REVIEW ARTICLE

Acetobacter xylinum: Biochemical synthesis production and regulation of cellulose with c-di-GMP-binding protein

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In consideration to BC, *Acetobacter xylinum* is considered as the known producer of cellulose and used in a lot of experiments. Variety of microorganisms and plants produce cellulose with several distinct mechanical and structural properties. However, Bacterial Cellulose (BC) has some unique properties of natural polymer and therefore considered as important candidate for biomedical applications, as it is free of impurities unlike to that of Plant Cellulose (PC), which use also cause environmental disaster. The initial discovery of c-di-GMP was in 1987, which controls variety of cellular processes and known as second messenger of bacteria. Cellular procedure like formation of the biofilm, virulence, motility, surface adapation and also bacterial pathogenicity are controlled by c-di-GMP.

This review explains our current understanding about the biochemical synthesis of *Acetobacter xylinum* with morphological properties of cellulose. It highlights the pathway and activity of c-di-GMP with numerous biomedical applications and forthcoming plans.

Keywords: *Acetobacter xylinu*; Bacterial cellulose; Plant cellulose; Biosynthesis; Micro fibrils

INTRODUCTION

Cellulose is considered as an essential biological polymer that is found in profuse amount on earth. In higher plants and some algae, cellulose is considered as one the main components of their cell walls. Organisms that are synthesizing cellulose are not only algae and plants but also it can be synthesized in fungi, bacteria, protests and tunicates. This shows that these organisms are extensively distributed all over the biological kingdom. Therefore, biosynthesized cellulose from various strains of microorganisms and also cellulose isolated from plants are then used for obtaining purified form of cellulose. In consideration to its structure, both the Bacterial Cellulose (BC) and Plant Cellulose (PC) have the same biochemical composition, however plant cellulose consists of some impurities as bacterial cellulose is impurity free (they have no lignin, pectin or hemicellulose) [1]. They also exhibit a finer structure of 10 to 100 nm width, biodegradable, higher crystalanity and extended fiber length.

Because of that, BC content is considered as purer than that of plant cellulose. An example of cellulose production from various organisms in various fibril forms are *Vallonia* (algae), *Acetobacter* and *Rhizobium* (bacteria) and *Saprolegnia* (fungi). In additon, considering the growth of BC in various fermentation vessels under static cultivation conditions, it can be grown into different shapes. This point is showing that BC has better chemical properties. Usually, due to low cost and physical properties of cellulose, it is extensively use as a reinforcement agent, in textile and paper making industries and in a lot of medical applications [2]. However, this extensive use of cellulose becomes the reasons for a lot of environmental issues due to deforestation and by product of wood pulping. For that reason, bacteria based cellulose works as a substitute to plant cellulose.

Basically, the synthesis of purest form of cellulose like an extracellular product is founded in *Acetobacter xylinum*, which retains high level of Cellulose Synthase Activity (CSA) which is exposed to a composite called multi component regulatory system. Regulation mainly depends on a reversible allosteric activator and a regulatory enzymes known as (bis-(2′, 5′)-cyclic diguanylic acid (c-di-GMP). Not only in bacteria but several other species also constitute c-di-GMP. That novel nucleotide c-di-GMP stays crucial for provoking a cellular function as these protein act as a c-di-GMP receptors.

LITERATURE REVIEW

Aiming the study for providing an overview of BC structure along with morphological and physiological properties, this study discuss the biochemical synthesis of cellulose by *Acetobaacter xylinum*. c-di-GMP being considered as a universal regulator for various cellular processes of bacteria, this review emphasize on regulation of cellulose by discussing the pathway and activity of c-di-GMP [3]. Finally, some BC based biomedical applications and recent improvements for enhancing the process of regulation with upcoming plans are also discussed, that will help in understanding the depth of this universal second messenger of bacteria.

Acetobacter xylinum and its role in cellulose biosynthesis

Among the bacteria the capability for the production Bacterial Cellulose (BC) is widespread to the strains. Though, *Komagataeibacter xylinus* is recongnized producer of BC. The *Koamagataeibacter xylinus* belong to the group of bacteria known as Acetic Acid Bacteria (AAB), which is well-defined with in the family *Acetobacteraceae*. AAB have the capability of oxidizing ethanol in to acetic acid. AAb is further classified into α-Proteobacteria and considered as strongly aerobic gram-negative bacteria. Some of bacteria are considered as a strong producers of cellulose *i.e. Komagataeibacter oboediens*, *i.e. Komagataeibacter medellinensis*, *Komagataeibacter hansenii*, *Komagataeibacter naticola*, *Komagataeibacter Komagataeibacter rhaeticus* and *Komagataeibacter pomaceti*. While some of the genera according to the recent classification of AAb that are known to synthesize cellulose with chemically identical but some superior feature over that of plant cellulose are *Acidomonas*, *Gluconobacter*, *Acetobacter*, *Saccharibacter*, *Gluconacetobacter*, *Asaia* and *Swaminathania* [4]. AAB basically considered as harmless as they have the characteristic of being Generally Recognized As Safe (GRAS) bacteria or food grade, therefore it is used for production of cellulose.

Acetobacter xylinum is probably the wildely used organism intended for bacterial cellulose synthesis. The ability of this bacterium is that through only one process of synthesis, this bacterium polymerizes glucose into

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cellulose. It is also known as *Gluconacetobacter xylinus* is a gram negative microorganism which is basically rod shaped, along with the length of about 2 to 10 microns and width ranging from 0.5 to 1 micron. It is purple non photosynthestic and common vinegar bacterium that that converts any organic substance *i.e.* glycerol, sugar and glucose into pure cellulose. The molecular formula of *A. xylinum* is ($CH_2O_{0.52}N_{0.23}$). *A. xylinum* is aerobic; they need oxygen to survive.

In late nineteenth century, *A. xylinum* was discovered and from then, they are used for many years in different practical applications. These bacteria do not create pigment endospore and live by combining 6 to 8 cells. However there some factor or sudden changes to which *A. xylinum* shows resistance *i.e.* pH, in media composition, low level of water, pathogenic organisms and existence of toxic substance, and also some of the weather conditions [5]. However, in case of weather for *A. xylinum*, it can grow and exhibit cellulose synthesis in an envelope. Though, after exposure to UV light, about 23% of cellulose coated bacterial cells survive. If the protective layer of polysaccharides cellulose is removed from bacterial cells, there survival chance is decrease to the level of 3% causing extreme reduction of cells.

Generally, polysaccharide (cellulose) has the molecular formula $(C_6H_{10}O_5)_n$ and comprising of β-1,4-d-glucose units. This renewable polymer is extensively used in medical and food industries. For instance, it possesses several exclusive properties *i.e.* high capacity for absorption of water, high crystalnity, high mechanical strength with extremely fibrous and super fine network structure. Furthermore, as we know that *A. xylinum* is one of the most efficient and studied BC producer that give high level of cellulose by assimilating several sugars in a liquid medium. Therefore, there large scale production of cellulose is essential which was discovered by researchers in this bacterium as micro fibrils. It is considered as a model organism due to all its all feature being aerobic, gram negative and rod shaped bacteria for cellulose synthesis for commercial fermentation. From each synthetic site, micro fibrils are merged to form long ribbon shaped cellulose in growth medium, which is basically non motile because of floating pellicles formation with the tangled associated cells. Besides, cellulose synthesis by bacteria also aid in attachments of organisms to the plants *i.e.* pathogen *Agrobacterium tumefaciens* and symbiotic *Rhizobium. Agrobacterium tumefaciens* which is host cells of plants and known as a tumor forming bacterium also shows cellulose fibrils secretion. Thus, for survival and cell attachment, this secretion surrounds the cell. Another way is to act as an obstacle to yeast, fungi and few other microorganisms.

On the top of liquid medium (static culture), the bacteria secretes cellulose fibrils which produce a thick gel called pellicle under special culturing conditions. This pellicle is composed of 97% of water and cellulose micro fibrils. The actual reason for cellulose production is unknown. However, it is considered important for their survival or to give bacteria necessary precautions *i.e.* to protect them from UV light. Apart from that, adjusting culturing conditions is the main advantage of cellulose micro fibrils derived from bacteria for changing the formation and crystallization of micro fibrils. In this bacterium, the cellulose micro fibrils association leads to the assemblage of a multicellular pellicle in static liquid cultures. Due to unique properties of BC *i.e.* integration with nanoparticles, non-toxicity, hydrophilicity and biocompatibility is used in a lot of contexts [6].

Moreover, for providing a better environment to access nutrients easily, the cells within the pellicles will stay near to the surface which may be necessary for these obligate aerobes growth. For attachment of bacteria to the decaying plant material, cellulose synthesis helps in it because on this materials, they grow and provide protection from other competitors which also dependent on the same source nutrients.

DISCUSSION

Cellulose structure

Cellulose is comprised of glucose monomer which is joined by β–(1,4) linkage, regarded as unbranched hompolymer. Glucose monomers of cellulose form the inter and intramolecular hydrogen bonds which includes 3 hydroxyl groups which are positioned at the c2 and c3 for secondary hydroxyl groups and at c6 for the primary hydroxyl groups. For bacterial polymerization into β-1,4-glucan chain, and bacteria uses several carbon sources from the medium [7]. These chains are then transported further outside of the cell through pores present in the outer membrane. Outside of the cell, these linear β-1,4-glucan chains assembled to form sub fibrils. The sub fibrils consist of 10-15 new β-1,4-glucan chains. Afterwards, these sub fibrils crystalized into microfibrils, further followed by bundles and then far along the bundles transformed into ribbons which are formed of 1000 of glucan chains individually. Thus cellulose is often seen in highly ordered structure due to this spatial conformation and chemical structure. Thus chemical, mechanical and enzymatic treatments with the raw materials are needed for the isolation cellulose isolation (Figure 1).

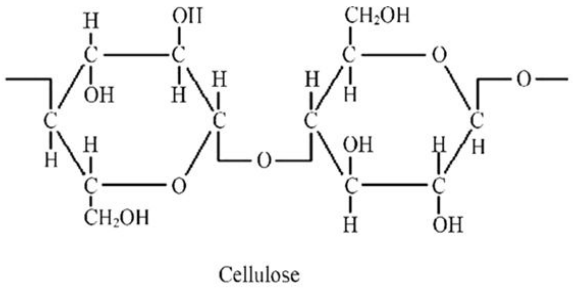

Figure 1: Chemical structure of bacterial cellulose.

Morphological and physiological properties of bacterial cellulose

Comparing BC to PC, BC shows higher range of crystalinity which is above 80%. In plant, cellulose crystilanity range from 40-49% and consists of a lot of amorphous regions. BC structure shows that it is similar to that of plant cellulose chemically but still they have some differences in degree of polymerization. For plants, the degree of polymerization is (2000-6000) and for BC, it is (13,000-14,000) individually. Besides, cellulose shows six diverse polymorphs in terms of consideration of crystalinity. These several polymorphs are named as I, II IVII (α and β), IIII, IIIII, IVI and IVII (α and β). There are two categories of cellulose type 1 and cellulose type 2. The type one is found in profuse amount in nature and is mostly synthesized by bacteria. Bacteria can also synthesize type II of cellulose. There is variation in the ratio of abundance of the two polymorphs which depends basically on the source of cellulose from which they are obtained. Iα is the main constituent of bacterial cellulose as BC is rich in that allymorph while cellulose from plants mainly constitutes Iβ allymorph. In fact, the swollen state value of 1.5 MPa and 8 MPa of young's modulus and tensile strength can be observed respectively because of the high affinity for water shown by BC.

Because of the strong affinity or capacity for absorption of water, BC can also be considered as hydrogel. As it can take water and reach 100 times more than of its own weight. BC hydrogel having amorphous structure, which helps it in absorbing water at a rate of 99% of its own weight. The BC exceptional properties are because of 3D network structure that makes it an appropriate podium for its use in numerous medicinal applications *i.e.* its use for the regeneration of some tissues. Additionally, because of this water up taking ability and hydrophilcity, BC is considered as bio degradable material. As we know that, the polymers degradation, allowing the material colonization by fungi and microorganisms that require to food *i.e.* carbon is referred as the process of hydrolysis. Consequently, hydrophilic materials are more vulnerable to hydrolysis.

Biochemical mechanism of cellulose synthesis

About more than 100 years, *Acetobacter xylinum* has been known to be a good producer of pure cellulose. A lot of research deals mostly with the elucidation of biosynthesis of cellulose with *A. xylinum*. However, only pellicle or skin are produced so far under agitated conditions from most of the strains. Cellulose is produced by bacteria through catalytic process. *A.*

xylinum organized the subunits on the main axis of the cell wall in a linear fashion. Three of the subunits are known as triplets while the latter is known as Terminal Complex (TC). The outer surface of bacteria is covered by each subunit. Then each subunit gathered in to sub fibrils which consist of cellulose molecules.

As cellulose biosynthesis is a complicated process. Firstly, it includes glucose residues polymerization into β1-4 glucan chain. Then, secrete the chains extracellularly finishing the crystallization and linear arrangement of glucan chains van der Waals forces and hydrogen bridges and arranged in strips hierarchically. Thus, a tough three dimensional structure is formed known as micro fibrils. *A. xylinum* synthesized molecular cellulose possess some unique characteristics *i.e.* variable thickness and unidirectional polarity. Basically, phosphofructokinase is an enzyme which is accountable for phosphorylation of fructose-6-phosphate and fructose 1-6 biophosphate by catalyzing the reaction. This process will prevent glycolysis. The four bacterial enzymes are involved basically to catalyze glucose conversion which is transported into cytoplasm from external environment. In bacterial cellulose synthesis, a huge amount of individual enzymes and a complex of regulatory and catalytic proteins are involved. Therefore it is considered as a multi-step regulatory process specifically. Firstly, the enzyme glucokinase is responsible for carbon 6 of glucose phosphorylation. This phosphorylation yields glucose-6-phosphate. Secondly, the isomerization reaction of glucose-phosphate into glucose-1-monophosphate is catalyzed through another enzyme known as phosphoglucomutase. Third is UDPG-pyrophosphorylase enzyme which is responsible for UDP-Glucose (UDPG) synthesis. It is also recognized as glucose-1-phosphate uridylyltransferase [8]. Lastly from UDP-glucose, the polymerization of glucose is instigated through Cellulose Synthase (CS). Figure 2 presents the model for biochemical sysnthesis of cellulose.

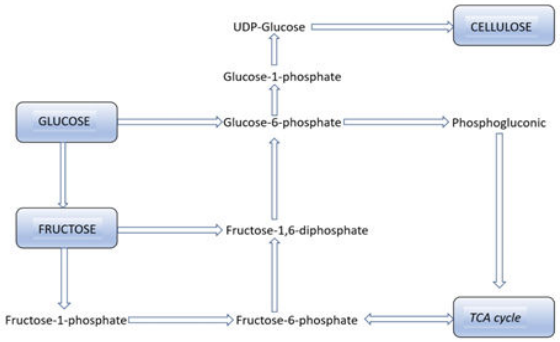

Figure 2: Biochemical pathways for the synthesis of cellulose.

Apart from all the above discussion, the growth of *Accetobacter xylinum* is affected by some factors. Temperature, pH, availability of oxygen, nitrogen and carbon sources are some of them [9].

Cellulose regulating c-di-GMP-binding protein

History: The secretion and synthesis of Bacterial Cellulose (BC) depends vastly on the concentration of cellular c-di-GMP which is known as universal second messenger. It helps in regulating diverse number of functions a huge variety of bacterial species *i.e.* formation of biofilm, virulence and motility. Though c-di-GMP is scientifically of a great importance. It is obvious that the discovery of c-di-GMP was unexpected for numerous scientific discoveries. Furthermore, its discovery was underrated in the earlier days. In that time, another model that is cellulose biosynthesis by acetic acid bacteria, including G. *xylinus*, was considered an efficient model in plants for studying cellulose biosynthesis. The innovation of cyclic dimeric (3'→5') GMP was passed thirty five years. c-di-GMP is occurred as the furthermost significant and common second or subsequent messenger of bacterium obtained from a comparatively insignificant allosteric activator of a BC synthase [10].

According to Ross, et al., 1987, C-di-GMP was discovered in Jerusalem as an allosteric factor at Hebrew university. This factor is needed in *Alphaproteobacterium (Gluconacetobacter xylinus)* for cellulose activation biosynthesis. Basically, *Gluconacetobacter xylinus* is referred as *Acetobacter xylinum* at the time of its discovery. In 1947, Moshe Benzimans and the coworkers discovered c-di-GMP in gram-negative bacterium *Komagataeibacter xylinum* while studying the cellulose biogenesis. The study resulted in c-di-GMP identification as a second messenger of bacterium. This bacteria is also previously recognized as *Acetobacter xylinum*. The genes encoding for enzymes was identified later by them which *in Komagataeibacter xylinus* aids in c-di-GMP regulation. The innovation of cyclic dimeric (3'→5') GMP was passed thirty five years. c-di-GMP is occurred as the furthermost significant and common second or subsequent messenger of bacterium obtained from a comparatively insignificant allosteric activator of a BC synthase [11].

In Figure 3 given below, the chemical structure is depicted for c-di-GMP. Through membrane preparation of *K. xylinus*, it was shown by *in vitro* studies that biosynthesis of cellulose is increased because of c-di-Gmp existence from 50 up to 200 folds [12]. Also, there are some indications about cellulose synthase promoter activation and their binding both at transcriptional level which suggest that the c-di-GMP may have effect on cellulose biogenesis. In *K. xylinus*, through itself activating the complex allosterically, the major activation mechanisms appear. The complex is known as known as cellulose synthase complex. c-di-GMP also controls several aspects of bacterial physiology.

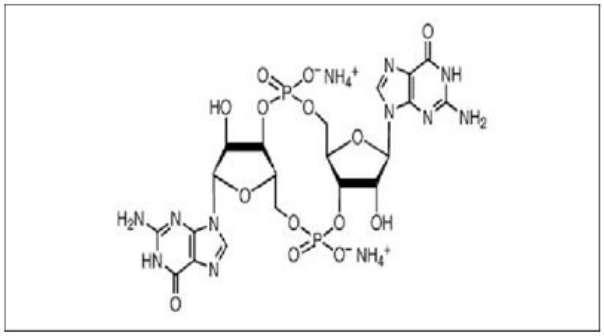

Figure 3: Chemical structure of c-di-GMP.

Pathway of c-di-GMP- binding protein

In microbiology, comparative genome analysis made it to possible to identify bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) as a universal second messenger [13]. In *Acetobacter xylinum* (cellulose producing bacterium), cyclic-di-GMP (c-di-GMP) work as a reversible and extremely precise positive effector of (1,4-β-d-glucan) cellulose synthase. Cyclic di-GMP has the capability to behave as a rescindable and precise allosteric activator of cellulose synthase [14]. The GGDEF and EAL domains which were discovered by Moshe Benziman and co-workers previously to be involved in turnover of c-di-GMP are considered to be amongst the huge amount of domains, which are encoded in genome of bacteria and recommend the extensive spread of c-di-GMP dependent regulation in the bacterial world. It is depicted in the reports that cyclic-di-GMP connected to the B-subunit of cellulose synthase.

The primary catalytic proteins are existed in BcsA protein for cellulose polymerization with the use of UDP-Glc. It uses UDP-Glc as a forerunner and also for membrane transport. Two transmembrane domains which are located in the internal membrane of bacteria are existed in it. It flanks the cytosolic Glycosyltransferase family 2 (GT2) domain that is accountable for polymerization of cellulose trailed by a C-terminal PilZ domain. This domain interacts immediately with c-di-GMP. The earlier proteins containing c-di-GMP binding motifs were the PilZ domains [15]. A six stranded β-barrel topology was adopted by them, and *via* preserved RXXXR and DXSXXG motifs, they got interacted with intercalated dimeric c-di-GMP. On the

other hand, through a C-terminal transmembrane, the BcsB localize mainly to the periplasm and secured in the inward membrane and got interaction with stabilizes BcsA. Through the main mechanism of allosteric activation of BcsA protein, c-di-GMP regulates biosynthesis of cellulose. The main signaling process is presented below in Figure 4. The C-terminal PilZ domain of BcsA through its conserved RXXXR Motif, form a straightaway bonds to dimeric c-di-GMP. Whereas, the BscA adopts an auto inhibited conformation in the absence of c-di-GMP binding. Hence, a gating loop sits directly above the active site of BcsA. It prevents entry to the UDP-Glc substrate. For getting access by the UDP-Glc to the active site, a conformational change is needed. In the gating loop, this change is induced by second messenger binding to the PilZ domain of BcsA. Consistently, intracellular high levels of c-di-GMP and greater level of BcsA actitvity individually support the production or the synthesis of abundant cellulose and cellulose polymers.

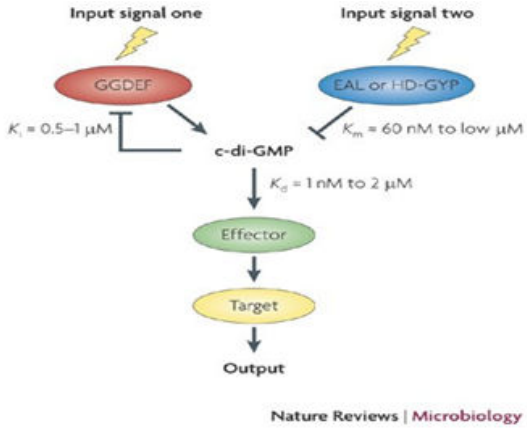

Figure 4: Basic signaling of c-di-GMP.

Activity of c-di-GMP

The turnover of cyclic di-GMP consists of two protein domains which were before known of unidentified purpose *i.e.* EAL and GGDEF. There are two enzymes which are included in the production and degradation of c-di-GMP. The Diguanylate Cyclase (DGCs) is known for c-di-GMP production and Phosphodiesterases (PDEs) comprising either one (HD-GYP or EAL domains) is for c-di-GMP degradation. Basically, GGDEF domains offer Diguanylate Cyclases (DGCs) activity while HD-GYP or EAL domains provide the activity of PDE. It is revealed in several bacterial species by biochemical and genetic evidences that c-di-GMP is degreaded to 5'-Phosphoguanylyl-(3'-5')-Guanosine (pGpG) by EAL or HD-GYP motif [16]. Multiple DGCs and PDEs encodes usually genomes of diverse number of bacteria. However under specific conditions, few of them basically affect biofilm formation. These (amino acid motifs) domains perform their enzymatic activity particularly. Activities of both PDEs and DGCs are controlled by these amino terminal sensory domains. On other side, some of the studies confirmed that the hydrolyzing activity of c-di-GMP comprising HD-GYP domain in contrast to EAL, result in both pGpG and GMP. On the other hand, because of ineffective purification of protiens comprising catalytically active HDGYP domain, theres is restriction in its biochemical validations. Apart from all the mechanisms of c-di-GMP synthesis and degradation, those proteins are also very crucial for provoking precise cellular activities which function as c-di-GMP receptors. The expressions are controlled by cellular and environmental signaling mechanisms. By binding to effector modules the c-di-GMP start it's functioning thus affecting the activity of effector components allosterically. This mechanism includes numerous types of proteins and RNA. The RNA acts as riboswitch. For regulation of mRNA expression, the ability of non protien coding sequence to bind directly minor molecule effector without the support of accessory proteins in which it is embedded is known as riboswtich. A lot of targeted processes are controlled directly by the C-di-GMP-binding effectors. As discussed above that c-di-GMP starts its function by binding to the effectors, so if the effector factor is transcriptional, it will initiate transcription. If the effector is riboswtich, then it will trigger either translation or transcriptional elongation. Some of the other process *i.e.* enzymatic activities and regulated proteolysis are controlled by it. Synthesis of specific EPS or mechanisms of excretions are few of complex cellular structures directed or comtolled through c-di-GMP-binding effectors. Some spps of both gram postive and gram negative bacteria consist of profuse amount of EAL and GGDEF domain containing protiens, as this indication is verified by Whole Genome Sequencing (WGS) [17]. Both (GGDEF and EAL domains) proteins activities show quick reaction to environmental signals because they also contain some subsidiary input signaling domains.

Biological importance of c-di-GMP

Environmental signals strongly effect the production of bacterial Exopolysaccharides (EPS) as they regulate this process. c-di-GMP domains are known to involve in EPS synthesis, which shows the significant role of c-di-GMP in responding or sensing cellular signals. A number of procedures are controlled by c-di-GMP *i.e.* synthesis of virulence factor, development of biofilm, motility and cell differentiation [18]. In consideration of plants, animals and human beings, this virulence factor synthesis and motility are promoted by lower level of c-di-GMP. The synthesis or the activation of cellulose which are essential component of matrix is also stimulated by c-di-GMP. Though, some studies show the promotion of aggregative behavior, sessility and surface adapation by higher c-di-GMP cellular levels. As high level of c-di-GMP inhibits motility and encourage or increase formation of biofilm, On the other hand, its low level affect the process by increasing motility and lowering the formation of biofilm.

Many aspects of bacterial behavior *i.e.* the three main features of biofilms like formation, maturation and dissolution are undoubtedly affected by the extracellular environment. Sensing of specific environmental signals is linked by signal transduction system to suitable changes in gene expression and bacterial physiology. The level of second intracellular signals (second messenger) is changed by perception of initial signal as it is one of the signal transduction mechanisms. Structurally, the c-di-GMP binding protein is connected with cellulose synthase. This association or connection looks to have main part in intracellular modulation c-di-GMP concentration. Therefore in *in vivo*, it may consider as an important factor for regulating synthesis of cellulose. One of the strategies to improve cellulose biosynthesis can be the use of mutagenisation with chemical and physical agents. Moreover, the ability of bacteria for interaction with eukaryotic or bacterial cells and also with abiotic surfaces are controlled by signaling pathways dependent on c-di-GMP [19].

Additionally, at the time of changing in lifestyle of various bacteria, cyclic c-di-GMP play major role in many areas. Some of them includes motile to sessile state transition. This transition helps in the development of multicellular biofilm communities. In acute infections, it also aids in the formation of less virulent state from virulent state with characteristics of more tough state of diseases (chronic infections). c-di-GMP is also considered as auspicious vaccine adjuvant because it is known by mammalian immune system as it takes part in inter-kingdom signaling. Delivery of GMP together with influenza vaccine through micro needles resulting in hummoral and cellular immunity enhancement. In comparison to influenza vaccine alone, it shows completes immunity against lethal or contaminated infections. Significantly, the role for many numbers of proteins has uncovered by molecular analysis of biofilm formation and communal behavior *i.e.*, in developmental transitions, EAL and GGDEF domains. In short terms, by taking all the consideration together, it specify the biological significance of cyclic-di-GMP dependent signaling as second messenger during formation of biofilms in several bacteria.

Future prospects

Generally in biofilms, the exopolysaccharide matrix substances are stimulated by c-di-GMP. In many cellular processes of bacteria, c-di-GMP performs work as a universal second messenger or regulator [20]. Taking the advantage of this fact, the aim for improving the production of bacterial

cellulose can be achieved. Furthermore, it is necessary to study different effectors or targets which are included in the metabolic pathways of c-di-GMP. Therefore, in diversity of bacteria, it is important to find out the crucial features from signaling pathways distribution of c-di-GMP. Apart from that, as machine learning approaches have two advantages therefore it can be used. Firstly, this can be used for identification of the supporting pathways that are associated with c-di-GMP signaling proteins. Secondly, this information can be used to limit the behavior of a novel strain in synthetic biology. Furthermore, a predictive model is needed in order to identify some relevant features that accomplished the connection among pathways and genes encoding domains.

CONCLUSION

Cellulose is considered as a biological polymer. *Acetobacter xylinum* is known to be good producer of cellulose which can be used in many medicinal and pharmaceutical applications. c-di-GMP discovered firstly and later on considered as a universal second messenger molecule taking the control of profuse amount of cellular activities like biofilm formation showing its versatility. GGDEF and EAL are the main domain proteins triggering a lot of environmental signals and helping in regulation of process thus controlling numerous pathways and providing several functions. For cellulose synthesis by bacteria, instigation by c-di-GMP is main crucial step to fulfill the process.

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