Germination of spore and decomposition of apple fruit tissue by hypha in Penicillium expansum

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Summary

On Penicillium expansum, germination of the spore (conidia), growth using fruit components, production of various enzymes and enzyme action for apple fruit were examined. Xylan- and pectin-degrading enzymes were easily liberated in the buffer solution from the spore. The levels of these enzyme activities were largely affected by carbon sources in the sporogenesis culture medium. The spore harvested from the culture of the xylan medium showed the high xylanase activity and the spore from the pectin medium showed the high pectinase activity. The carbon source of germination medium and the enzyme level of the spore were related to the elongation of germ tube. The mold grew well in the medium containing xylan or pectin, and abundantly formed the spore. Unlike this, its growth using soluble sugars (glucose, fructose, sucrose) was poor. The mold produced gluconate from glucose, and glucose oxidase and catalase were produced in the culture broth. The fruit tissue remarkably browned, when it was incubated with glucose oxidase and catalase. This browning reaction was promoted in the presence of xylan-degrading enzyme. Two xylanases and β-xylosidase were purified from the culture filtrate as homogeneous proteins. The reducing sugar was liberated from the fruit when it was incubated with these enzymes.

Key words: Penicillium expansum, apple blue mold, xylanase, pectinase

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Many reports on an apple blue mold, Penicillium expansum, concerning the fruit spoilage under storage have been made from various angles. However, information on the relation between the component of the fruit and the growth characteristic of the mold is limited. Therefore, this report deals with germination of the spore (conidia) of P. expansum, growth using fruit components, production of various enzymes and enzyme action for the fruit.

It was enabled that the spore was abundantly obtained in the dry state. The Japanese paper was stuck on agar plate media containing various carbon sources, and the mold was inoculated on those surfaces, respectively, and they were cultivated, until spores were sufficiently formed. This Japanese paper was torn off from the agar plate and dried. By rubbing the paper on a 200-mesh sieve, the spore was harvested. Xylan-degrading enzyme (xylanase) and pectin-degrading enzyme (pectinase) of the spore were easily liberated in the buffer solution (Table 1). The cellulose-degrading enzyme activity was not detected in either spore. The activity levels of these enzymes which were liberated from the spore were largely affected by the carbon source in the sporogenesis culture medium. The
spore harvested from the culture of the xylan medium showed the high xylanase activity and the spore from pectin medium showed the high pectinase activity. These spores could not germ in the inorganic salt medium without the carbon source. They could bud out in the inorganic salt medium adding the carbon source (Table 2). The type of the carbon source and the enzyme level of the spore hardly affected the germinating rate. The carbon source of the germination medium and the enzyme level of the spore were related to the elongation of germ tube (Fig. 1). On the spore possessing the high activity of xylanase, the elongation of the germ tube was good in the xylan medium. On the spore of the high pectinase activity, the elongation of the germ tube was good in the pectin medium.

The mold grew well using main fiber component [hemicellulose (the xylan was used), pectin] in the apple fruit, and abundantly formed the spore. Unlike this, the growth using main soluble sugar (glucose, fructose, sucrose) was poor and the spore was not formed in most. In the culture using these soluble sugars, pH was lowered, and organic acids were produced, such as gluconate from glucose.

From the culture which used xylan as a carbon source, the filtrate including the xylan-degrading enzyme without pectinase activity was obtained. From the culture which used pectin, the filtrate including the pectin-degrading enzyme without xylanase activity was obtained. When the apple fruit was incubated with these culture filtrates, it was proven that the xylan-degrading enzyme destroyed cell wall of the apple fruit tissue and there was an action of separating the cell from the fruit organization on the pectin-degrading enzyme. Besides, glucose oxidase and catalase were produced, when the mold was incubated in the medium containing glucose as a carbon source. The fruit tissue

### Table 1. Effects of growth substrate on polysaccharide degrading enzymes of conidia

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Xylanase</th>
<th>Pectinase</th>
<th>Cellulase</th>
<th>Amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylan</td>
<td>0.157</td>
<td>0.047</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pectin</td>
<td>0.032</td>
<td>0.293</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*1 Conidia was harvested from agar medium containing 2.0% substrate as a carbon source.
*2 Conidia was suspended with acetate buffer (0.1M, pH 5.0).

### Table 2. Effects of carbon sources on germination rates of conidia

<table>
<thead>
<tr>
<th>Carbon source for germination</th>
<th>Germination rate (%)*1</th>
<th>Conidia-X*2</th>
<th>Conidia-P*3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal medium</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Basal medium + xylan</td>
<td>47 ± 2.1*4</td>
<td>41 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Basal medium + pectin</td>
<td>46 ± 2.7</td>
<td>42 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>D. W. *5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D. W. + xylan</td>
<td>33 ± 3.8</td>
<td>38 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>D. W. + pectin</td>
<td>31 ± 3.1</td>
<td>37 ± 3.0</td>
<td></td>
</tr>
</tbody>
</table>

*1 Germination rate was estimated after 24 h incubation at 30°C
*2 Conidia were formed on xylan medium
*3 Conidia were formed on pectin medium
*4 Mean of three experiments (± SD)
*5 Distilled water
remarkably browned, when glucose oxidase and catalase worked to the fruit. This browning action was promoted in the presence of the xylan-degrading enzyme (Table 3).

Two xylanases and β-xyllosidase of were purified from the culture filtrate of the mold. Xylanase I (Mr 21 kDa) formed xylose and various xylooligosaccharides from xylan. On the other hand, xylanase II (Mr 40 kDa) mainly formed xylose and xylobiose from the initial reaction stage. Although the apple fruit organization was not almost affected only by xylanase I and II, the reducing sugar was liberated from the fruit tissue when simultaneously, these xylanases and β-xyllosidase were incubated (Fig. 2). From these facts, it was indicated that the tissue of the fruit was spoiled by the interaction of β-xyllosidase and xylanases.

The spore and hypha of P. expansum have the physiological characteristics which adapted to the component of the apple fruit like the above. A knowledge obtained in this study seems to be a foothold in clarifying why P. expansum is mainly isolated from rotted apple fruit under storage.
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References


**Penicillium expansum** の胞子の発芽と菌糸によるリンゴ組織の分解

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**Penicillium expansum** が形成する分生胞子からは、これを緩衝液に懸濁するだけで、キシラン分解酵素とベクチン分解酵素が容易に溶出してきた。セルロース分解酵素活性は検出されなかった。キシランおよびベクチン分解酵素の活性レベルは、胞子形成培地中の炭素源により大きく影響を受け、キシラン培地の培養から収穫した胞子はキシラン分解酵素の、ベクチン培地からの胞子はベクチン分解酵素の高い活性を示した。胞子の酵素活性レベルと発芽管の伸長との間には相関が見られた。キシランとベクチンを炭素源とした培地をそれぞれ用いて、本菌を培養して得られた培養液をキシラン分解酵素液、ベクチン分解酵素液として果実に作用させた結果から、キシラン分解酵素は果実組織の細胞壁を破壊し、ベクチン分解酵素は果実組織より細胞を相互に切り離す作用があることが分かった。本菌の培養液から、キシラン分解酵素として 2 種のキシラナーーゼと β-キシロシダーゼが単一なまでに精製された。

キーワード：*Penicillium expansum*, リンゴ青カビ病菌, キシラナーーゼ, ベクチナーーゼ