The mechanism of the increased cell growth and cellulose production of *Acetobacter xylinum* subsp. *sucrofermentans* BPR3001E, a sulfaguanidine (SG)-resistant mutant, was investigated. We found that adding *p*-aminobenzoic acid (PABA) to cultures of the parent strain, BPR2001, led to increased levels of intracellular adenosine-related purine compounds and increased cellulose production. Furthermore, adding ATP increased the cellulose production by permeabilized BPR2001 cells. On the other hand, the intracellular levels of PABA and adenosine-related purine compounds in BPR3001E cells were higher than those in BPR2001 cells. These results suggest that SG resistance increases enhance cellulose production through increased levels of intracellular high-energy compounds caused by increased PABA biosynthesis, reflecting the promoted supply of cellulose precursors.

**Key words:** cellulose; sulfaguanidine; *p*-aminobenzoic acid; ATP

*Acetobacter xylinum* often occurs as a contaminant during vinegar fermentation, and is known to produce bacterial cellulose. Bacterial cellulose has many useful physical properties that plant cellulose does not have, and it is expected to become a new chemical commodity with many industrial uses. One of the problems hindering the application of bacterial cellulose to industrial uses is low productivity. In a previous study, we found that *p*-aminobenzoic acid (PABA) increases the cell growth and cellulose production of *A. xylinum*, and we isolated a sulfaguanidine (SG; an analog of PABA)-resistant mutant, BPR3001E, from the BPR2001 strain. This mutant strain was found to have increased growth and increased cellulose production compared to the parent strain, BPR2001. In this study, we investigated the mechanism of the increased cell growth and cellulose production of BPR3001E.

ATP is required for cell growth and cellulose production, so we first investigated the effects of PABA on intracellular ATP-related compounds. We found that adding PABA increased the intracellular levels of adenosine-related purine compounds in BPR2001 cells (Table I). Furthermore, we investigated the relationship between the levels of adenosine-related purine compounds and cellulose production. In a previous study of a continuous cellulose production system, we found that adding lactate increased both the intracellular ATP level and cellulose production. Therefore, we expected that the increased levels of intracellular adenosine-related purine compounds, such as AMP, ADP, and ATP, in BPR2001 cells would be positively correlated with cellulose production. We next examined the effects of ATP on cellulose production using permeabilized cells (Fig. 1). Increased cellulose production was observed after the addition of ATP. This suggests that addition of PABA increases cellulose production by BPR2001 cells by increasing the intracellular levels of high-energy compounds such as AMP, ADP, and ATP.

Next, we investigated the levels of PABA and adenosine-related purine compounds in the SG-resistant mutant BPR3001E. It has been reported that the intracellular level of chorismate (a precursor of PABA) in a SG-resistant mutant strain of a tryptophan producer is higher than that in the parent strain. Therefore, we measured the intracellular level of PABA in BPR3001E cells (Table II), and found that it was higher than that in BPR2001 cells. Furthermore, the intracellular levels of adenosine-related purine compounds in BPR3001E cells were also increased. These results are compatible with the observations of a previous study concerning a proline producer. Thus, the intracellular levels of PABA and adenosine-related purine compounds in the SG-resistant mutant were higher than those in the parent strain.

Concerning the relationship between PABA and

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**Table 1. Effects of PABA on Intracellular Concentrations of Adenosine-Related Purine Compounds in BPR2001 Cells**

<table>
<thead>
<tr>
<th>Addition of PABA</th>
<th>AMP (× 10⁻⁷ mol/mg of protein)</th>
<th>ADP (× 10⁻⁷ mol/mg of protein)</th>
<th>ATP (× 10⁻⁷ mol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>14.0±0.5</td>
<td>4.6±0.3</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>PABA</td>
<td>16.0±0.6</td>
<td>5.7±0.1</td>
<td>2.2±0.1</td>
</tr>
</tbody>
</table>

The cell extracts for adenosine-related purine compounds analysis were prepared using cellulose producers cultured for two days in synthetic medium. The cellulose was removed by gauze filtration, and the cells were harvested, washed, and resuspended in 50 mM phosphate buffer (pH 7.0). The cells were disrupted using a French pressure cell (1,200 psi). Acetonitrile was added to the supernatant of the cell extract (final concentration: 30%). Adenosine-related purine compounds were analyzed by HPLC by the procedure described in a previous study. The intracellular levels of adenosine-related purine compounds are given per milligram of protein in the cell extract, as measured using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, USA). To examine the effects of PABA, 0.015 mM PABA (final concentration) was added to the culture.

*Adenosine-related purine compounds concentrations are the average of three experiments.*

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† Present address: Nakano Central Institute, Nakano Vinegar Co. Ltd., 2-6 Nakamura-cho, Handa 475-0873, Japan.
Fig. 1. Effects of ATP on Cellulose Production.
Closed circles: cellulose production.
BPR2001 cells cultured for one day in CSL-Fru\(^9\) containing 1% cellulase were used. The cells were harvested, washed, and resuspended in 50 mM acetate buffer (pH 5.0). Cellulose production using permeabilized cells\(^9\) was done as described previously.\(^9\) The reaction mixture contained 50 mM acetate buffer (pH 5.0), 250 mM fructose, 5 mM CaCl\(_2\), 5 mM MgCl\(_2\), ATP (0–30 mM) and permeabilized BPR2001 cells, and the final volume was 100 ml. The reaction mixture was incubated at 28°C for 24 hr. The cellulose produced was measured by a previously described method.\(^9\)

Table II. Intracellular Concentrations of Adenosine-Related Purine Compounds in BPR2001 and BPR3001E Cells

<table>
<thead>
<tr>
<th>strain</th>
<th>PABA conc. (\times 10^{-12}) mol/mg of protein</th>
<th>Adenosine-related purine compounds conc. (\times 10^{-6}) mol/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMP</td>
<td>ADP</td>
</tr>
<tr>
<td>BPR2001</td>
<td>4.4 ± 0.4</td>
<td>14.0 ± 0.5</td>
</tr>
<tr>
<td>BPR3001E</td>
<td>7.3 ± 0.4</td>
<td>18.2 ± 0.6</td>
</tr>
</tbody>
</table>

The methods used to measure the adenosine-related purine compounds are described in Table I. The PABA concentration of each extract was analyzed by HPLC by the procedure described in a previous study.\(^10\)

Intracellular levels of adenosine-related purine compounds and PABA are given per milligram of protein in the cell extract, as measured using a Bio-Rad Protein Assay Kit (BIO-RAD Laboratories, USA).

* The concentrations of PABA and adenosine-related purine compounds are the averages of three experiments.

Adenosine-related purine compounds, folic acid derived from PABA seems to be an essential factor for the synthesis of adenosine-related purine compounds. For example, folic acid is a cofactor of some enzymes involved in the biosynthetic pathway of adenosine-related purine compounds such as glycaminamide ribonucleotide transformylase and 5-aminoimidazole-4-carboxamide ribonucleotide transformylase.\(^10\)

In this study, the strain BPR3001E, which shows increased cell growth and cellulose production, was found to have increased intracellular high-energy compounds, such as AMP, ADP, and ATP, due to increased PABA biosynthesis.

In other words, the level of ATP seems to be a key factor in cell growth and cellulose production.

Recently, we confirmed that ATP played significant roles in cellulose biosynthesis. Figure 2 shows the biosynthetic pathway of cellulose. First, ATP is used for fructose phosphorylation, the first step of fructose metabolism. It has also been reported that fructose hexokinase, catalyzing this step, requires ATP.\(^8\) Therefore, the increased level of ATP may increase the supply of fructose-6-phosphate (F6P). Next, glucose-6-phosphate (G6P) is a common substrate for phosphoglucomutase in the cellulose biosynthetic pathway and also for glucose-6-phosphate dehydrogenase (G6PD) in the pentose cycle. Moreover, G6PD is inhibited by ATP.\(^10\) Accordingly, the flow of G6P into the cellulose biosynthetic pathway is expected to become abundant due to the inhibition of G6PD activity. As a result, glucose-1-phosphate (G1P) may increase. Furthermore, we have found that UDP-glucose pyrophosphorylase (UGP) activity in the cellulose producers is higher than that in cellulose non-producers, indicating that this enzyme plays a key role in the pathway of cellulose biosynthesis.\(^9\) This enzyme requires UTP as a substrate, and ATP has an important role in UTP synthesis. Therefore, the higher ATP level is expected to promote the UGP reaction.

In conclusion, these results suggest that SG resistance increases cellulose production, reflecting the fact that the increased level of intracellular adenosine-related purine compounds due to increased PABA promotes the supply of F6P, G6P, G1P and UDP-G as precursors of cellulose.

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References


