Physiology and Biochemistry

Regulation of Cercosporin Accumulation in Culture by Medium and Temperature Manipulation

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ABSTRACT


The ability to manipulate cercosporin accumulation in specific isolates of Cercospora in culture is a necessary prerequisite for studying the regulation of toxin accumulation at a molecular level. This study defined medium, temperature, and light conditions for maximum and minimum cercosporin accumulation in isolates of C. asparagi, C. beticola, C. kikuchii, C. nicollei, and C. zeae-maydis. A simple method was developed for the extraction and measurement of cercosporin in cultures of Cercospora spp. grown on solid medium. Of six growth media, malt and potato-dextrose agar were generally favorable for cercosporin accumulation, but the effects of medium and isolate on cercosporin accumulation interacted significantly. The ratio of carbon to nitrogen in a defined medium affected cercosporin accumulation in four of the eight isolates tested but not in any consistent manner. Cercosporin accumulation also was regulated by temperature in four of the eight isolates, higher levels accumulating at 20°C than at 30°C. Two isolates of C. kikuchii accumulated more cercosporin when grown in light than when grown in darkness, but the effect of light interacted with those of medium and isolate. Patterns of regulation of cercosporin accumulation differed markedly among species and even isolates of the same species of Cercospora, making generalizations about the regulation of cercosporin production by environmental factors of limited use. However, the present study did identify certain isolates for future investigation of cercosporin regulation. Our data also show that screening isolates of Cercospora for cercosporin production under a single set of cultural conditions is unreliable and question the reliability of correlating toxin production in vitro to the virulence of a Cercospora isolate.

The genus Cercospora includes species that cause many plant diseases, some of which are economically important, such as Cercospora leaf spot on corn (30) caused by C. beticola Sacc.,

Sigotoka disease of banana caused by Mycosphaerella fijiensis Morelet var. difformis Mulder and Stover (18), early leaf spot of peanut caused by C. arachidicola Hori (13), gray leaf spot of corn, caused by C. zeae-maydis Tehon & Daniels (15), and purple-seed stain of soybean, caused by C. kikuchii Matsu & Tomoyasu (16).
The nonspecific toxin, cercosporin, has been isolated from at least 34 Cercospora species in vitro (1,3,7,14,19), but attempts to isolate the toxin from another 51 species have been unsuccessful (1,3,4,7). The only production in vitro varied widely among cercosporin-producing isolates grown under the same conditions (1,3,7). We observed similar variations among the isolates in our collection and also with variations over time in the level of toxin production by individual isolates. The ability to regulate cercosporin production in vitro is vital for the investigation of the genetic control of toxin production in this fungus. Also, large numbers of isolates of Cercospora are often screened for toxin-producing ability on a medium, under growth conditions that favor toxin detection in one good producer with no consideration of variation among isolates (1,3,7). To avoid a premature conclusion about the ability of an isolate to produce toxin, it is important to learn the effect of media and environmental conditions on cercosporin accumulation by different isolates. This is particularly important because toxin production has been used as a criterion for predicting pathogenicity (10) and has been proposed as a taxonomic criterion (3).

Some of the previous studies on effects of light, temperature, and medium on cercosporin accumulation in culture have aimed to optimize in vitro conditions for cercosporin production. Fajola found that light, temperature, and the six media tested affected cercosporin accumulation in C. cucurbitae (3). Kayama and Tamura (5) found that the five media they tested affected cercosporin accumulation in C. cucurbitae, and Assante et al (1) found that the effect of amendments to the medium on the accumulation of cercosporin and other secondary metabolites differed among the 23 species of Cercospora tested and even between isolates of C. cucurbitae. Other workers have studied regulation of cercosporin accumulation to shed light on the biochemical pathway of cercosporin production and its regulation. Extensive studies with C. beticola isolates have found light (2,8) and growth medium components (1,2,6,8,9) to have marked effects on cercosporin accumulation. The effects on cercosporin accumulation of amino acids, carbon-nitrogen ratio, oxygen, reducing agents, carbon dioxide, trace elements, an uncoupler of oxidative phosphorylation, and an inhibitor of protein synthesis were examined by Lynch and Geoghegan (8). Mumma et al (11) studied the influence of nutrient source on cercosporin accumulation in one isolate each of C. halii and C. cucurbitae. The thrust of our study was to define for specific isolates conditions that stimulate or suppress cercosporin accumulation to allow a molecular investigation of the genetic regulation of toxin production in this fungus. Media, light conditions, and temperatures were varied to determine both conditions that would suppress toxin accumulation and conditions that would enhance it. Isolates of several species of Cercospora were included in all experiments to assess the generality of the results.

MATERIALS AND METHODS

Isolates of Cercospora. C. asparagi Sacc. was isolated from asparagus in North Carolina in 1983 and provided by C. Cooperman (North Carolina State University, Raleigh). Isolates IN, IN, and PR of C. cucurbitae were isolated from soybeans in Illinois, Indiana, and Puerto Rico, respectively, and were provided by J. B. Sinclair (University of Illinois, Urbana), who also provided isolate ATCC 86864 of C. cucurbitae. F. M. Latterell, USDA, provided the C. zeae-maydis Troy (Type A). The other isolates used were C. beticola (ATCC 24080) and C. nicotianae (ATCC 18366).

Media. Malt medium contained 15 g of malt extract, 3 g of peptone, 30 g of glucose, and 15 g of agar per liter. Cercospora sporulation medium (CSM) was an adaptation of that reported by Stavelly and Nimmro (17) to induce sporulation in C. nicotianae and contained the major and minor salts of Murashige and Skoog (12), 1.6 g of sucrose, 3.6 g of yeast extract, 0.5 mg of thiamine, 1 g of nicotinic acid, 10 mg of biotin, and 18 g of agar per liter. Potato-dextrose agar (PDA) contained 24 g of Bacto dehydrated potato dextrose broth (Difco Laboratories, Detroit, MI) and 15 g of agar per liter. Minimal medium (MM), complete medium (CM), minimal medium with soybean leaves (SBL), sucrose sorbose medium (SS), and sucrose medium (S), all contained 1 g of Ca(NO₃)₂·4H₂O, 10 ml of a solution containing 2 g of KH₂PO₄, 2.5 g of MgSO₄·7H₂O, and 1.5 g of NaCl in 100 ml of H₂O, adjusted to pH 5.3 with NaOH; and 15 g of agar per liter. Additional ingredients per liter were: for MM, 10 g of glucose; for CM, 10 g of glucose, 1 g of yeast extract, and 1 g of casein hydrolysate; for SBL, 10 g of glucose and 2 g of dried soybean leaves ground in a blender; for SS, 10 g of sucrose and 1.7 g of sorbose; and for S, 10 g of sucrose. V-8 juice agar with soybean leaves (V8 + SBL) contained 300 ml of V-8 juice (clarified by centrifuging with 4.5 g of CaCO₃ for 10 min at 850 g) and 15 g of agar per liter. The medium for the carbon:nitrogen (C:N) ratio experiments was the basal medium of Lynch and Geoghegan (8), containing 20 g per liter of sucrose as sole carbon source and varying quantities of NaNO₃ as nitrogen source.

Cercosporin assay. Plugs of solid media (6-mm diameter) with fungal colonization were removed with the wide ends of sterile Pasteur pipets and soaked in 5 N KOH in the dark for 4 hr, after which absorbance of the soaking solution was measured at 480 nm (the visible absorbance maximum of cercosporin in base) in a Beckman model 25 spectrophotometer. To compare the amounts of cercosporin detected by this method and a more exhaustive extraction procedure, four plugs (6-mm diameter) were taken from 10-day-old cultures on malt agar of six of the eight isolates of Cercospora used in the other experiments. These were either soaked in 8 ml of 5 N KOH, as described, or ground with sand in 8 ml of acetone, filtered, and the absorbance read at 473 nm (the visible absorbance maximum of cercosporin in acetone). To determine if any interfering compounds were present in the extract, 100 μl each of the extracts in 5 N KOH and in acetone were subject to thin-layer chromatography on silica gel 60 plates (Merek), pretreated in 2% H₃PO₄, and dried overnight at 60°C, using hexane/isopropanol (8:2) as the solvent. Spots with the same Rf as the cercosporin standard were scraped off the plate, resuspended in 2 ml of acetone, and their absorbance read at 473 nm. After correcting for opacity by subtracting the absorbance at 650 nm, absorbances of acetone and KOH extracts were well correlated (R² = 0.99, P < 0.0001, Fig. 1). The acetone extracts also contained compounds that fluoresced blue under long-wavelength ultraviolet light. These were resuspended in acetone and found to have no significant absorbance at 473 nm, indicating that they do.

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![Fig. 1. Amount of cercosporin detected in extracts made by soaking mycelial plugs (6-mm diameter) from 10-day-old cultures of six different isolates of Cercospora isolates in 5 N KOH versus amount of cercosporin detected in extracts made by grinding plugs from the same cultures in acetone. The coefficient for correlation between the two methods was 0.99, significant at P < 0.001.](image-url)
not affect the estimation of cercospordin concentration obtained from reading the unpurified extract at 473 nm. No interfering compounds were detected in the KOH extracts.

**Isolate/media experiments.** Plugs (6-mm diameter) taken from the margin of each isolate of *Cercospora* growing on malt medium were placed in the center of Petri plates containing 25 ml of the test media. Two plates were used for each isolate/medium combination, and the experiment was performed twice. The plates were incubated at 25°C in a chamber with 16 hr of light (13.5 μE·m⁻²·sec⁻¹) and 8 hr of dark. Every 3 days, the diameters of the cultures were measured, and 6-mm-diameter plugs taken from 2 mm behind the margin of growth (one from each plate) were extracted together in 4 ml of 5 N KOH. The correlation between colony diameter and mycelial weight was investigated by inoculating three plates of PDA as described above with each of the eight isolates. After 7 days, colony diameter was measured, and the mycelial mat was cut out and gently scraped to remove the medium. Mycelial mats were placed on weighed filter paper, dried for 72 hr in an oven at 80°C, and then weighed.

To investigate the effect of C:N ratio on cercospordin production, two plates per isolate containing the medium of Lynch and Geoghegan (8) with molar C:N ratios of 10:1, 50:1, 100:1, 200:1, or 500:1 were inoculated as described above and incubated at 25°C in a chamber with 16 hr of light (27 μE·m⁻²·sec⁻¹) and 8 hr of dark. After 12 days, the diameters of the cultures were measured, and for each isolate/medium combination, four 6-mm-diameter plugs taken from 2 mm behind the margin of growth on each plate were extracted in 2 ml of 5 N KOH. The experiment was performed three times.

**Temperature experiments.** Suspensions of aerial mycelium were made by adding sterile deionized water to cultures growing on V8+SBL and gently scraping the surface with a sterile Pasteur pipet. Suspensions were spread on plates of solid medium, and these were incubated at either 20 or 30°C, with continuous illumination at 1.5 μE·m⁻²·sec⁻¹. Plates were shifted from 30 to 20°C after various periods, and four plugs (6-mm diameter) were taken at random from each plate and extracted in 2 ml of 5 N KOH. Eight isolates were included in the experiment, in which plates were shifted from 30 to 20°C after 1, 5, 7, 11, and 14 days and cercospordin was assayed after 2, 6, 8, 12, and 15 days. The experiment was performed three times.

**Light experiments.** Plugs (6-mm diameter) taken from the margin of isolates of *C. kikuchii* II and PR growing on malt medium were placed in the center of Petri plates containing 25 ml of PDA or malt medium. Half of the plates were wrapped with aluminum foil to exclude light, and all were placed in chambers at 20 or 25°C with 16 hr of light (4 μE·m⁻²·sec⁻¹) and 8 hr of dark. Three plates were used per isolate/medium/temperature/light combination, and the experiment was performed twice. After 8 days, four 6-mm-diameter plugs from each plate were extracted in 2 ml of 5 N KOH.

**RESULTS**

Media effects on cercospordin production and growth. In preliminary experiments, several isolates of *Cercospora* produced less red pigment, assumed to be cercospordin, when grown on any other medium tested. Omission of individual ingredients of the medium, however, revealed that no one component was responsible for pigment suppression. In *C. beticola* and *C. kikuchii* ATCC 86864, omission of sucrose or thiamine restored the red pigment, but in *C. asparagi* and *C. kikuchii* II, these components had no effect. Omission of yeast extract partially restored red pigmentation in *C. kikuchii* II. Because peak time of accumulation of cercospordin varied among both media and isolates (Fig. 2), the best measure of the cercospordin-producing ability of an isolate/medium combination was the highest amount of cercospordin extracted on any harvest date. The numbers in Table 1 are the means of the levels of cercospordin in each trial on the harvest date with the highest level of cercospordin for each isolate/medium combination in that trial. Comparison of measured amounts of cercospordin produced on

![Graph](image-url)

**Fig. 2.** Mean cercospordin accumulation per plug (6-mm diameter) over time over all eight isolates of *Cercospora* on each medium (A) and over all six media for each isolate (B). Media: malt medium (MALT), potato-dextrose medium (PDA), *Cercospora* sporulation medium (CSM), minimal medium (MM), complete medium (CM), and minimal medium with soybean leaves (SBL). Isolates: *C. asparagi* (CA), *C. beticola* (CB), *C. kikuchii* ATCC 86864 (AT), *C. kikuchii* II (IL), *C. kikuchii* IN (IN), *C. kikuchii* PR (PR), *C. nicotianae* (CN), and *C. zea-maydis* (CZM). Means are from two replicates for each isolate/medium combination in each of two trials.

**TABLE 1. Maximum production of cercospordin (nmol per plug) by eight isolates of *Cercospora* on six media**

<table>
<thead>
<tr>
<th>Medium</th>
<th>C. beticola</th>
<th>C. zeae-maydis</th>
<th>C. asparagi</th>
<th>C. kikuchii ATCC 86864</th>
<th>C. nicotianae</th>
<th>Medium mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALT</td>
<td>65</td>
<td>120</td>
<td>69</td>
<td>37</td>
<td>7</td>
<td>45</td>
</tr>
<tr>
<td>PDA</td>
<td>112</td>
<td>74</td>
<td>28</td>
<td>30</td>
<td>64</td>
<td>45</td>
</tr>
<tr>
<td>CM</td>
<td>50</td>
<td>9</td>
<td>43</td>
<td>20</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>SBL</td>
<td>47</td>
<td>11</td>
<td>18</td>
<td>5</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>MM</td>
<td>9</td>
<td>27</td>
<td>24</td>
<td>9</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>CSM</td>
<td>3</td>
<td>3</td>
<td>43</td>
<td>6</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>

| Isolate mean | 48 abc | 41 ab | 38 abc | 18 bcd | 16 bcd | 13 cd | 10 cd | 5 d |

*Extracted by soaking 4 hr in 5 N KOH from a 6-mm diameter plug of agar taken from 2 mm behind the margin of growth.*

*MALT = malt medium; PDA = potato-dextrose medium; CM = complete medium; SBL = minimal medium with soybean leaves; MM = minimal medium; CSM = *Cercospora* sporulation medium.*

*Mean of the amounts of cercospordin extracted per 6-mm diameter plug on the harvest date with the highest cercospordin level for each isolate/medium combination in each of the two trials.*

*Means followed by the same letter do not differ significantly *P* ≤ 0.05 by Tukey's studentized range test (HSD).*
different growth media confirmed that CSM was unfavorable for
toxin production in any isolate tested (Table 1, Fig. 2). The
medium on which the highest mean level of cercosporin was
produced was malt for C. asparagi, C. beticola, C. nicotianae, C.
kikuchii ATCC 86864, IL, and PR and was PDA for C. zeae-
maydis and C. kikuchii IN (Table 1). Averaged over all isolates,
malt and PDA were significantly more favorable for cercosporin
accumulation than the other four media tested (Table 1). When
averaged over all isolates, peak levels of cercosporin were found
after 8 days on PDA, dropping to less than 50% of the maximum
by day 29, whereas levels of cercosporin on malt agar remained
fairly constant throughout the experiment (Fig. 2).
Overall, C. kikuchii IN and IL accumulated the most cer-
kosporin and C. nicotianae the least, but differences among isolates
were less distinct than among media (Fig. 2, Table 1). Peak times
of accumulation averaged over all media types differed among
isolates, but cercosporin level declined after 21 days in all isolates.
Analysis of variance of maximum cercosporin content showed
isolate and medium effects to be significant at \( P < 0.0001 \) and the
isolate \( \times \) medium interaction to be significant at \( P = 0.03 \).

The correlation between dry weight of mycelium and colony
area as calculated for the eight isolates on PDA was significant
\( (R = 0.54, P = 0.0072) \). The most favorable medium for overall
growth of the eight isolates of Cercospora was PDA and the least
favorable SBL (Fig. 3). When averaged over the different media, C.
asparagi and C. nicotianae had the largest areas of mycelium by 20
days and C. kikuchii IN the smallest (Fig. 3). Because isolates that
accumulated the most cercosporin had grown the least by 20 days,
the correlation between maximum cercosporin accumulation and
area at 20 days was investigated. The overall correlation was not
significant, but when data for each medium were examined
separately, area at 20 days and cercosporin accumulation were
found to be significantly negatively correlated on CM \( (R = -0.73,
P = 0.0012) \) and PDA \( (R = -0.79, P = 0.002) \), two media favorable
for cercosporin accumulation. When data for each isolate were
examined separately, area of mycelium at 20 days and maximum
cercosporin accumulation were found to be significantly correlated
for C. asparagi \( (R = 0.67, P = 0.0241) \) and C. beticola \( (R = 0.66,
P = 0.0198) \). Analysis of variance of area at 20 days showed isolate
and medium effects to be significant at \( P < 0.0001 \), but the isolate \( \times
\) medium interaction to be nonsignificant at \( P < 0.05 \).

C:N ratio over the range of 10:1 to 500:1 was found by analysis of

![Fig. 3](image)

**Fig. 3.** Mean increase in colony area over time, calculated from diameter (assuming colony to be circular), over all eight isolates of Cercospora on each medium (A) and all six media for each isolate (B). Media: malt medium (MALT), potato-dextrose medium (PDA), Cercospora sporulation medium (CSM), minimal medium (MM), complete medium (CM), and minimal medium with soybean leaves (SBL). Isolates: C. asparagi (CA), C. beticola (CB), C. kikuchii ATCC 86864 (AT), C. kikuchii II (IL), C. kikuchii IN (IN), C. kikuchii PR (PR), C. nicotianae (CN), and C. zeae-maydis (CZM). Means are from two replicates for each isolate/medium combination in each of two trials.

![Fig. 4](image)

**Fig. 4.** Mean cercosporin accumulation per plug (6-mm diameter, open stars) and growth (filled squares) by eight isolates of Cercospora on media with different C:N ratios. Means are from two replicates in each isolate/C:N ratio combination in each of three trials. Note that ordinate scales are not equivalent.
and 500:1 in *C. kikuchii* PR (Fig. 4).

The most dramatic effects of C:N ratio were on *C. nicotianae* (Fig. 4), which accumulated 36 times as much cercosporin at a C:N ratio of 10:1 as at 500:1. In the other isolates that were significantly affected by C:N ratio, two to four times as much cercosporin accumulated at the most favorable ratio as at the least. In a single trial, media containing no nitrogen or a C:N ratio of 1:1 were included. All isolates of *Cercospora* grew poorly on both these media and all accumulated low or average quantities of cercosporin except *C. beticola*, which accumulated more cercosporin on these media than on those with any other C:N ratio.

**Temperature shift experiments.** An ultraviolet light-induced mutant of *C. kikuchii* PR, white when grown on SS at 30°C, became red when placed at 4°C overnight. On the assumption that the color change was indicative of rapid toxin accumulation, the effect of temperature on toxin accumulation in eight isolates of *Cercospora* grown on SS was investigated. Temperature conditions during the growth period did not significantly affect levels of cercosporin measured at 2 or 6 days in any isolate or at 8 or 15 days in *C. zeae-maydis* or isolates AT, IN, or PR of *C. kikuchii*. Temperature did have a significant ($P \leq 0.05$) effect on levels of cercosporin measured at 8 days in *C. nicotianae*, at 15 days in *C. beticola*, and at 8 and 15 days in *C. asparagi* and isolate IL of *C. kikuchii*. In *C. asparagi*, significantly more ($P \leq 0.05$) cercosporin accumulated by 8 days in cultures grown at 20°C or switched from 30 to 20°C after 1 day than in cultures grown at 30°C (Fig. 5). In *C. kikuchii* IL, significantly ($P \leq 0.05$) more cercosporin accumulated by 8 days in cultures switched from 30 to 20°C after 1 day, and by 15 days in cultures grown at 20°C or switched from 30 to 20°C after 1 day, than in cultures grown at 30°C (Fig. 5). Similar trends were apparent in *C. beticola* and *C. nicotianae*, but differences were not significant.

In a single experiment, in which other media were used to grow cultures at different temperatures, higher levels of cercosporin were found in cultures grown for 5 days at 20°C than at 30°C on PDA (for *C. asparagi*, *C. beticola*, and isolates ATCC 86864, IL, and PR of *C. kikuchii*), on malt (for *C. asparagi* and *C. kikuchii* IL and PR), and on 5 medium (for *C. asparagi*, *C. zeae-maydis*, and *C. kikuchii* IL).

Cultures of *C. kikuchii* IL and PR grown in low light accumulated significantly ($P \leq 0.05$) more cercosporin than those grown in darkness under any set of temperature and medium conditions tested (Fig. 6). There were, however, significant interactions between light and isolate ($P = 0.0022$), light and medium ($P = 0.0001$), and light and temperature ($P = 0.0037$). Darkness did not completely inhibit cercosporin accumulation; for example, as much cercosporin accumulated in dark-grown cultures of PR on PDA as in light-grown cultures of IL on malt medium (Fig. 6).

**DISCUSSION**

The simple extraction method employed in this study facilitates the measurement of cercosporin accumulation in large factorial experiments. This method is a great improvement on visual estimation of cercosporin content since masking of the red pigment by darker pigments can occur, and all red pigments seen in cultures of *Cercospora* are not cercosporin (4).

Growth media had large effects on the production of cercosporin in agar cultures of the isolates of *Cercospora* tested, and these effects differed among isolates. The best media for cercosporin production in most of the isolates tested were PDA and malt.

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Fig. 5. Mean cercosporin accumulation per plug (6-mm diameter) in eight isolates of *Cercospora* grown continually at 20°C (20), 30°C (30) or shifted from 30 to 20°C after 1 day (1), 5 days (5), 7 days (7), or 14 days (14) after the start of the experiment. Cercosporin levels were determined at 2, 6, 8, and 15 days after the start of the experiment. Lines on bars represent the standard error of the mean. Shaded bars represent means that differ significantly at $P \leq 0.05$ by Tukey's studentized range test from the mean at 30°C of the same isolate on the same harvest date. Means are from one observation for each isolate/temperature treatment combination in each of three trials.
Cercosporin accumulation was also regulated by temperature in some isolates. On several media, these isolates showed inhibition of cercosporin accumulation at 30 C and no inhibition at 20 C. Shifting cultures from 30 to 20 C often increased cercosporin content of the cultures, but after a certain age, which differed among isolates, cultures could no longer be induced to increase cercosporin content. Fajola (3) tested growth temperatures between 10 and 35 C and found 22.5 C to be optimum for cercosporin production in C. ricinellla. Although temperatures between 20 and 25 C do favor cercosporin accumulation in some isolates of Cercospora, this was not even true of all eight isolates tested in this study and should not be assumed for untested isolates.

Light is known to be a strong regulator of cercosporin accumulation in Cercospora (2,3,8). When all other conditions were held constant, light-grown cultures had much higher levels of cercosporin than those grown in darkness. However, the effect of light interacted significantly with those of isolate, medium, and temperature.

Our examination of the regulation of cercosporin accumulation by medium, temperature, and light has revealed much specific information about the eight isolates of Cercospora tested but little information generally applicable to the species. Patterns of regulation differed markedly even among isolates of the same species. Although temperatures from 20 to 25 C seem to favor cercosporin accumulation, this may not be true for every isolate and may also depend on the growth medium. Although malt medium and PDA favor accumulation in general, there are also exceptions. The C:N ratio in a medium is very likely to affect cercosporin accumulation, but how it will do so is unpredictable. Although light strongly affects cercosporin production, its effect is modified by isolate, medium, and temperature. These data cast doubt on failures to detect cercosporin in Cercospora species screened by using a single set of cultural conditions and suggest the unreliability of correlating toxin production in vitro to the virulence of an isolate of Cercospora, supporting the statements of Yoder (21).

The specific knowledge gained about the regulation of cercosporin production in these eight isolates of Cercospora will be used to study the mechanism of the regulation of this toxin in specific isolates. Proteins from cultures grown under sets of conditions that favor or suppress cercosporin accumulation are being subjected to SDS polyacrylamide gel electrophoresis. At 20 C, which favors cercosporin accumulation, C. kikuchii II produces proteins with a banding pattern different from that produced at 30 C, which suppresses cercosporin accumulation. Proteins in cultures grown on media or in light conditions that regulate cercosporin accumulation are under study. Although some of the factors regulating cercosporin production differ among isolates of Cercospora isolates, the underlying mechanism of such regulation may prove common to all.

LITERATURE CITED


