Vitamin Requirements of *Cercospora beticola* Sacc.

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A number of workers have experienced difficulty in obtaining rapid growth and sporulation of some *Cercospora* species in artificial media with the result that they have resorted to the use of semisynthetic media for the same (Lewis, 1940; Diachun and Valleau, 1941; Murakishi, 1951; Kilpatrick and Johnson, 1956; Plakidas, 1956) while others, using synthetic media, found that species worked out by them had very exacting nutritional requirements (Shanta, 1956; Rangaswamy and Chanderasekaran, 1962; and Landers, 1964).

The author has already done some work in culture and nutrition of some *Cercospora* species (Thind and Mandahar, 1964; 1965) and in view of the statement of Rangaswamy and Chanderasekaran (1962) that out of 1500 *Cercospora* species a very limited number have been cultured artificially and studied physiologically, it was decided to extend this work to other *Cercospora* species. *Cercospora beticola* disease on spinach (*Spinacea oleracea* Mill.) was, therefore, selected. Its growth responses in artificial medium do not seem to have been investigated so for. Vitamin requirements of the pathogen, as worked out by the authors, are reported here.

Materials and Methods

Several monosporic isolates of the pathogen were made on PDA slants from infected leaves. One of them was selected for further study. All the studies were carried out in liquid culture media at 26° C for 10 days at 6.0 pH using 25 ml of the basal medium in 125 ml pyrex glass Erlenmeyer flasks in triplicate. Composition of the synthetic basal medium used was: glucose, 20 g; KNO_3, 5 g; KH_2PO_4, 2.5 g; $MgSO_4 . 7H_2O$, 0.5 g; and water to make 1000 ml. Concentrations of vitamins used per liter of the medium were: thiamine hydrochloride, 1 mg; biotin, 0.05 μg; pyridoxine, 500 μg; nicotinic acid 500 μg; ascorbic acid, 500 μg; riboflavin, 500 μg; choline, 5 μg; and p-amino benzoic acid 5 gm. Vitamins were added aseptically to the partially cooled medium after autoclaving.

All the sterilizations were carried out at 10 lbs per square inch steam pressure. Only the C. P., or AnaLR grade reagents were used. Inoculation of the liquid medium was always done by using 1 ml standardized mycelial suspension. Dry weights, sporulation and final pH were determined after the experiments were over and are given in various tables as the mean of three replicates in each case. As the degree of sporulation, when it occured was determined empirically in a drop of spore suspension prepared by shaking the culture flasks, was always poor (1—5 spores per drop) no special meathod for harvesting and counting the spores was used. Poor sporulation is represented by the sign (+) while no sporulation is represented by (—).

Results

In the first experiment, a single vitamin was excluded each time from the basal medium containing all the rest of 7 vitamins. Two controls, one with all the vitamins and the second with none, were also set up. The data, summarized in table 1, indicate that *C. beticola* showed poor growth and no sporulation when all the vitamins or thiamine alone was omitted from the basal medium. Omission of choline and pyridoxine resulted in slight decrease in dry weight of mycelium while sporulation occurred poorly. Omission of any of the remaining vitamins from the medium did not effect growth of the pathogen. Omission of the riboflavin resulted in slight increase in its growth. This, however, can not be taken as an indication of a clear inhibition of its growth.

Effect of individual vitamin on growth and sporulation of the pathogen was studied next. To the basal medium lacking all vitamins only one vitamin was added at a time. The data are summarized in table 2 which indicate that addition of thiamine significantly increased growth of the pathogen, that of choline and pyridoxine did so only to a little extent while that of any of the remaining vitamins practically made no difference. Sporulation, although poor, occurred only in the presence of thiamine. It is clear thus from tables 1 and 2 that the pathogen is partially deficient for thiamine, choline and pyridoxine and that deficiency for thiamine is more than for the other two vitamins.

Effect of three vitamins (thiamine, pyridoxine and choline) for which the pathogen is partially deficient were studied in combination. Results summarized in table 3, show that growth of the fungus on medium containing all the three vitamins was the best while its growth in media containing any of the two vitamins was not equally good. As usual, sporulation although poor, occurred only in medium containing thiamine. The fact that sporulation never took place in the absence of thiamine indicates that it is absolutely essential for its sporulation.

Discussion

C. beticola is partially deficient for thiamine, pyridoxine and choline. Out of the various *Cercospora* species studied so for, a number of them have been found to be totally or partially deficient for thiamine (Robbins and Kavangh, 1942; Steinberg, 1950; Shanta, 1956; Sethi and Munjal, 1963; and Landers, 1964). It seems, therefore, that total or partial thiamine deficiency is very common in *Cercospora* species.

C. beticola sporulated only in the presence of thiamine. Favourable effect of thiamine on sporulation of some other *Cercospora* species has also been reported (Shanta, 1956; Landers, 1964). McDowell and De Hertogh (1968) have shown that the activity of Embden-Meyerhof-Parnas (EMP) and hexose monophosphate shunt (HMP) appears to be stimulated during sporulation of *Endothia parasitica*, with the HMP being stimulated to the greatest extent, and further that thiamine is an essential requirement for some enzymes of HMP. This explains the essentiality of thiamine for sporulation of *C. beticola*.

Besides *C. beticola* possibly only one other *Cercospora* species *C. viticola* (Sethi and Munjal, 1963) has been found to be partially deficient for choline and pyridoxine. According to Wickberg (1959) pyridoxine has been found in a variety of tissues ?? from plants and animals and that wide distribution of pyridoxine indicates that it is an important, perhaps indispensable, metabolite for all living things and also that probably all microorganisms which do not need this vitamin for growth are able to synthesize it themselves. The fact that only two *Cercospora* species and only a couple of other fungi have been reported to be deficient, completely or partially, for pyridoxine is viewed in this context, it seems that a very great majority of fungi have the genetic ability to form pyridoxine while only a few fungi have lost it.

It is known that pyridoxine is active only as pyridoxal phosphate and pyridoxamine phosphate, and that the former acts as a coenzyme for a number of enzymes like transaminases, racemases, amino acid decarboxylases, etc., while the latter participates only in transamination (Wickberg, 1959). This role of pyridoxine in metabolic processes explains its essentiality and also, because its derivatives are needed as coenzymes in so fundamentally important enzymes, why only a couple of fungi have lost the genetic capability of synthesizing this vitamin.

Summary

Cercospora beticola Sacc. is a common and a serious disease of spinach (*Spinacea oleracea*). The pathogen was found to be partially deficient for three vitamins: thiamine, pyridoxine and choline. The

deficiency of thiamine was more pronounced than the deficiency for pyridoxine and choline. Thiamine only was found to be essential for the sporulation of the pathogen; pyridoxine and choline did not affect sporulation which could take place even in their absence. Role of thiamine for sporulation is discussed.

Table 1

Effect of omission of a single vitamin from basal medium containing all the vitamins on the growth and sporulation of *Cercospora beticola*

Vitamin omitted	Dry wt. of mycelium in mg	Sporulation
All	70	—
None	200	+
Thiamine	95	—
Biotin	196	+
Pyridoxine	140	+
p-aminobenzoic acid	210	+
Riboflavin	195	+
Choline	135	+
Nicotinic acid	205	+
Ascorbic acid	201	+

Table 2

Effect of addition of individual vitamin to the basal medium lacking all the vitamins on the growth and sporulation of *Cercospora beticola*

Vitamin added	Dry wt. of mycelium in mg	Sporulation
None	65	—
All	220	+
Thiamine	180	+
Biotin	100	—
Pyridoxine	150	—
p-aminobenzoic acid	105	—
Riboflavin	70	—
Choline	145	—
Nicotinic acid	72	—
Ascorbic acid	75	—

Table 3

Effect of addition of thiamine, pyridoxine and choline singly and in combination to the basal medium on the growth and sporulation of *Cercospora beticola*

Vitamin added	Dry wt. of mycelium in mg	Sporulation
None	60	—
All the 8	215	+
Thiamine	172	+
Pyridoxine	140	—
Choline	144	—
Thiamine + Pyridoxine	198	+
Choline + Pyridoxine	170	—
Thiamine + Choline	190	+
Thiamine + Choline + Pyridoxine	235	+

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