Bacterial cellulose & Microalgae: engineered tissue for biomedical applications

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BACKGROUND

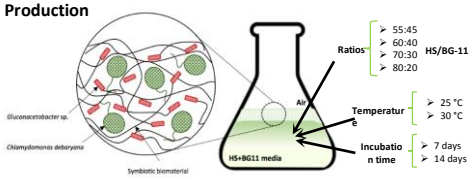
Bacterial cellulose (BC) has been a subject of much recent research, not only for its environmental friendly synthesis (biocompatible and biodegradable), but also for its high potential in areas such as biomedicine. BC is a by-product of *Gluconacetobacter* sp. bacteria. *Chlamydomonas debaryana* is a unicellular green microalga with attractive compounds, e.g. oxygen, which are able to be applied on a wide range of fields.

OBJECTIVES

Promote an association between BC and microalgae and obtain a life material (biofilm) by *C. debaryana* immobilization into a BC 3D network produced by a *Gluconacetobacter* sp. strain during their biosynthesis.

MATERIALS AND METHODS

Production



Analysis

- Fluorescence microscopic analysis
- Scanning electron microscopy (SEM)
- Elemental analysis
- Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR)

RESULTS

In order to allow and study adaptation of both microorganisms to new symbiotic media, two periods were study: 7 and 14 days. Increasing the incubation time all ratios present biofilm production and an increase in the biofilm was observed (Figure 1).

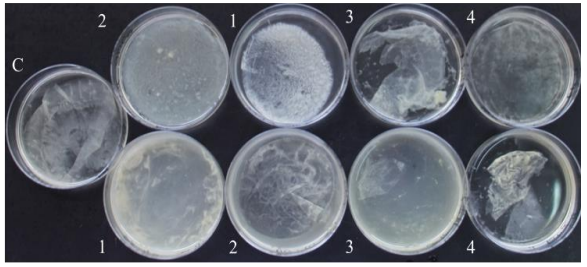


Figure 1. Optical photographs of symbiosis results after 14 days of incubation, at 30°C. Number of *C. debaryana* cells added was fourteen (bottom line) and fifty-two (top line) million. C– only bacteria; 1 – 55:45 (HS:BG-11); 2 – 60:40 (HS:BG-11); 3 – 70:30 (HS:BG-11); 4 – 80:20 (HS:BG-11).

The higher presence of microalgae cells shows a better BC production (visible shape and uniformity), which show the greater release of oxygen produced by the microalga in the *Gluconacetobacter* sp. metabolic processes.

The highest incorporation rate was registered when 80:20 (HS:BG-11) media were used (Table 1). This result is coherent with the biofilm produced where the most uniform membrane was obtained.

Table 2. Incorporation rates (%) of *C. debaryana* inside bacterial cellulose produce by *Gluconacetobacter* sp. in seven-days and fourteen-days long incubations.

Sample	*C. debaryana* cells ($\times 10^6$)	Incorporation rate (7 days)/ %	Incorporation rate (14 days)/ %
55:45	14	44.4	62.9
biofilm	52	50.6	41.3
60:40	14	41.5	44.8
biofilm	52	53.2	77.7
70:30	14	44.2	25.0
biofilm	52	55.1	65.5
80:20	14	69.4	33.3
biofilm	52	62.4	67.7

The best incorporation registered in all experiments was the biofilm produced in 60:40 (HS:BG-11) media with an incubation of fourteen days.

SEM images (Figure 2) confirm that all biofilms were constituted by BC around microalgae cells.
In 70:30 (HS:BG-11) media biofilm image, it is very easy to distinguish bacteria (small cylinders) and microalgae (larger egg-shape volume). Besides that, some cellulose fibres are well represented and evolving both microorganisms.

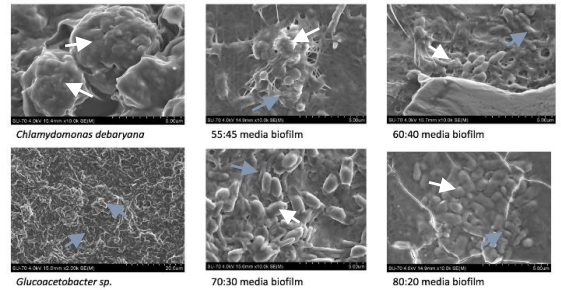


Figure 2. Scanning electron micrograph of microalgae *C. debaryana* (white arrow); bacteria *Gluconacetobacter* sp. (blue arrow); and biofilms produced by both microorganisms in different symbiotic media at 30 °C and after 14-day incubation.

SUMMARY

Biofilms were obtained and allowed the report, for the first time, of a successful symbiotic interaction developed in situ between an alkaline photosynthetic microalgae and an acetic acid bacterium. Optimal temperature and incubation duration for exopolymer production was, respectively, 30 °C and 14 days. High microalgae concentration, 2.6 million cells per millilitre, and high percentage, 80 per cent, of HS medium induces the finest biofilm production. Microalgae incorporation capacity of the synthetized de novo biofilms were ranged between 65 and 78 % at optimum conditions. These results were promising and open a new window to BC applications in medical fields until now not explored.

CONCLUSIONS

- Symbiotic relationship is possible between a fairly alkaline microalga, *C. debaryana*, and an acid cellulose producer bacterium, *Gluconacetobacter* sp., under different media compositions and conditions.
- Optimal temperature and incubation time for exopolymer production is, respectively, 30 °C and 14 days after bacteria addition.
- High microalgae concentration and high percentage of HS medium induces to biofilm production.

These results were promising and open a new window to BC applications in medical fields until now not explored.

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