) specificity of the 2-glucan bond³¹. The study found that an operon encoding two ORFs was located 3.0 kb upstream of the *bcs* operon in *A. xylinum*, which is same as

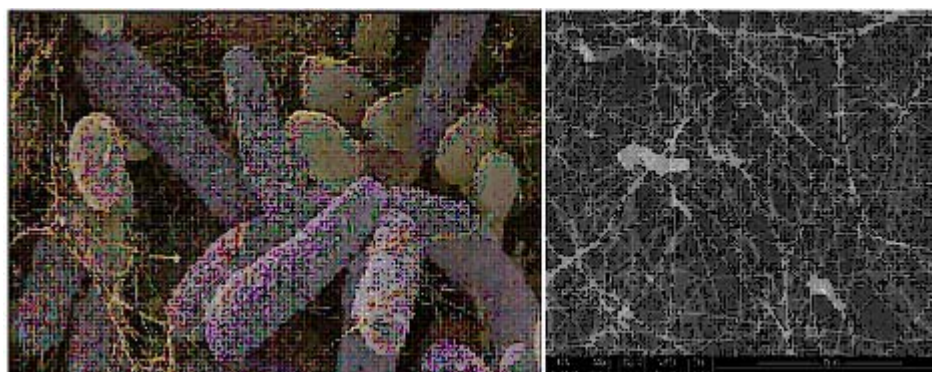


Fig.1. SEM images of *Acetobacter xylinus* [45,46]

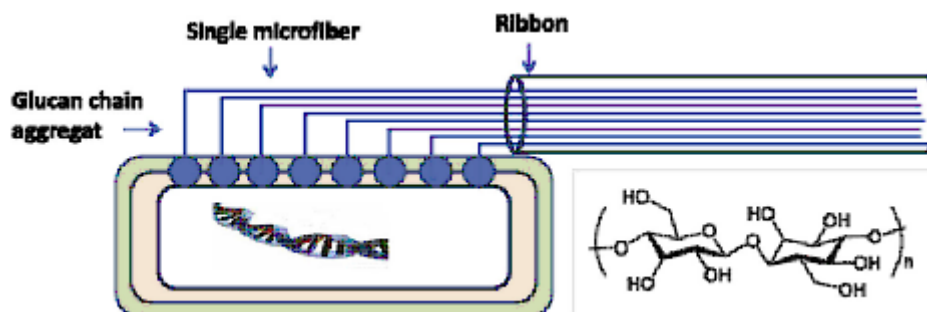


Fig.2. Formation of Bacterial cellulose [45,46]

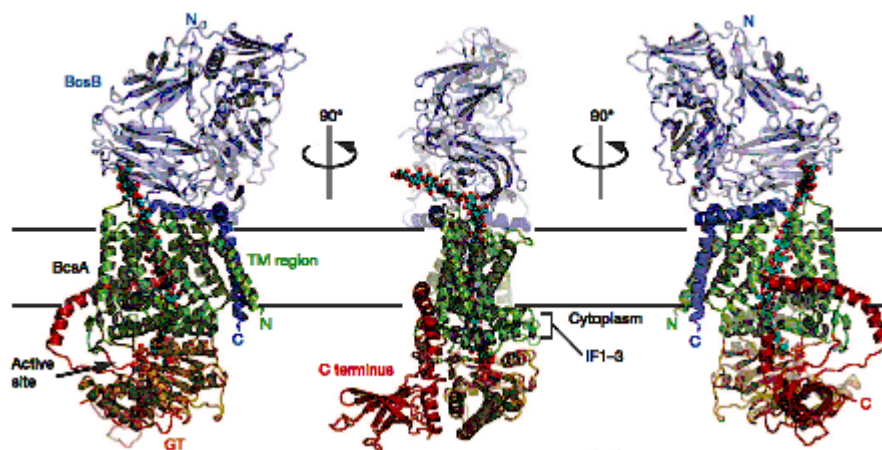


Fig. 3. Architecture of the BcsA-BcsB complex. BcsA and BcsB form an elongated complex with large cytosolic and periplasmic domains. The transmembrane helices of BcsA are coloured green, the glycosyltransferase (GT) domain beige, and the C-terminal domain red. BcsB is shown in light and dark blue for its periplasmic and membrane-associated regions, respectively. The N and C termini of both subunits are indicated and the translocating glucan and UDP are shown as cyan and violet spheres. Horizontal bars indicate the membrane boundaries. IF, amphipathic interface helices of BcsA

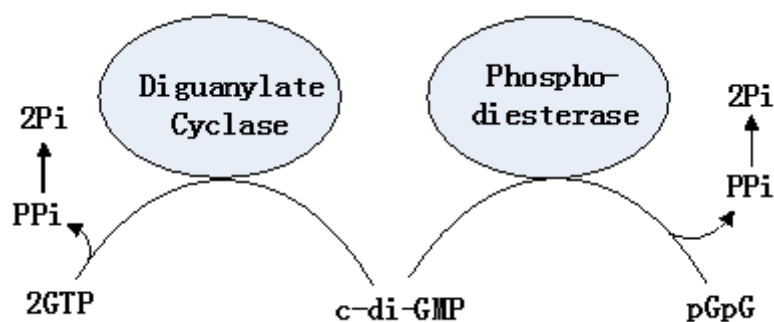


Fig.4. The schematic of reactions for c-di-GMP synthesis and degradation. 2 GTP condensation reaction occurs, the first release of 1 molecules of P_{Pi} is converted into a linear dinucleotide pyrophosphate three phosphorylation of pppGpG, then release the 1 P_{Pi} molecules, and condensed to form c-di-GMP; PDE cuts a single phosphodiester from c-di-GMP, and turns c-di-GMP to be active to do not have the activity of two linear polymer pGpG

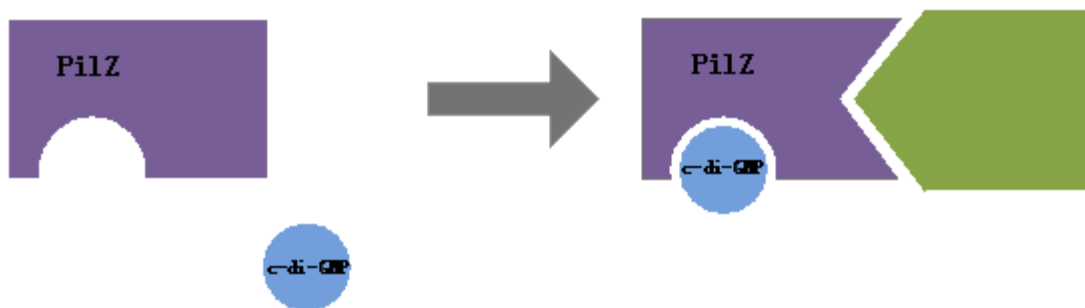


Fig. 5. Possible c-di-GMP effector mechanisms. c-di-GMP (the small ball) binding domains act as molecular switches through interaction with protein partners

Salmonella typhimurium and *Escherichia coli*. In order to define the influence of these two ORFs, Standal introduced the upstream regions into a shuttle vector pSA19. The presence of the first could enhance the production of cellulose and the transposon mutation at the second ORF which caused the inability to produce BC in vivo³⁸. While in *Gluconacetobacter* and *G. xylinus* NBRC3288, three ORFs code for the CS³⁷, and only one ORF contained in the strains ATCC 23769 and closely-related ATCC 53582⁴¹.

Regulation and control of the synthesis of bacterial cellulose is influenced by so many different factors. The experiment proved that approximately eight enzymes involved in the biosynthesis of bacterial cellulose, including cellulose synthase which is the characteristic and key enzyme in the process of cellulose synthesis⁴². The last step catalyzed by the CS from glucose into polyglucan chain is the unique rate-limiting reaction. In 1987, Ross *et al*³⁵ found a kind of guanosine acyl oligonucleotide analogues which had evidently allosteric effect in cellulose synthase when they investigated the pathway of cellulose synthesis in *A. xylinum*. Two years later, they identified the active principle was c-di-GMP and put forward the concept of the cyclic diguanylic acid regulatory system (Fig.4). *A. xylinum* is known to produce c-di-GMP and to synthesize cellulose fibrils in response to the change of intracellular c-di-GMP concentration. The possible mechanisms of c-di-GMP have showed in Figure.3. Amikam⁴³ confirmed that the C-terminal part of *acsA* contained the active site binding c-di-GMP, it was highly homologous to the PilZ domain. So we predict that c-di-GMP binds to *acsA* act as molecular switches through interaction with protein partners (Fig.5).

In order to examine the action of the activator c-di-GMP which confirmed as a bacterial second messenger, Peter and Raphael³⁴ researched the kinetics of activation, the result showed that in the range of 0.3-4.0 μ M, the effector concentration, the activity of the CS manifested a typical Michaelis-Menten relationship via the experiments in vitro. The CS activity fell off rapidly with the concentration of the c-di-GMP below this range. And the intracellular content of cyclic diguanylic acid is dependent on a balance between the cellular levels of diguanylate cyclases (DGC) and the

opposing action of the Ca^{2+} -sensitive phosphodiesterases (AxPDEA1)⁴⁴.

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REFERENCES

1. Brown, A.J., An acetic ferment which forms cellulose. *J. Chem. Soc.*, 1886; **49**: 432-439.
2. Jahn, C.E., Selimi, D.A., Barak, J.D., Charkowski, A.O., The *Dickeya dadantii* biofilm matrix consists of cellulose nanofibres, and is an emergent property dependent upon the type III secretion system and the cellulose synthesis operon. *Microbiol.*, 2011; **157**: 2733-2744.
3. Morgan, J.L., Strumillo, J., Zimmer, J., Crystallographic snapshot of cellulose synthesis and membrane translocation. *Nat.*, 2013; **493**(7431): 181-186.
4. Sani, A., Dahman, Y., Improvements in the production of bacterial synthesized biocellulose nanofibres using different culture methods. *J. Chem. Technol. Biotechnol.*, 2010; **85**(2): 151-164.
5. Czaja, W., Krystynowicz, A., Bielecki, S., Brown Jr, R.M., Microbial cellulose the natural power to heal wounds. *Biomater.*, 2006; **27**(2): 145-151.
6. Ross, P., Mayer, R., Benziman, M. Cellulose biosynthesis and function in bacteria. *Microbiol. Rev.*, 1991; **55**(1): 35-58.
7. Jonas, R., Farah, L.F. Production and application of microbial cellulose. *Polym. Degrad. Stab.*, 1998; **59**(1): 101-106.
8. Keshk, S.M., Haija, M.A., A new method for producing microcrystalline cellulose from *Gluconacetobacter xylinus* and kenaf. *Carbohydr. Polym.*, 2011; **84**(4): 1301-1305.
9. Yang, Y., Jia, J., Xing, J., Chen, J., Lu, S., Isolation and characteristics analysis of a novel high bacterial cellulose producing strain *Gluconacetobacter intermedius* CIs26. *Carbohydr. Polym.*, 2013; **92**(2): 2012-

- 2017.
10. Tanskul, S., Amornthathree, K., Jaturonlak, N., A new cellulose-producing bacterium, *Rhodococcus* sp. MI 2: Screening and optimization of culture conditions. *Carbohydr. Polym.*, 2013; **92**(1): 421-428.
11. Castro, C., Zuluaga, R., Álvarez, C., Putaux, J.L., Caro, G., Rojas, O. J., Gañán, P., Bacterial cellulose produced by a new acid-resistant strain of *Gluconacetobacter* genus. *Carbohydr. Polym.*, 2012; **89**(4): 1033-1037.
12. Rani, M.U., Udayasankar, K., Appaiah, K.A., Properties of Bacterial Cellulose Produced in Grape Medium by Native Isolate *Gluconacetobacter* Sp. *J. Appl. Polym. Sci.*, 2011; **120**(5): 2835-2841.
13. Wu, R.Q., Li, Z.X., Yang, J.P., Xing, X.H., Shao, D.Y., Xing, K.L., Mutagenesis induced by high hydrostatic pressure treatment: a useful method to improve the bacterial cellulose yield of a *Gluconacetobacter xylinus* strain. *Cell*, 2010; **17**(2): 399-405.
14. Hestrin, S., Schramm, M., Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochem. J.*, 1954; **58**(2): 345-352.
15. Ha, J.H., Shah, N., Ul-Islam, M., Khan, T., Park, J.K., Bacterial cellulose production from a single sugar $\pm$ -linked glucuronic acid-based oligosaccharide. *Process. Biochem.*, 2011; **46**(9): 1717-1723.
16. Tonouchi, N., Tsuchida, T., Yoshinaga, F., Beppu, T., Horinouchi, S., Characterization of the biosynthetic pathway of cellulose from glucose and fructose in *Acetobacter xylinum*. *Biosci. Biotechnol. Biochem.*, 1996; **60**(8): 1377-1379.
17. De Wulf, P., Joris, K., Vandamme, E.J., Improved cellulose formation by an *Acetobacter xylinum* mutant limited in (keto) gluconate synthesis. *J. Chem. Technol. Biotechnol.*, 1996; **67**(4): 376-380.
18. Velasco-Bedrán, H., López-Isunza, F., The unified metabolism of *Gluconacetobacter entanii* in continuous and batch processes. *Process. Biochem.*, 2007; **42**(8): 1180-1190.
19. Shoda, M., Sugano, Y., Recent Advances in Bacterial Cellulose Production. *Biotechnol. Bioprocess. Eng.*, 2005; **10**(1): 1-8.
20. Son, H.J., Heo, M.S., Kim, Y.G., Lee, S.J., Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter* sp. A9 in shaking cultures. *Biotechnol. Appl. Biochem.*, 2001; **1**(1): 1-5.
21. Zhao, J., Yu, H., Luo, J., Cao, Z.W., Li, Y., Complex networks theory for analyzing metabolic networks. *Chin. Sci. Bull.*, 2006; **51**(13): 1529-1537.
22. Vallino, J.J., Stephanopoulos, G., Metabolic flux distributions in *Corynebacterium glutamicum* during growth and lysine overproduction. *Biotech. Bioeng.*, 1992; **41**(6): 633-646.
23. Christensen, B., Nielsen, J.: *Metabolic network analysis. In: Bioanalysis and Biosensors for Bioprocess Monitoring.* Denmark: Springer Berlin Heidelberg, 2000; 209-231.
24. Li, Y., Tian, H., Zhang, J., He, X., Ping, W., Lei, H., Improvement of bacterial cellulose production by manipulating the metabolic pathways in which ethanol and sodium citrate involved. *Appl. Microbiol. Biotechnol.*, 2012; **96**(6): 1479-1487.
25. Okuda, K., Structure and phylogeny of cell coverings. *J. Plant. Res.*, 2002; **115**(4): 283-288.
26. Stanislaw, B., Alina, K., Marianna, T., Halina, K. (ed): *Biopolymers.* Wiley-VCH Verlag GmbH 2005: 381-434.
27. Kudicka, K., Wardrop, A., Itoh, T., Brown Jr, R.M., Further evidence from sectioned material in support of the existence of a linear terminal complex in cellulose synthesis. *Protoplasma*, 1987; **136**(2-3): 96-103.
28. Lin, F.C., Brown Jr, R.M., Drake Jr, R.R., Haley, B.E., Identification of the uridine 5'-diphosphoglucose (UDP-glc) binding subunit of cellulose synthase in *Acetobacter xylinum* using the photoaffinity probe 5-azido-UDP-glc. *J. Biol. Chem.*, 1990; **265**(9): 4782-4784.
29. Saxena, I.M., Brown Jr, R.M. (ed): *Cellulose biosynthesis in Acetobacter xylinum: a genetic approach.* New York: John Wiley and Sons, 1989; pp 537-557.
30. Saxena, I.M., Brown, R.M., Cellulose Biosynthesis: Current Views and Evolving Concepts. *Ann. Bot.*, 2005; **96**(1): 9-21.
31. Römling, U., Molecular biology of cellulose production in bacteria. *Res. Microbiol.*, 2002; **153**(4): 205-212.
32. Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., Henrissat, B., The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic. Acids. Res.*, 2009; **37**(suppl 1): 233-238.
33. Saxena, I.M., Kudlicka, K., Okuda, K., Brown Jr, R.M., Characterization of genes in the cellulose-synthesizing operon (acs operon) of *Acetobacter xylinum*: implications for cellulose crystallization. *J. Bacteriol.*, 1994; **176**(18): 5735-5752.
34. Ross, P., Mayer, R., Weinhouse, H., Amikam, D., Huggiratt, Y., Benziman, M., de Vroom, E.,

- Fidder, A., de Paus, P., Sliedregt, L.A., The cyclic diguanylic acid regulatory system of cellulose synthesis in *Acetobacter xylinum*. Chemical synthesis and biological activity of cyclic nucleotide dimer, trimer, and phosphothioate derivatives. *Biol. Chem.*, 1990; **265**(31): 18933-18943.
35. Ross, P., Weinhouse, H., Aloni, Y., Michaeli, D., Weinberger-Ohana, P., Braun, S., de Vroom, E., van der Marel, G.A., van Boom, J.H., Benziman, M., (1987) Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid. *Nat.*, 1987; **325**(6101): 279-281.
 36. Nakai, T., Nishiyama, Y., Kuga, S., Sugano, Y., Shoda, M., ORF2 gene involves in the construction of high-order structure of bacterial cellulose. *Biochem. Biophys. Res. Commun.*, 2002; **295**(2): 458-462.
 37. Wong, H.C., Fear, A.L., Calhoon, R.D., Eichinger, G.H., Mayer, R., Amikam, D., Benziman, M., Gelfand, D.H., Genetic organization of the cellulose synthase operon in *Acetobacter xylinum*. *Proc. Natl. Acad. Sci.*, 1990; **87**(20): 8130-8134.
 38. Raphael, M., Peter, R., Haim, W., Dorit, A., Gail, V., Patricia, O., Roger, D.C., Hing, C.W., Ann, W.E., Moshe, B., Polypeptide composition of bacterial cyclic diguanylic acid-Polypeptide composition of bacterial cyclic diguanylic acid-dependent cellulose synthase and the occurrence of immunologically crossreacting proteins in higher plants. *Proc. Natl. Acad. Sci.* 1991; **88**(12): 5472-5476.
 39. Sunagawa, N., Fujiwara, T., Yoda, T., Kawano, S., Satoh, Y., Yao, M., Tajima, K., Dairy, T., Cellulose complementing factor (Ccp) is a new member of the cellulose synthase complex (terminal complex) in *Acetobacter xylinum*. *J. Biosci. Bioeng.*, 2013; **115**(6): 607-612.
 40. Pear, J.R., Kawagoe, Y., Schreckengost, W.E., Delmer, D.P., Stalker, D.M., Higher plants contain homologs of the bacterial celA genes encoding the catalytic subunit of cellulose synthase. *Proc. Natl. Acad. Sci.*, 1996; **93**(22): 12637-12642.
 41. Ogino, H., Azuma, Y., Hosoyama, A., Nakazawa, H., Matsutani, M., Hasegawa, A., Otsuyama, K., Matsushita, K., Fujita, N., Shirai, M., Complete genome sequence of NBRC 3288, a unique cellulose-nonproducing strain of *Gluconacetobacter xylinus* isolated from vinegar. *J. Bacteriol.*, 2011; **193**(24): 6997-6998.
 42. Lei, L., Li, S., Gu, Y., Cellulose synthase complexes: composition and regulation. *Front. Plant. Sci.*, 2012; **3**(75): 1-6.
 43. Amikam, D., Benziman, M., Cyclic diguanylic acid and cellulose synthesis in *Agrobacterium tumefaciens*. *J. Bacteriol.*, 1989; **171**(12): 6649-6655.
 44. Ryjenkov, D.A., Tarutina, M., Moskvina, O.V., Gomelsky, M., Cyclic diguanylate is a ubiquitous signaling molecule in bacteria: insights into biochemistry of the GGDEF protein domain. *J. Bacteriol.*, 2005; **187**(5): 1792-1798.
 45. Brown Jr, R.M., (1992). Emerging technologies and future prospects for industrialization of microbially derived cellulose. In: Harnessing biotechnology for the 21st century, proceedings of the 9th International Biotech Symposium and Exposition, 1992: 76-79.
 46. Pae, N., Rotary discs reactor for enhanced production of microbial cellulose. Universiti Teknologi Malaysia, Faculty of Chemical and Natural Resource Engineering, 2009.