Inhibition of Deoxyribonucleic Acid Synthesis in *Flavobacterium aurantiacum* by Aflatoxin B₁

E. B. LILLEHOJ AND A. CIEGLER

Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

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Aflatoxin B₁ inhibits growth of *Flavobacterium aurantiacum* and initiates production of filamentous cellular forms (E. B. Lillehoj, A. Ciegl, and H. H. Hall, J. Bacteriol. 93:464, 1967). In the course of investigating the mechanism of action of the toxin on such bacterial cells, it was found that DNA synthesis was selectively inhibited.

Cells grown in a defined medium [Yeast Nitrogen Base, pH 6.8 (L. J. Wickerham, U.S. Dept. Agr. Tech. Bull. 1029:1, 1951)] were harvested during the logarithmic-growth phase and suspended in a similar fresh medium with and without aflatoxin B₁. B₁ was transferred from chloroform into the growth medium by evaporating the chloroform under vacuum. The concentration of toxin in the growth medium was then determined by thin-layer chromatography (O. L. Shotwell et al., Appl. Microbiol. 14:425, 1966). Amounts of 10 ml of the bacterial suspension, containing 2.0 × 10⁶ cells, were incubated in 50-ml flasks at 30°C with shaking, and were harvested at various intervals. The cells were sedimented, washed, and extracted with perchloric acid (G. Berrah and W. A. Koneztka, J. Bacteriol. 83:738, 1962). DNA was determined by the diphenylamine technique (K. Burton, Biochem. J. 62:315, 1956), and ribonucleic acid (RNA), by the orcinol reaction (W. Mejbam, Hoppe-Seyler's Z. Physiol. Chem. 258:117, 1939). The pellet from the hot perchloric acid extract was dissolved in 1 N NaOH at 90°C for 30 min, and protein was determined by the Folin method (O. H. Lowry et al., J. Biol. Chem. 193:265, 1951).

Effects of various levels of aflatoxin B₁ on the synthesis of DNA, RNA, and protein are presented in Table 1. A concentration of 50 µg of B₁ per ml completely blocked DNA synthesis during a 4-hr incubation while reducing the RNA and protein accumulation less than 15% (relative to the control with no aflatoxin).

Alterations in DNA, RNA, and protein synthesis during a 10-hr incubation period are shown in Fig. 1. The DNA synthesis was inhibited within the first 2 hr by the toxin and did not recover during the experiment, whereas RNA and protein continued to increase throughout the test interval.

Table 1. Effect of various levels of aflatoxin B₁ on the synthesis of DNA, RNA, and protein by *Flavobacterium aurantiacum*

<table>
<thead>
<tr>
<th>Aflatoxin B₁ µg/ml</th>
<th>DNA µg</th>
<th>RNA µg</th>
<th>Protein µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33</td>
<td>70</td>
<td>190</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>60</td>
<td>190</td>
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<tr>
<td>25</td>
<td>21</td>
<td>60</td>
<td>180</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>60</td>
<td>170</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>50</td>
<td>165</td>
</tr>
</tbody>
</table>

*Mean of three observations indicating the increase in DNA, RNA, and protein in 10 ml of culture medium over the amount added as inoculum after a 4-hr incubation.*
J. Bacteriol.


Assuming that accumulation of thymidine and uracil represents DNA and RNA synthesis, respectively, and that leucine incorporation is a measure of protein synthesis, DNA synthesis was inhibited 80% (Table 2) by 50 μg of B1 per ml, RNA production was reduced 48%, and protein synthesis, 32%.

Although the incorporation of labeled thymidine into DNA was not entirely blocked by 50 μg of aflatoxin B1 per ml, the DNA synthesis was more sensitive to B1 than was the production of either RNA or protein. There was an inconsistency in the degree of inhibition of DNA synthesis by 50 μg of aflatoxin B1 per ml determined by the colorimetric and radioisotope techniques. Since it appeared that the isotope uptake method was more sensitive than the colorimetric measurements, it was concluded that 50 μg of aflatoxin B1 per ml could not be used as a uniquely specific inhibitor of DNA production in the F. aurantiacum test system.

Reports indicate that aflatoxin reacts with DNA in vitro, and it has been suggested that this reaction is responsible for the observed toxin-induced inhibition of RNA synthesis in liver cells (J. I. Clifford and K. R. Rees, Nature 209:312, 1966; M. B. Sporn et al., Science 151: 1539, 1966). Since RNA synthesis in F. aurantiacum seems less sensitive to aflatoxin than DNA, the toxin may not be inhibiting the same process in bacterial and liver cells. Studies are being continued to elucidate the mode of action of aflatoxin on microbial processes.

### Table 2. Effect of aflatoxin B1 on the incorporation of radioactive constituents into nucleic acids and protein in Flavobacterium aurantiacum

<table>
<thead>
<tr>
<th>Aflatoxin B (μg/ml)</th>
<th>Isotope incorporation (counts/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>³H-thymidine</td>
</tr>
<tr>
<td>0</td>
<td>7,595</td>
</tr>
<tr>
<td>5</td>
<td>5,308</td>
</tr>
<tr>
<td>10</td>
<td>4,139</td>
</tr>
<tr>
<td>25</td>
<td>2,775</td>
</tr>
<tr>
<td>50</td>
<td>1,528</td>
</tr>
</tbody>
</table>

*Activity determined after 1 hr of incubation on 1-ml samples containing 2.0 × 10⁸ F. aurantiacum cells/ml and 0.2 μc of ³H-thymidine/ml, 0.01 μc of ¹⁴C-uracil/ml, or 0.05 μc of ¹⁴C-L-leucine/ml.