Biosynthesis of Microbial Cellulose from the Antarctic Microorganisms

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Abstract:

Microbial cellulose is characteristic in that it has a much finer structure than plant cellulose and potential as a new material for various applications. However, because it is produced by culture approximately at 30°C, its production requires antifungal measures. Therefore, microbial cellulose-producing microorganisms were collected in Antarctica, an extremely cold region, and cultured at low temperatures. As a result, in *Rhodotolura glacialis* (No.3), *Cryptococcus gastricus* (No.4) and *Cryptococcus victoriae* (No.5), which were collected in Antarctica, the culture at 4°C clearly formed pellicles. However, the culture at 30°C did not form any pellicles, showing that this microbe was clearly psychrophilic. As mentioned above, psychrophilic microorganisms have made it possible to produce microbial cellulose in low-temperature culture, which is believed to have big industrial advantages.

1. Introduction

Cellulose is a useful biological resource that is extensively used in our lives. Much of cellulose is produced by higher plants, such as trees. However, microbes are also capable of producing cellulose, which is called "microbial cellulose". Because it has different properties from plant cellulose, it has been keenly noted as a new material[1)]. Microbial cellulose and plant cellulose differ considerably in fine structure. In plant cellulose, many cellulose chains collect to form very fine fibers called microfibrill. These microfibrills further form bundles to develop fibrils, lamellae, and fiber cells in steps for conformation. In contrast, because microfibrills made of cellulosed microbial cells secrete form mesh, microbial cellulose has a very fine structure[1,2)]. In addition, whereas plant cellulose forms complexes with hemicellulose and lignin, microbial cellulose has no components other than cellulose. Therefore, microbial cellulose is also characteristic in that it is made of high-purity cellulose.

In general, fungi readily grow in microbial culture approximately at 30°C. Such microbial culture requires various antifungal measures, such as thorough air cleaning and sterilization.

In fact, fungi often grow in food products, which are often disposed of for the reasons of adulterated quality, damaged appearance, and mycotoxin contamination. If microbial cellulose production is possible in low-temperature culture, it would be very advantageous industrially.

Antarctica, which is cold and oligotrophic, has extreme environments. Regions around Syowa Station have been influenced by global changes of environment, or repeated glacial and interglacial periods. There are more than 100 lakes of various sizes that were formed as the Antarctic ice bed receded after the last glacial period of about 20 thousand years ago. Most of them are oligotrophic fresh water lakes. However, there are some lakes that have plant communities (Algae, Cyanobacteria, and Bryophyte) all over the lake bottoms.

Figure 1 shows a five-meter-deep underwater photograph of Namazu Ike in Skarvsnes, Antarctica. Antarctic lakes are very clear because they are oligotrophic and have little influx of soil and sand. Figure 2 shows a photograph of the bottom of Naga Ike in Skarvsnes, over which crest-like mosses spread out. The crest-like mosses are believed to draw nutrition from a community they form with Cyanobacteria, which can fix atmospheric nitrogen, and the like. Figure 3 is an enlarged photograph of the crest-like mosses. A large amount of transparent fibrous materials, which are believed to be cellulose, are attached to the crest-like mosses. Therefore, attempts were made to collect microbial cellulose-producing microorganisms from deposits in lakes and lake shore soil in Antarctica, which is an extremely cold region[3,4]. A low-temperature culture was

Fig. 1　A five-meter-deep underwater photograph of Namazu Ike in Skarvsnes, Antarctica (Photographer: S. Kudoh, Diver: S. Imura)

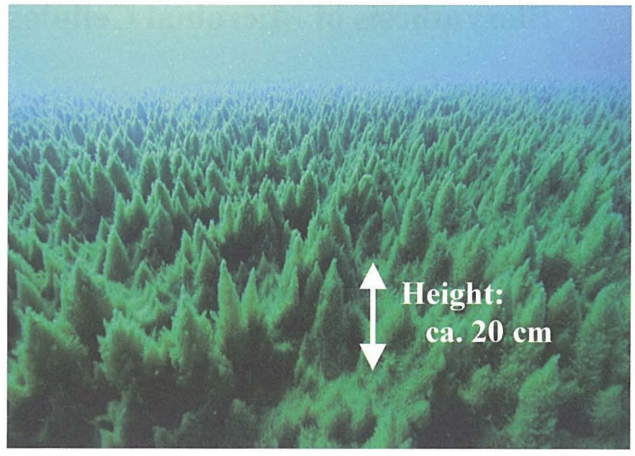

Fig. 2　A photograph of the bottom of Naga Ike in Skarvsnes, Antarctica

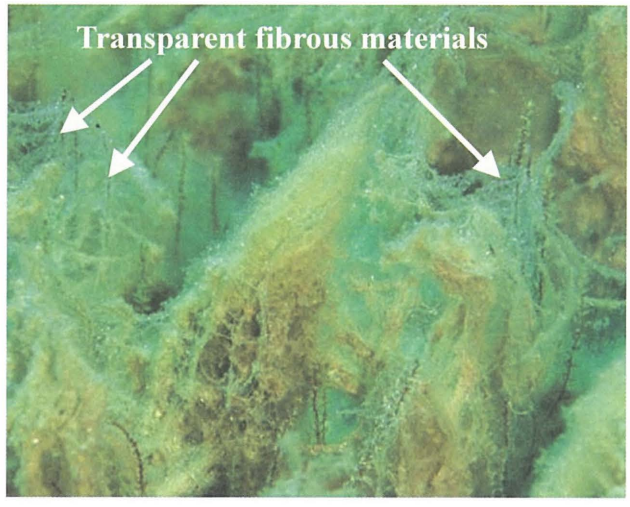

Fig. 3　An enlarged photograph of the crest-like mosses

Table 1 The microbial strains used in this experiment

Sample	Source	Place
No.1 *Microdochium nivale*	winter wheat	Sapporo, Hokkaido
No.2 *Mrakia blollopis*	Bottom deposits	Naga Ike in Skarvsnes, Antarctica
No.3 *Rhodotorura glacialis*	Lakeshore soils	Abi Ike in Skarvsnes, Antarctica
No.4 *Cryptococcus gastricus*	Lakeshore soils	Tokkuri Ike in Skarvsnes, Antarctica
No.5 *Cryptococcus victoriae*	Lakeshore soils	Arisa Ike in Skarvsnes, Antarctica
No.6 *Leucosporidium antarcticum* S4-B strain	Soils	East Ongul Island, Antarctica
No.7 *Leucosporidium antarcticum* NBRC1917 strain	Sea water	Southern Ocean
No.8 Acetic acid bacterium		(Cont.)

applied to the microbes that were collected in Antarctica and Hokkaido, which were expected to produce polysaccharides. As a control, acetic acid bacterium was also treated with a similar culture method.

2. Experimental

2.1 Microbial strains

Table 1 shows microbial strains used in this experiment. Strain No. 1 collected in Hokkaido, strains No.2-6 collected in Antarctica, and strain No. 7 collected in the Antarctic Ocean were used as test samples. For comparison, No. 8, an acetic acid bacterium, was also used. Difco potato dextrose broth was diluted to a concentration of 2.4 wt% to be used as the liquid culture medium.

2.2 Culture method

As culturing conditions, at three levels of temperatures (4°C, 15°C, and 30°C), a shaking culture was applied for 35 days using potato dextrose as a liquid culture medium.

2.3 Turbidity of culture

A spectrophotometer was used to measure the liquid cultures with pellicle removed for absorbance at 600 nm.

2.4 Dry weight of microbial cellulose

The liquid cultures were centrifuged to obtain precipitates (2,500rpm, 10mins) , which were dried and weighed.

3. Results and Discussion

3.1 Evaluation based on the appearance of culture

The microbial strains were added to the liquid medium and kept in shaken culture for 35 days. Figure 4 shows photographs of the samples cultured at 4°C and Figure 5 shows those cultured at 30°C. As a result, in *Microdochium nivale* (No.1) collected in Hokkaido, the culture at 4°C clearly formed pellicles, and the culture at 15°C formed a large amount of pellicles. However, the culture at 30°C did not form any pellicles, showing that this microbe was clearly psychrophilic. In *Rhodotolura glacialis* (No.3), *Cryptococcus*

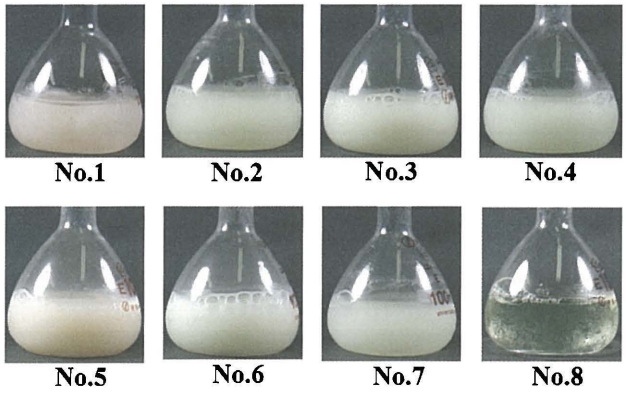

Fig. 4 Applied shaking cultures for 35 days using potato dextrose as a liquid culture medium at 4°C

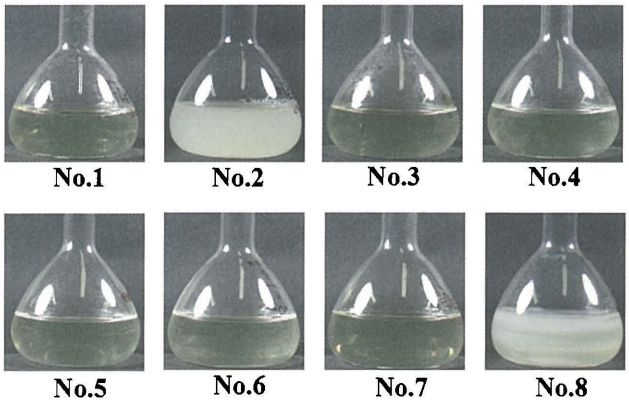

Fig. 5 Applied shaking cultures for 35 days using potato dextrose as a liquid culture medium at 30°C

gastricus (No.4) and *Cryptococcus victoriae* (No.5), which were collected in Antarctica, a similar pattern was observed: Although the culture at 4°C formed pellicles, cultures at 15°C and 30°C formed little pellicles. On the other hand, in acetic acid bacterium used as a control, while the cultures at 4°C and 15°C did not form pellicles at all, the culture at 30°C formed a large amount of pellicles.

As described above, the microbial strains (No.2-7) collected in Antarctica and *Microdochium nivale* (No.1), which was collected in Hokkaido, were found to be clearly psychrophilic. Strains No.3-7 in particular showed unique properties, such as no growth at all when cultured at 30°C.

3.2 Quantitative evaluation of culture

Changes after 35-day culture were examined quantitatively. Table 2 shows the state of pellicle formation in the upper part, dry weights of the products in the middle part, and pH values in the lower part. Not all the samples were measured for the pH value of liquid culture.

When cultured at 4°C, strains No. 1 and No. 3 produced the largest amounts of polysaccharides, or about 8.5 mg/ml. They were followed by No. 4 and No. 5, which produced about 4.9-5.4 mg/ml. However, dry weight measurement revealed that those strains produced little polysaccharides when cultured at 30°C. In addition, the liquid cultures were found to have turned acidic to pH values ranging 5.1-5.9. In contrast, when cultured at 30°C, strain No. 8, the acetic acid bacterium, produced a large amount of polysaccharides as shown by a relatively large dry weight of 7.26 mg/ml. Its culture liquid had a pH value of 4.28, quantitatively showing the most intensive acidification.

As described above, cellulose-producing strains were collected from sediment and lakeshore soil of Antarctic lakes. A large amount of transparent materials, which were believed to be microbial cellulose, were attached to crest-like mosses on the bottom of lakes. These transparent materials are judged to play a role as scaffolds for mosses, algae, and Cyanobacteria to grow in symbiosis.

4. Predicted applications of microbial cellulose

Because microbial cellulose is produced in

Table 2 States of growing bacterial cultures for 35 days

Sample	Culturing temperature		
	4 ℃	15 ℃	30 ℃
1. *Microdochium nivale*	++, Formed pellicle 8.56 5.9	+++, Formed pellicle 8.54	— 3.23
2. *Mrakia sp.*	+ 2.95	+ 0.66	+ 0.64
3. *Rhodotolura glacialis*	++ 8.53 5.39	— 5.95	— 1.51
4. *Cryptococcus gastricus*	++ 4.90 5.1	+ 4.12	+, Formed pellicle (a little) 1.25
5. *Cryptococcus victoriae*	++ 5.37 5.59	+ 4.78	+, Formed pellicle (a little) 0.63
6. *Leucosporidium antarcticum* S4-B strain	++ 3.29	+ 6.35	— 1.18
7. *Leucosporidium antarcticum* NBRC1917 strain	++ 1.95	— 0.72	— 2.23
8. Acetic acid bacterium	— 0.30	— 1.93	+++, Formed pellicle 7.26 4.28

Upper: Culture state
Middle: Dry weight / medium (mg/ml)
Lower: pH value

culture containers, it is believed possible to plan the production of well quality-controlled microbial cellulose products in manufacturing plants. Applications include separation membranes, medical pads, cosmetic pads, and graphite film which take advantage of the fine mesh structure and moisture holding capacity of microbial cellulose membrane[5,6]. When mechanically crushed to fine fibers, microbial cellulose can also be used as a raw material for making paper. When mixed with wood pulp, microbial cellulose improves paper in strength and modulus[5,7]. If microbial cellulose can be mixed with functional agents, such as photocatalytic titanium dioxide, paper products of high strength and multiple functions would be obtained. Industrial advantages of low-temperature culture offer great expectations for commercial applications of microbial cellulose.

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