Production and Secretion of 5-n-Alkylresorcinols by Fusarium culmorum

Robert Zarnowski*, Teresa Lewicka, and Stanislaw J. Pietr

Department of Agricultural Microbiology, Agricultural University, Grunwaldzka 53, 50-375 Wroclaw, Poland.
Fax: ++48 (71) 328 28 68. E-mail: robert@ozar.wroc.pl

* Author for correspondence and reprint requests

Z. Naturforsch. 55c, 846–848 (2000);
received April 19/May 22, 2000

Fusarium culmorum, 5-n-Alkylresorcinols, Resorcinolic Lipids

Fusarium culmorum F1 was found to produce and secrete into the culture medium several of 5-n-alkylresorcinols. The amount of resorcinolic lipids was 5.3 μg/g and 0.9 μg/l in mycelium and in post-culture liquid, respectively. First of all Fusarium culmorum F1 produces saturated homologues with C15 to C25 side chains. The extract from the medium contained only homologues with shorter carbon chains (C13 to C17).

Introduction

Several strains of Fusarium culmorum Sacc. are one of the most abundant and aggressive cereal pathogens. These strains are able to cause different cereal diseases including seedling blight, brown foot rot and ear blight (Blakeman and Williamson, 1994). The ability of F. culmorum to produce mycotoxins has been studied extensively (Chelkowski, 1989). Although there are many miscellaneous fungal toxins, the most important are fumonisins, trichothecenes and zearalenone. Their occurrence in agricultural products is a worldwide problem. Therefore it is important to restrict the Fusarium expansion, thus lowering the possibilities of food contamination. In our previous report (Zarnowski et al., 1999), the considerable resistance of F. culmorum to rye 5-n-alkylresorcinols (ARs) was shown. The basis of the presented research was the assumption that tolerance of Fusarium to ARs is due to its ability to biosynthesis of ARs – long-chain, odd-numbered homologues of orecinol (1,3-dihydroxy-5-methylbenzene).

Results and Discussion

Application of extraction with organic solvent systems along with chromatography on silica gel plates revealed the presence of 5.3 μg of ARs per 1 g dry weight of F. culmorum as well as 0.9 μg of ARs per litre of post-culture liquid. Analysis of ARs provided evidence of their basic skeletal structure. Use of GC and EI/MS methods enabled us to determine alkyl chain length and its unsaturation degree. The isolated material showed the base ion peaks characteristic for alkylresorcinols. Unambiguous identification was disclosed by the occurrence of peaks at m/z 123 and 124 and their mutual ratio 1:4 or 1:5. In the mycelium extract, occurrence of six parent molecular ions with m/z masses from 320 to 460 confirmed the presence of homologues from C15 to C25. Only spurious amounts of mono-unsaturated homologues were found. The extract prepared from the post-culture medium contained homologues with short side chains (C13 to C17). ARs content is summarized in Table I.

Undoubtedly, the ARs in fungal cells cause resistance to the action of these natural phenols. ARs biosynthesis may result from the decarboxylation of resorcinolic acids, which were recognized as direct precursors of toxic zearalenone derivatives (Schaafsma et al., 1998). Secretion of only small amounts of ARs into culture liquid medium apparently is due to their amphiphilic character. ARs molecules rather exhibit an affinity to biological membranes because their partition coefficient in an octanol/water system are over 7.4 (Kozubek and Tyman, 1999). It was found that ARs in the medium contained only short homologues. We assume that the process of ARs secretion has to be preceded by shortening of the carbon side chain. ARs released outside may cause direct negative effects on microorganisms in different environmental niches. Moreover ARs of cereal waxes ori-
gin have been reported as antifungal and antibacterial compounds active versus certain species (Kozubek and Tyman, 1999; Garcia et al., 1997; Zarnowski et al., 1999). The limitation of microbial growth due to the presence of ARs in such a specific niche, like the surface of the grain, could favour the growth of plant pathogens like F. culmorum. Our results clearly confirm the finding that plant pathogens which constitutively produce antifungal compounds (such as ARs) are to some extent tolerant to these compounds. Fungi contain subtoxic levels of ARs and only higher concentrations may arrest their growth.

**Experimental**

The fungus *F. culmorum* F1 was isolated from winter wheat grains and identified at the Plant Pathology Department (Agricultural University, Wroclaw, Poland). The voucher specimen is kept in the culture collection of the Agricultural Microbiology Department (Agricultural University, Wroclaw, Poland). The culture (1.61) was grown on a liquid potato medium (LPM) with 1% glucose at 28 °C. LPM was prepared by autoclaving of 200 g of potato dry puree at 121 °C for 10 min. The extract was filtered through cotton wool filter and 2 g of casamino acid (Difco, Michigan, USA) and 10 g of glucose were added. Then, the volume was filled up to 1 litre with distilled water and pH was adjusted to 6.4 and autoclaved. The 5-day-old culture was centrifuged (7500×g, 10 min) and the separated mycelium was lyophilized. Afterwards the dry material (8.6 g) was extracted twice with acetone. The supernatant was extracted twice with EtOAc. Combined extracts were concentrated *in vacuo*, redissolved in CHCl₃ and applied to 20 × 20 cm preparative TLC silica gel 60 plates (Merck, Darmstadt, Germany). Two-dimensional chromatograms were developed in CHCl₃/EtOAc (85:15, v/v) and then in hexane/Et₂O/HCOOH (70:30:1, v/v). Spots on the gel of the gel containing tested compounds were scrapped off the plates and then re-extracted with CHCl₃/AcOEt (85:15, v/v) for 30 min. After filtration and removal of the solvent the residues were dissolved in CHCl₃ and used for further analyses. The microcolorimetric method (Tluscik et al., 1984) was used for quantitative determination of ARs. All determinations were made at least in triplicate and the results were analysed statistically. Homologue composition was determined by GC (HP 5890 II) and EI/MS (AMD Intectra, Harpstedt, Germany). Identification of each AR homologues was done by the comparison of retention times (GC), molecular ions and common two base peak ions at *m/z* 123 and 124, which are characteristic of AR standard molecules (EI/MS). Additionally, detection of ARs was achieved thanks to their characteristic reddish-violet colour by reaction with the diazonide salt fast blue B (Lachema, Prague, Czech Republic) and chromatographic mobility (Kozubek and Tyman, 1995). The standard of 5-n-pentadecylresorcinol was provided by Aldrich Co. (Steinheim, Germany). Other chemicals were from POCh (Gliwice, Poland).

---

**Table I. Alkylresorcinols in *Fusarium culmorum* F1.**

<table>
<thead>
<tr>
<th>Source</th>
<th>Content [µg/g and µg/l±SE]</th>
<th>Percentage composition of homologuesa</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>saturated</td>
<td>monounsaturated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C13</td>
<td>C15</td>
</tr>
<tr>
<td>Mycelium</td>
<td>5.3±0.10</td>
<td>n.d.</td>
<td>t</td>
</tr>
<tr>
<td>Medium at harvest</td>
<td>0.9±0.02</td>
<td>26.6</td>
<td>40.5</td>
</tr>
</tbody>
</table>

a The data are means from three independent determinations. The standard errors did not exceed 3%.
SE, standard error,
n.d., not detected.
t, trace.