キシロースを炭素源として培養したFusarium graminearumによるトリコテセンの生産

<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
</tr>
</thead>
<tbody>
<tr>
<td>誌名</td>
<td>JSM Mycotoxins</td>
</tr>
<tr>
<td>ISSN</td>
<td>02851466</td>
</tr>
<tr>
<td>巻/号</td>
<td>661</td>
</tr>
<tr>
<td>掲載ページ</td>
<td>p. 17-19</td>
</tr>
<tr>
<td>発行年月</td>
<td>2016年1月</td>
</tr>
</tbody>
</table>
Trichothecene production in axenic liquid culture of *Fusarium graminearum* using xylose as a carbon source

Keywords
arabinoxylan; *Fusarium graminearum*; sucrose; trichothecene; xylanase; xylose

We examined the effect of using xylose as a carbon source in the medium on trichothecene production by *Fusarium graminearum*. By frequently adjusting the pH of the submerged culture in response to the pH changes of the known trichothecene-inducing sucrose-agmatine culture, trichothecene became detectable from the xylose liquid culture initiated at neutral pH. In contrast to the low pH requirement of the xylose medium for trichothecene production, the fungus produced the mycotoxin in sucrose liquid culture at neutral pH.

*Fusarium graminearum* is a causal pathogen of head blight, a devastating disease of wheat and barley\(^1\). The fungal species contaminates grains with trichothecene mycotoxins and threatens food safety. The floral cavity between the lemma and palea of the floret is the initial site of infection, where arabinoxylans, the major hemicellulosic component constituting a flexible matrix with porosity, and cellulose, the cell wall microfibrils embedded in the hemicellulose matrix, are degraded\(^2\). In the infected tissues, the pathogens produce trichothecenes that function as a virulence factor\(^3\). In axenic submerged cultures, it was reported that trichothecene production is effectively induced by sucrose, but not by xylose, when used as a carbon source of the medium\(^4\). Evolutionary data from wheat xylanase inhibitor genes suggests that arabinoxylan degradation is an important event during infection for the pathogen\(^5,6,7\), although the indispensability of xylanase in the infection process is controversial\(^8\). Typically, xylose is expected to be the major carbon source that is available at the early stage of infection in wheat flowers, but sucrose is not. Thus, we were interested in seeing whether *F. graminearum* can produce trichothecenes in an axenic culture containing xylose as a carbon source.

We examined whether xylose can be used as a carbon source for trichothecene production in submerged cultures of *F. graminearum* JCM 9873, which accumulates 15-acetyldeoxynivalenol (15-ADON) in the liquid medium. Decreasing the extracellular pH of fungal culture during growth is known to promote trichothecene biosynthesis, and as such, a sucrose-agmatine medium (Supplementary Table 1) is often used for trichothecene production\(^9\). For this reason, we monitored pH changes of the sucrose-agmatine culture as a reference for pH adjustment, and the pH of fungal cultures to be inspected (cultures with xylose-glutamine, xylose-agmatine, and sucrose-glutamine medium; Supplementary Table 1) was frequently adjusted to that of the reference. Thirty milliliters of each of the above media were inoculated with 1% (v/v) of the pre-culture, which was prepared by incubating 50 µL of conidial suspension (1 x 10^7 /mL) in 50 mL of YG medium (Supplementary Table 1) with reciprocal shaking (125 strokes/min) at 25 °C for 16 h. The main culture was incubated at 25 °C with gyratory shaking (135 rpm) under two conditions—one with initial pH adjustment of the medium only and the other with frequent pH adjustment to the reference trichothecene-inducing culture. The pH was monitored using a compact pH meter LAQUAtwin B-712 (Horiba Ltd., Kyoto), and adjusted to the reference pH by adding 0.25 N or 0.5 N of HCl solution (see Supplementary Table 2).

When the xylose-glutamine medium was used without pH adjustment, the pH of the fungal culture remained over 6.4 during the incubation period of 120 h (Fig.1A). No trichothecenes accumulated in the medium, as demonstrated by thin-layer chromatography (TLC) analysis (Fig.1B; lane 1). With frequent pH adjustment to that of the continuously decreasing reference culture (Fig.1A), the same negative result was obtained; 15-ADON production was not observed in the xylose-containing medium with glutamine as a sole nitrogen source (Fig.1B; lane 2). Similarly, use of xylose did not induce trichothecene production when using agmatine as a sole nitrogen source (Fig.1B; lane 3), which is in agreement with a previous report\(^10\). However, when the pH of the xylose-agmatine culture was frequently adjusted to that of the reference trichothecene-inducing culture (Fig.1A), the mycotoxin became detectable on a TLC plate after 5 days of incubation (Fig.1B; lane 4). The result indicates that xylose, with an appropriate nitrogen source such as agmatine, is also used for trichothecene production in the submerged culture if the pH of the culture is frequently controlled during fungal growth. Compared to the use of the medium containing sucrose with glutamine (Fig.1B; lane 5 and 6), 15-ADON accumulation induced by the xylose-agmatine medium was significantly less (Fig.1B; lane 3 and 4) despite the presence of agmatine in the medium.

The results obtained in this study demonstrate that *F. graminearum* produces trichothecenes by using...
xylose as the sole carbon source under certain conditions. At the infection sites of wheat spikelets, growth conditions for the fungus differ significantly from that of the axenic submerged culture\textsuperscript{10}. Because aerial hyphae formation generally serves as a stimulus for the fungus to produce trichothecenes\textsuperscript{11}, the quality of carbon sources and pH are not the sole determinants for the trichothecene productivity in infected spikelets. It may be possible that trichothecene biosynthesis is induced with xylose and other carbon sources available at the site of infected tissues.

Acknowledgements

This work was supported by the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry and by a Grant from Noda Institute for Scientific Research.

Supplementary Materials

Supplementary materials may be found in the online version of this article:
Supplementary Table 1 Media used in this study.
Supplementary Table 2 Time course of pH changes of the culture and frequent pH adjustments with HCl. Blue and green letters denote the pH of the culture before and after pH adjustment, respectively. The amount of an HCl solution (\textmu L) necessary for the pH adjustment is shown on a shaded background.

References


Fig. 1 Relationships between pH changes and trichothecene production by \textit{F. graminearum} under different culture conditions. (A) Time course changes of the culture pH with (+) or without (-) manual pH adjustments to that of the known trichothecene-inducing sucrose-agmatine culture (reference). For details regarding manipulation of pH adjustment, refer Supplementary Table 2. (B) TLC analysis of 15-ADON that accumulated in fungal cultures 3 and 5 days after initiation of the main culture. 15-ADON was extracted from 600 \textmu L of the medium with ethyl acetate and analyzed as described previously\textsuperscript{11}. Toxin amount/mycelial dry weight (\textmu g/mg) is shown on the TLC panel. The experimental scheme is summarized on the right.


キシロースを炭素源として培養したFusarium graminearumによるトリコテセンの生産

鬼頭 良幸1, 古崎 貴大1, 前田 一行1, 棚橋 義和1, 中嶋 佑一1, 金丸 京子1, 小林 哲夫1, 木村 真1
1名古屋大学大学院農学研究科（〒464–8601 愛知県名古屋市千種区不老町）

Fusarium graminearumにおけるトリコテセン産生に対する炭素源としてのキシロースの影響を解析した。中性pHから培養を開始し、pH変化をトリコテセン誘導培地であるスクロース-アグマチン培地で培養したものに合わせることで、キシロースにおいてもトリコテセン産生が確認された。キシロースを炭素源とした培地でのトリコテセン産生には低pH条件が必要なのに対し、スクロースを炭素源とした培地では中性でも産生量が増大した。

キーワード：アラビノキシラン：キシラナーゼ：キシロース：スクロース：トリコテセン：Fusarium graminearum