Screening of Bacterial Cellulose-producing *Acetobacter* Strains Suitable for Sucrose as a Carbon Source

Akira Seto, Yukiko Kojma, Naoto Tonouchi, Takayasu Tsuchida, and Fumihiro Yoshinaga

Bio-Polymer Research Co., Ltd., 3–2–1 Sakato, Takatsu-ku, Kawasaki 213, Japan

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For potential application in the economic production of bacterial cellulose, cellulose-producing *Acetobacter* strains capable of using sucrose as a carbon source were screened for using corn steep liquor as a nitrogen source. A total of 1500 strains were isolated and four were selected through static and shaking flask cultures. Much higher cellulose accumulation was observed in a jar fermentor using these strains in sucrose-CSL medium than seen using the previously isolated strain, BPR2001, in fructose-CSL medium. It was found that these strains also had high cellulose production from glucose. The pH of the cultures of the isolated strains did not decrease, and it was suggested that these strains have low glucose-oxidizing ability.

Key words: *Acetobacter*; cellulose; sucrose; screening; glucose

Some strains of *Acetobacter* are known to produce cellulose in culture. This bacterial cellulose has many useful properties in comparison with plant cellulose, and is expected to become a new industrial material. Therefore, an economic production system involving agitation culture is required for the commercial exploitation of bacterial cellulose. In a previous study, using extensive screening of material from natural sources, we succeeded in isolating a strain, *Acetobacter xylinum* subsp. *saccharofemmentans* BPR2001, which is suitable for use in agitated culture using fructose as a carbon source.

However, the most suitable carbon source for economic cellulose production is sucrose, since it is readily available throughout the world at a low price. In the previous screening, we found no strain capable of producing cellulose from sucrose at a high level. One possible reason for this result may have been the nitrogen source of the cellulose production medium. CSL (corn steep liquor) was used as the nitrogen source of the screening medium containing fructose, while YE (yeast extract) was used for the medium containing sucrose. In addition, our subsequent study found that CSL is the most suitable nitrogen source for cellulose production by *Acetobacter*.

Therefore, we tried to isolate strains that produce bacterial cellulose efficiently from sucrose by screening with CSL-Suc medium, which contains 4% sucrose and 4% CSL. Other screening and culture methods for cellulose production were as described previously. Strain BPR2001 was also used as a control.

A total of 346 samples from many sources were collected and screened, and the results are shown in Table I. The cellulose producers were efficiently isolated from fruits, as was the case of BPR2001. Efficient cellulose producers were also found in samples collected from a vinegar factory. No cellulose producers were found in samples of soil or activated slurry.

Fifteen hundred isolated strains that had been found to produce thick pellets in static culture with CSL-Suc were further evaluated using shaking flask culture. Figure 1 shows the distribution of cellulose production in these strains. Cellulose accumulation of more than 5 g/liter was observed in 108 of these isolates.

Several colonies were collected from these strains and further evaluated (Table II). Four strains showing high cellulose production were selected, and then finally evaluated using a jar fermentor (Fig. 2). High levels of cellulose production were observed with strains 757, 771, and 184, which had been isolated from melon, cherry, and grape, respectively. The amount of accumulated cellulose was greater than that observed with BPR2001 cultured in CSL-Frc medium.

Cellulose production by BPR2001 (used as a control) in a shaking flask culture was only 1.8 g/liter, and the pH in the culture fell to 3.0. It is known that sucrose is first degraded into glucose and fructose (and an appreciable amount of levan, which is a polymer of fructose) and then metabolized. Most *Acetobacter*
Table II. Cellulose Production by Isolated Strains in Flask Culture

<table>
<thead>
<tr>
<th>Strains</th>
<th>Sucrose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose</td>
<td>pH</td>
</tr>
<tr>
<td>184</td>
<td>9.1</td>
<td>5.5</td>
</tr>
<tr>
<td>747</td>
<td>8.0</td>
<td>5.2</td>
</tr>
<tr>
<td>757</td>
<td>9.1</td>
<td>5.3</td>
</tr>
<tr>
<td>771</td>
<td>9.7</td>
<td>4.8</td>
</tr>
<tr>
<td>BPR2001</td>
<td>1.8</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Sucrose (40 g/liter) or glucose (20 g/liter) was used as the carbon source. Cellulose was indicated as the final accumulation (g/liter). The final pH of the culture was also shown. The initial pH was adjusted to 5.0.

![Cellulose Production by Isolated Acetobacter Strains in a Jar Fermentor](image)

Fig. 2. Cellulose Production by Isolated Acetobacter Strains in a Jar Fermentor.

Strains are known to oxidize glucose and produce gluconate. It is possible that this oxidation reaction led to a reduction in cellulose yield and the decrease in pH inhibited both cell growth and cellulose production. An approach for selecting mutants that have decreased or deficient activities of the relevant enzyme has been reported. However, the pH of the culture of the isolated strains in this study did not decrease, and it is considered that their ability to oxidize glucose is low. In fact, a primitive analysis of glucose dehydrogenase (GDH) activity showed that the isolates have little GDH activities: only 0.01 U/mg of the activity was detected in strain 757, while 0.89 U/mg of that was detected in BPR2001. It has also been reported that a strain of Acetobacter polyoxyxogenes had low oxidation ability. Table II also shows that these isolated strains produce cellulose efficiently from glucose in glucose-CSL medium (glucose 20 g/liter). On the other hand, 4.2 g/liter and 4.1 g/liter of cellulose were produced in fructose-CSL medium using 757 and 184 respectively, which were the same amounts as that using BPR2001 (4.4 g/liter).

In conclusion, by using CSL as the screening medium, several strains showing high cellulose production from sucrose were selected in this study. In our previous study, it was found that cellulose production is associated with cell growth, CSL or lactate, which is contained in CSL, was shown to increase cell growth and cellulose production. These results confirm that CSL is a suitable nitrogen source for cellulose production.

We are now investigating the characteristics of the selected strains and the production of cellulose using them.

References