Expression of genes involved in the biosynthesis of bacterial cellulose by symbiotic culture of Medusomyces gisevii, depending on the available carbon source

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Abstract. Scientific and practical interest in the design of symbiotic cultures is determined by their potential for use in biotechnological processes for the production of a wide range of biogenic molecules. We have determined the expression levels of genes involved in the biosynthesis of cellulose by the symbiotic culture Medusomyces gisevii that had been statically grown either on glucose or sucrose or fructose, considered as three most economically justified carbon sources. 16S ribosomal RNA was used as a reference gene. The PCR primers used were bcsA1, bcsA2, bcsA3, bcsA4, bcsB, DGC, PDE, UDP GT. Overall higher gene expression levels were observed when cultivating a symbiotic culture of Medusomyces gisevi on glucose or sucrose substrates compared with fructose. Furthermore, the expression of these genes in the synthesized BC gel films is significantly higher compared to that in the bacterial culture medium.

1 Introduction

Cellulose, found in both plants and algae, is also synthesized by certain microorganisms [1]. The term "nanocellulose" denotes cellulose possessing a fibrillar structure, which can be categorized into three distinct types: cellulose nanocrystals, cellulose nanofibrils, and bacterial nanocellulose (BC) [2]. Bacterial cellulose is synthesized by a number of microorganisms in the form of exopolysaccharides, the most effective in this regard are of the genera Acetobacter, Gluconacetobacter and Komagataeibacter. BC stands out as a unique natural nanopolymer composed of β-(1 → 4) glucan chains, produced by various bacterial species [3, 4, 5].

The production of BC encompasses three distinct stages: a) glucose polymerization and the formation of β-1,4-glucan by cellulose synthase, b) secretion of the glucan chains into the culture medium, and c) assembly and crystallization of the excreted chains through hydrogen bonding, resulting in the formation of a ribbon-like structure [6, 7].

The production of microbial cellulose and the organization of genes responsible for its biosynthesis have been extensively examined in Gluconacetobacter xylinum [8]. The bcsABCD operon, housing four genes responsible for the BC biosynthesis pathway, was initially identified in Acetobacter xylinus.

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Interestingly, most of the studied bacteria (taxa) have only two subunits, bcsA and bcsB (*Gluconacetobacter diazotrophicus PAI5*), that are sufficient for the formation of a polysaccharide chain.

The bcsA gene product of bacterial cellulose synthase (CSC) performs a catalytic function and is located on the cytoplasmic side, while the regulatory bcsB subunit faces the periplasmic side - they together determine the overall activity of the CSC [9].

However, to date, a variety of bacterial CSC operons and the bcs genes influencing enzymatic activity and product yield have been discovered in individual proteobacteria genomes. So, it was found that the other two subunits bcsC and bcsD can modulate the expression of the biosynthetic apparatus, the export of the nascent β-D-glucan polymer to the cell surface and the organization of cellulose fibers into a higher-order structure, which leads to maximum BC formation.

The operon, which includes four types of genes, bcsA, bcsB, bcsC и bcsD, is surrounded by accessory genes (cmcA, ccpA и bgIА). They complement the bcs operon in the structuring of cellulose polymer chains and determine the organization of the superstructure of fibrils.

Similar operons (bcsA) are found in other bacteria, such as *Agrobacterium tumefaciens* and *Rhizobium leguminosarum*, each including two genes (bcsA and bcsB).

The catalytic process controlled by CSC is the limiting stage that determines the rate of biosynthesis of bacterial cellulose. However, in certain strains (such as *Acetobacter*), the level of UGPase, which is less process-specific, can also act as a limiting factor. The permeability of the cell wall to glucose in nutrient-poor environments may represent the slowest stage, while the rate of gluconeogenesis may become a bottleneck when glycerol is used as a substrate. Thus, the determination of which specific factor constrains the speed also relies on a given set of physiological conditions [10, 11, 12].

A primary and less resource-intensive method for enhancing BC production and reducing costs is to optimize crop growing conditions, which entails refining the type of the culture medium [5, 13], as well as fine-tuning parameters such as incubation time, temperature, inoculation level, and air-liquid interface [14, 15]. Typically, the technological process of BC production must be tailored to each strain to maximize product yield [16-17]. Despite the undoubted advantages of using isolated strains in BC biotechnology, fermentation in symbiotic communities can provide a greater yield of cellulose in the future for its industrial production. Of particular relevance is the examination of gene activation among cellulose-synthesizing bacteria, contingent upon process conditions.

### 2 Materials and Methods

#### 2.1 Culture medium composition and biosynthetic process

The symbiotic microbial consortium *Medusomyces gisevii* was obtained from the Scientific Center Kurchatov Institute–Research Institute for Genetics and Selection of Industrial Microorganisms (Russia). This stable microbial community comprises 15–30 genera, predominantly yeasts (especially *Zygosaccharomyces sp.*.) and acetic acid bacteria (primarily *Gluconacetobacter sp.* and *Acetobacter sp.*). The precise species composition of the culture is elaborated elsewhere [18, 19].

The culture medium utilized consisted of glucose, sucrose, or fructose at w/w concentrations of 7.5%, dissolved in a 0.4% extract of plain green tea. The tea solution was prepared by steeping 4 g of dry green tea in 1L of hot (80°C) tap water for 15 minutes, resulting in approximately 1.21 g L⁻¹ of green-tea extractives in the medium. This medium
is considered "classical" for *Medusomyces* as it does not require further sterilization, allowing cultivation under non-sterile conditions.

### 2.2 Determination of gene expression

To assess the activation of genes involved in BC biosynthesis, total RNA was isolated from microorganisms in two series: firstly, from the culture liquid of the symbiotic culture *Medusomyces gisevii*, and secondly, from the BC gel film produced over 7 days at a temperature of 29°C, under static conditions, utilizing substrates such as glucose, sucrose, and fructose.

The process for determining the expression of genes involved in bacterial cellulose biosynthesis entailed several preliminary steps. Firstly, cultural RNA was isolated as a precipitate using 80% ethanol, with a minimum yield of 10 micrograms per sample. To achieve this, the suspended cell suspension in PBS was lysed using the "Lyra" reagent, followed by phase separation in the presence of chloroform during centrifugation at 10,000 g with cooling to 4°C. The RNA from the aqueous phase was precipitated in the presence of isopropanol. Subsequently, the deposited RNA was treated with RNase -free DNase, as per the manufacturer's protocol, followed by purification using the "RealBestSorbitus" kit (Vector-Best).

Gene expression analysis was conducted via real-time RT-PCR. Reverse transcription was carried out using M-MuLV–RH reagents (Biolabmix), supplemented with primers containing random sequences. This mixture underwent a heating and cooling process, followed by the introduction of RNA-dependent DNA polymerase, OT buffer, and deoxynucleotide triphosphates. The reaction was terminated thereafter. Quantitative PCR with real-time detection utilized Biolabmix's HS-qPCR SYBR Blue (2×) BioMaster. To ensure accuracy, universal conditions were selected, allowing for the amplification of any genes under investigation. These conditions included several stages. Fluorescence was measured during each cycle, and a melting curve was constructed. Each point was subjected to three repetitions.

### 3 Results and Discussion

To assess the expression levels in both the liquid phase of the symbiotic culture of *Medusomyces gisevii* and the BC gel film produced under static cultivation conditions using different carbon sources, 16S ribosomal RNA was employed as a reference gene. The list of primers utilized is detailed in Table 1.

<table>
<thead>
<tr>
<th>The gene</th>
<th>Nucleotide sequences</th>
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<tbody>
<tr>
<td>bcsA1</td>
<td>CCGGTACGCAGCCTGAG</td>
</tr>
<tr>
<td></td>
<td>TCTCGATGGCATTTGCCC</td>
</tr>
<tr>
<td>bcsA2</td>
<td>CGGCATGAAGGGTCTACC</td>
</tr>
<tr>
<td></td>
<td>ATGACGCTTGCCCTCCAC</td>
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<tr>
<td>bcsA3</td>
<td>CGATGTTTCTTGTACGCG</td>
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<td>TCAGCGTCAGGGTGGAAC</td>
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<td>bcsA4</td>
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<td>CGAATACATCTGTATCTTG</td>
</tr>
<tr>
<td></td>
<td>ACTG</td>
</tr>
<tr>
<td>bcsB</td>
<td>CCCACCACAACGGCATTTG</td>
</tr>
<tr>
<td></td>
<td>GACAAGAACGTAAGGCA</td>
</tr>
<tr>
<td></td>
<td>AGAC</td>
</tr>
<tr>
<td>DGC</td>
<td>CGGCTTCCAGTTTTTCT</td>
</tr>
<tr>
<td></td>
<td>TAGAGCGGCTGGAATGCA</td>
</tr>
<tr>
<td></td>
<td>G</td>
</tr>
<tr>
<td>PDE</td>
<td>TCCGCTATGACGTACACATG</td>
</tr>
<tr>
<td></td>
<td>GTTGAACCGCCTGAGTATT</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>UDP GT</td>
<td>GTGGCGCTTTTGTAGC</td>
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<td></td>
<td>CATGGCAGTTCCTCCGTCA</td>
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3 BIO Web of Conferences 100, 02030 (2024) https://doi.org/10.1051/bioconf/202410002030 IFBioScFU 2024
It was found that the relative expression of genes within the bcsA operon is higher when utilizing sucrose and glucose substrates compared to fructose for BC biosynthesis. Additionally, it is noteworthy that genes positioned at the 5' end of the operon exhibit higher expression levels, potentially attributable to variations in PCR efficiency across different targets (Fig. 1).

Microorganisms isolated from synthesized BC gel films are characterized by a higher activity of the bcsA1 gene in comparison with microorganisms of the culture liquid. The smallest changes in the activity of this gene are characteristic of the fructose substrate.

A higher expression of bcsB was also determined in cases of sucrose and glucose (Fig. 2). Interestingly, for microorganisms in the culture fluid and BC, the difference between the expression of the bcsB gene remains the greatest for the sucrose substrate, for glucose and fructose it is minimal.

Higher expression of the diguanylate cyclase (DGC) gene was determined when using sucrose as a substrate in the biosynthesis process and less pronounced in the case of glucose (Fig. 3).
Fig. 3. Expression of the DGC gene (substrates: 1, 2 – fructose; 3, 4 - glucose, 5, 6 – sucrose; culture liquid -1, 3, 5; gel film BC – 2, 4, 6)

An increased expression level of phosphodiesterase (PDE) and glucose transferase (UDP GT) is also observed for sucrose (Figs. 4 and 5).

Fig. 4. Expression of the PDE gene (substrates: 1, 2 – fructose; 3, 4 - glucose, 5, 6 – sucrose; culture liquid -1, 3, 5; gel film BC – 2, 4, 6)

Fig. 5. Expression of the UDP GT gene (substrates: 1, 2 – fructose; 3, 4 - glucose, 5, 6 – sucrose; culture liquid -1, 3, 5; gel film BC – 2, 4, 6)

Conclusions

Under static cultivation conditions of the symbiotic culture of Medusomyces gisevii utilizing sucrose and glucose substrates in the nutrient medium, a higher level of gene expression is observed for most genes involved in bacterial cellulose biosynthesis.
Furthermore, the expression of these genes in the synthesized BC gel films is significantly higher compared to that in the bacterial culture medium.

Acknowledgments

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