An Optimum Environment for the Culturing of Coryneum carpophilum

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ABSTRACT

Three isolates of *Coryneum carpophilum* were subjected to variations of temperature, nutrition, and hydrogen ion concentration to identify optimum conditions for linear growth on an agar medium. Results showed that a temperature: carbon: nitrogen: pH interaction was present, with the best single set of conditions for most rapid growth of the fungus being use of a basal medium in which β-maltose served as the carbon source, L-asparagine provided the nitrogen, an initial pH of 5.4-5.8 was utilized, and the medium was incubated at 15 C. Phytopathology 61:829-830.

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Corneum blight, incited by *Coryneum carpophilum* (Lev.) Jauch, is relatively easy to control in stone fruit orchards if effective chemicals can be applied under proper conditions of concentration and timing. However, satisfactory control is achieved with difficulty, and serious losses continue in infested areas. These facts suggested that an effective systemic fungicide was needed, because such a chemical agent could reduce the seriousness of timing errors, washing off of fungicide by rains during critical periods, etc. To expedite the screening of potential systemics, a rapid in vitro assay procedure was needed in which direct toxicity to the fungus could be determined. Studies, therefore, were initiated to identify laboratory conditions that would promote most rapid linear growth of the assay fungus on a solid medium.

MATERIALS AND METHODS.—Three isolates of *C. carpophilum* (Co-1, Co-2, and Co-3) were obtained from active lesions on peach twigs collected from widely separated locations in Idaho. The synthetic D-glucose: L-asparagine medium described by Lilly & Barnett (3) was used, and agar was added to form a 2% agar preparation. Standard strength (1.5%) Difco malt agar was included in all tests as a reference medium.

All media were autoclaved for 20 min at 15-lb. pressure and dispensed at a rate of 20 ml/petri plate. In each experiment, except the temperature study, the pH of each treatment was measured after autoclaving.

Each isolate was inoculated onto five plates for each treatment. Inoculum was obtained with a No. 2 cork-borer from the periphery of 6- to 8-day-old cultures of the isolates maintained on Difco malt agar or the synthetic-agar (6).

In all studies, the plates were incubated until mycelium reached the edges of the first plates (ca. 25 days), except for the hydrogen ion test where measurements were made after 7 and 21 days. Since the temperature study indicated that 15 C was best for linear growth of the fungus, all other studies were conducted at that temperature.

Seven temperatures were tested, ranging from 5-35 C in 5-degree increments ± 1 C. Eighteen carbon sources were evaluated, plus one treatment without added carbon. All carbon sources were added to the basal medium at a rate of 4.0 g of actual carbon/liter. The 15 nitrogen sources tested were incorporated into the basal medium at the rate of 0.43 g actual nitrogen/liter of medium. Hydrogen ion concentration was not adjusted to predetermined levels in any medium of this study because utilization of the different nitrogen sources was assumed to be optimal at different pH levels, and because the test fungus was expected to change the hydrogen ion concentration of the medium (1).

The effects of pH were studied by adjusting six combinations of carbon and nitrogen to nine selected levels, ranging from 4.0 to 8.0 in half-unit increments, with 0.1 N NaOH or HCl. Since nitrogen utilization is dependent upon pH of the medium (1), and since a carbon: nitrogen interaction was indicated in the nitrogen-source experiment, the best two carbon sources were combined separately with either L-proline, L-asparagine, or L-tyrosine. Buffers were omitted in order to exclude possible effects of the nutritive elements which the buffers would add (1). To avoid contamination, the pH of each treatment was adjusted prior to autoclaving and checked upon removal from the autoclave. Colony diameters were recorded after 7 days in order to avoid possible staling effects, and again after 21 days.

RESULTS.—Colonies on standard Difco malt agar incubated at 15 or 20 C were the first to fill plates (after 25 days); most rapid growth on the synthetic agar medium occurred at 15 C, but the difference was not significant at the 5% level. Analyses revealed a temperature: medium interaction; i.e., the temperature range over which maximal linear growth occurred was different for the two media used. No significant differences among isolates were indicated for either medium.

Significantly more rapid growth occurred on the synthetic medium containing β-maltose than on any other medium. No evidence of selective utilization of any class of carbon compounds was observed, and differences among isolates were not significant.

Largest colony diameters were produced on the β-maltose:L-asparagine combination (after 23 days), but these colonies were not significantly larger than those on the D-glucose:L-proline combination. Media containing β-maltose generally yielded the best linear growth; isolate Co-1 generally produced significantly larger colony diameters than did the other two isolates. Statistical analysis showed a carbon:nitrogen interaction.

All initial (adjusted) pH levels were altered by autoclaving, but for simplicity, results will be expressed in terms of the adjusted pH levels. After 7 days, L-asparagine (with either of the carbon sources, β-maltose, or D-glucose) yielded most rapid linear growth, on media adjusted to pH 5.5-6.0. Statistical analysis showed that there was a carbon:nitrogen interaction except where L-asparagine supplied the nitrogen. Since linear growth varied with the hydrogen ion concentration, which in turn depended on the carbon source and the nitrogen source, a carbon:nitrogen:pH interaction was indicated and statistically verified. A significant difference between isolates also was found, with Co-1 and Co-2 growing more rapidly than Co-3.

After 21 days, growth was best with L-asparagine or L-tyrosine; pH 4.0-4.5 and 8.0 generally were best; no significant differences due to the two carbon sources were found. Statistical analyses indicated that a carbon:nitrogen:pH interaction occurred. Significant differences among isolates also were present, with Co-2 growing more rapidly than Co-1 and Co-3 in comparable situations.

Discussion and Results.—Growth response at different temperatures was dependent upon the medium used. The results of this study therefore are in agreement with Cochrane's (1) contention that temperature optima and ranges for growth of fungi in vitro are valid only under specified conditions of time, nutrient medium, and method of measurement.

Since the medium without an added carbon source in the present study allowed significantly more growth than that obtained with L-sorbose, L-sorbose apparently was inhibitory in some manner. This conclusion is in agreement with the contention of Lilly & Barnett (4) and Tatum et al. (5) that L-sorbose is toxic to some fungi.

The nitrogen source study shows that while amino acid utilization is superior, nitrate nitrogen definitely is better than inorganic ammonia forms and nearly as good as amino acid forms, depending upon the accompanying carbon source. This last fact supports Cochrane & Conn's (2) observation that the kind of carbon source, insofar as it affects pH, decisively influences the response of some organisms to nitrate. As was the case with the treatment containing no carbon, the treatment containing no nitrogen allowed relatively good growth. Either the fungus was able to reutilize nitrogenous materials already assimilated or the small quantity of nitrogen in the agar inoculum plug was sufficient for fungal growth for the duration of the test period. Results of the carbon test (which showed β-maltose to be the best carbon source) were verified by the nitrogen test in that β-maltose was found to interact better with more nitrogen sources than D-glucose.

Optimal pH varied with nitrogen source, carbon source, and isolate. After 7 days' incubation, maximum linear growth occurred on the L-asparagine treatments at pH 5.4-5.8, with no significant differences observed in the utilization of β-maltose or D-glucose. However, with L-proline and L-tyrosine, optimum pH was dependent upon carbon source.

Within the limitations of these studies, the following conclusions are in order: (i) linear growth rate of *C. carpophilum* varies with temperature, carbon nutrition, nitrogen nutrition, and hydrogen ion concentrations, and the interactions of these factors are statistically significant; (ii) specific growth optima occur at 15-20 C (depending upon the medium used), when β-maltose supplies the carbon, when L-asparagine or L-proline supply the nitrogen (provided the carbon source is β-maltose or D-glucose, respectively), and when the medium is adjusted to pH 5.4-5.8 (when L-asparagine supplies the nitrogen); (iii) the best single set of conditions for optimal linear growth of all isolates is use of a Lilly & Barnett (3) basal agar medium in which β-maltose supplies the carbon, L-asparagine provides the nitrogen, the pH is adjusted to 5.4-5.8 when the fungus is introduced, and the cultures are incubated at 15 C.

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