EFFECT OF VARIOUS CARBON AND NITROGEN SOURCES ON THE GROWTH AND SPORULATION OF *MICROXYPHIELLA HIBISCIFOLIA*

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Abstract

The carbon and nitrogen nutrition of *Microxyphiella hibiscifolia* Bat., Nasc., and Cif., isolated from the leaves of cotton (*Gossypium hirsutum* L.), was studied at 28° C for 14 days at pH 4·0. Out of forty-one carbon compounds tested, the pathogen showed excellent growth on dextrose, sucrose, starch, raffinose and maltose; good on inulin; fair on melibiose and galactose; and poor or no growth on rest of the carbon compounds. The pathogen showed excellent sporulation on all the carbohydrates, in general. Out of thirty-three nitrogen compounds tested, the pathogen showed excellent growth on peptone, asparagine, proline, ammonium oxalate, yeast extract, ammonium phosphate, ammonium sulphate; good on ammonium chloride, serine, ammonium nitrate, urea, sodium nitrate, potassium nitrate, histidine mono HCl and histidine di HCl; fair on glutamic acid, lysine mono HCl, arginine mono HCl, threonine, nor-valine and glycine; and poor or no growth on rest of the nitrogen compounds. The pathogen showed excellent or good sporulation, in general, on all the nitrogen compounds. The utilisation of potassium nitrite was conditioned by the pH of the medium.

Introduction

M. hibiscifolia was isolated from the cotton leaves obtained from Hissar (Haryana) in the months of October and November, 1969. Several monosporic isolates of the fungus were made separately on potato dextrose agar slant-(peeled and sliced potatoes 200 g, dextrose 20 g, agar 20 g, and distilled water 1,000 ml). No marked morphological variation among its various isolater was observed and, therefore, one representative isolate was selected to study the effect of carbon and nitrogen compounds on the growth and sporulation of *M. hibiscifolia.* No such studies have been carried out previously on this organism.

MATERIAL AND METHODS

The study on carbon and nitrogen nutrition of *M. hibiscifolia* was carried out with liquid basal medium of the following composition : dextrose 20 g, asparagine 3·740 g, KH_2PO_4 10 g, $MgSO_4$, $7H_2O$ 0·250 g, $Fe_2(SO_4)_3$. $6H_2O$ 0·005 g, and distilled water 1,000 ml. This basal medium was found to give excellent growth of this fungus in the preliminary experiments.

In order to study the effect of various carbon* and nitrogen** sources, they were substituted singly by dextrose and asparagine, respectively, in the basal medium. The amount of these substances was so adjusted as to furnish the same quantity of carbon and nitrogen, which was present in the basal medium. Twenty-five millilitres of medium were poured in 100 ml Pyrex glass Erlenmayer flasks and three replicates were taken in each case. The various media were sterilized at 7 lb./sq. inch steam pressure for half-an-hour. Initial pH of the various media was adjusted to 4·0 after autoclaving. However, a solution of various carbon compounds was prepared separately in distilled water, adjusted to pH 7·0 and then sterilized in the autoclave to minimise the possibility of their breakdown during autoclaving. The solution of remaining ingredients of the basal medium was sterilized in the autoclave without adjusting to the optimum pH. A carbon solution and the solution of remaining ingredients of the basal medium were then mixed together proportionally to get a normal strength of the basal medium and pH was adjusted to 4·0. The spore suspension was prepared in sterilized distilled water from 6 days old culture. The medium in each flask was inoculated with 1 ml standardised spore suspension (8–16 spores per low power field of the compound microscope). It may be mentioned here that pH 4·0, temperature 28° C and incubation period of 14 days were found to be optimum conditions for the growth of *M. hibiscifolia* by preliminary experiments. The cultures were filtered and dried at 60° C to a constant weight in hot air oven for 24 hours. The data were recorded in terms of final pH, sporulation and dry weight of the mycelium.

For the estimation of spore concentration in different experiments, each culture flask was shaken and the fungal growth was mixed thoroughly by

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* Soluble starch, pectin and inulin were added at the rate of 20 g each while oils were added at the rate of 20 ml each per litre of the basal medium.

** Peptone, yeast extract and casein hydrolysate were added at the rate of 5 g each per litre of the basal medium.

means of glass rod in order to get a homogenous mixture. After shaking thoroughly, drops were taken from this homogenate and the spore count was estimated under low power of the compound microscope. The degree of sporulation was measured on the basis of average number of spores present per low power field of the compound microscope and recorded in the following grades :

No. of spores per low power field of the microscope	Degree of sporulation	Symbol used
No spore	Nil	—
1 – 10	Poor	+
10 – 20	Fair	++
20 – 30	Good	+++
Above 30	Excellent	++++

The growth of the fungus on different media has been termed excellent, good, fair and poor on the basis of its following mycelial weights :

Category	*Dry weight, mg*
Excellent	Above 150
Good	100–150
Fair	50–100
Poor	Below 50

Experimental Work

Carbon nutrition.—A total of sixteen carbohydrates comprising three pentoses, five hexoses, four disaccharides, one trisaccharide and three polysaccharides were used singly as the sole source of carbon in order to observe their effect on the growth and sporulation of *M. hibiscifolia.* The data on dry weight, sporulation and final pH are summarised in Table I. *M. hibi-*

scifolia showed excellent growth on dextrose, sucrose, starch, raffinose and maltose; good on inulin; fair on melibiose and galactose; poor on lactose, pectin, fructose, arabinose, sorbose and mannose; and no growth on xylose and ribose. There was no sporulation on xylose, ribose and mannose; poor

TABLE I

Effect of different carbohydrates used singly as the sole source of carbon on the growth and sporulation of M. hibiscifolia *after* 14 *days of incubation at* 28° *C. Initial pH adjusted to* 4·0

Carbon source	Dry wt. in mg	Sporulation	Final pH
Control (without carbon)	0	—	4·0
Carbohyarates			
D (+) Xylose	0	—	4·0
L (+) Arabinose	18	+	4·5
D (−)-Ribose	0	—	4·5
Dextrose	186	++++	4·8
D (−)-Fructose	25	++++	4·5
D (−) Mannose	8	—	4·8
D (+)-Galactose	85	++++	5·6
L-Sorbose	12	+	4·3
Sucrose	180	++++	4·0
Lactose	30	++++	4·2
D-Maltose	150	++++	4·1
D (+) Melibiose	87	++++	5·2
Raffinose	160	++++	5·6
Inulin	125	++++	4·8
Starch	180	++++	5·3
Pectin	30	++++	3·6

on arabinose and sorbose while it was excellent on all other carbohydrates. The pH increased slightly to considerably in all the carbohydrates except pectin in which case the pH fell down slightly. However, there was no change in pH in the case of sucrose.

Sorbose is utilized poorly or not at all by the fungi, in general. It has been reported not only unutilisable for many fungi but also toxic for several others. The toxicity is evidenced by the death of hyphal tips followed by meagre branching of mycelium below the killed portions (Lilly and Barnett, 1953). It has been suggested that this sugar interferes with the respiratory pathway of these microorganisms. In order to find out whether the poor growth of *M. hibiscifolia* on sorbose is due to inhibitory effect or its nonutilisation, an experiment was set up as follows:

Equivalent amount of good carbon sources so as to furnish 8 g/l of carbon, which is present in 20 g/l of dextrose, was added to the basal medium containing sorbose. The mixture of two carbohydrates, therefore, furnished twice the amount, *i.e.*, equivalent to 40 g dextrose per litre. A control with 40 g/l dextrose was set up to study if there was any adverse effect on the growth of fungus due to the increased amount of total sugars. Twenty-five millilitres of medium were poured in each flask and three replicates were taken in each case. Rest of the procedure was as usual. The data on dry weight, sporulation and final pH are given in Table II. It is clear from the table that sorbose markedly inhibited the growth of *M. hibiscifolia* in the presence of dextrose and sucrose and to some extent in the presence of maltose, raffinose, inulin and starch. Therefore, it appears to be inhibitory to the growth of this pathogen.

Six alcohols, five oils and fourteen organic acids were used as the sole source of carbon for the growth and sporulation of *M. hibiscifolia.* The data on dry weight, sporulation and final pH are summarised in Table III. It is apparent from the data that *M. hibiscifolia* showed poor growth on mannitol, ethyl alcohol and succinic acid and no growth on rest of the carbon compounds. The pathogen showed good sporulation on mannitol and succinic acid; fair on ethyl alcohol; and no sporulation on rest of the carbon compounds.

Nitrogen nutrition.—Some inorganic and organic nitrogenous compounds were tested as the sole source of nitrogen for the growth and sporulation of this pathogen in one experiment. The data on dry weight, sporulation and final pH are summarised in Table IV. It is apparent from the data that

TABLE II

Effect of sorbose used singly and in combination with other carbon sources on the growth and sporulation of M. hibiscifolia *after* 14 *days of incubation at* 28° C. *Initial pH adjusted to* 4·0

Carbon source	Dry wt. in mg	Sporulation	Final pH
Dextrose 40 g/l	220	++++	5·0
Sorbose	15	+	4·3
Dextrose	190	++++	4·8
Dextrose + sorbose	30	++++	4·9
Sucrose	182	++++	4·0
Sucrose + sorbose	36	++++	4·5
Maltose	153	++++	4·1
Maltose + sorbose	89	++++	4·5
Raffinose	158	++++	5·6
Raffinose + sorbose	89	++++	5·1
Inulin	128	++++	4·8
Inulin + sorbose	78	++++	4·5
Starch	176	++++	5·3
Starch + sorbose	100	++++	5·0

M. hibiscifolia showed excellent growth on peptone, asparagine, ammonium oxalate, yeast extract, ammonium phosphate and ammonium sulphate; good on ammonium chloride, ammonium nitrate, urea, sodium nitrate and potassium nitrate; and poor on casein hydrolysate. However, there was neither any growth nor sporulation on potassium nitrite. The fungus showed excellent sporulation on all the inorganic and organic sources except casein hydrolysate which showed poor sporulation. There was a slight or no pH change

TABLE III

Effect of different alcohols, oils and organic acids used singly as the sole source of carbon on the growth and sporulation of M. hibiscifolia *after* 14 *days of incubation at* 28°C. *Initial pH adjusted to* 4·0

Carbon source	Dry wt. in mg.	Sporulation	Final pH
Control (without carbon)	0	—	4·0
Alcohols :			
Mannitol	20	+++	6·2
Dulcitol	0	—	4·0
Methyl alcohol	0	—	4·0
Ethyl alcohol	16	++	6·0
Isopropyl alcohol	0	—	4·0
n-Butyl alcohol	0	—	4·0
Oils :			
Castor oil	0	—	4·0
Olive oil	0	—	4·0
Cotton seed oil	0	—	4·0
Almond oil	0	—	4·0
Coconut oil	0	—	4·0
Organic acids :			
Formic acid	0	—	4·0
Acetic acid	0	—	4·0
Propionic acid	0	—	4·0
Butyric acid	0	—	4·0
n-Velaric acid	0	—	4·0
Stearic acid	0	—	4·0
Lactic acid	0	—	4·0
Oxalic acid	0	—	4·0
Maleic acid	0	—	4·0
Succinic acid	12	+++	4·6
Sebacic acid	0	—	4·0
Malic acid	0	—	4·0
Tartaric acid	0	—	4·0
Citric acid	0	—	4·0

in the case of nitrates, nitrites, ammonium oxalate, ammonium nitrate and asparagine. However, pH fell down considerably in the case of other ammonium salts and rose appreciably in the case of urea, peptone, yeast extract and casein hydrolysate. In all cases pH remained in the acidic range.

TABLE IV

Effect of different inorganic and organic nitrogenous compounds used singly as the sole source of nitrogen on the growth and sporulation of M. hibiscifolia *after* 14 *days of incubation at* 28° C. *Initial pH adjusted to* 4·0

Nitrogen source	Dry wt. in mg.	Sporulation	Final pH
Control (without nitrogen)	0	—	4·0
Potassium nitrate	130	++++	4·0
Potassium nitrite	0	—	4·0
Sodium nitrate	136	++++	4·0
Ammonium oxalate	169	++++	4·6
Ammonium sulphate	152	++++	2·5
Ammonium nitrate	137	++++	4·8
Ammonium chloride	148	++++	2·6
Ammonium phosphate	160	++++	2·6
Urea	137	++++	5·9
Asparagine	188	++++	4·7
Peptone	200	++++	6·0
Yeast extract	165	++++	5·8
Casein hydrolysate	18	+	5·6

H–ion concentration is known to influence markedly the utilisation of KNO_2 by different fungi. Therefore, an experiment was set up to find out the effect of whole range of pH on the growth and sporulation of *M. hibiscifolia* on KNO_2. It is clear from Table V that this pathogen did not make any growth on acid pH range of 3·0–6·0, while made some growth at pH

7·0–10·0 but no growth at pH 10·0. However, the fungus showed good sporulation at pH 7·0–10·0.

TABLE V

Effect of different hydrogen-ion concentration on the utilization of potassium nitrite for the growth and sporulation of M. hibiscifolia *after* 14 *days of incubation at* 28° *C*

Initial pH	Dry wt. in mg	Sporulation	Final pH
2·0	0	—	2·0
3·0	0	—	3·0
4·0	0	—	4·0
5·0	0	—	5·0
6·0	0	—	6·0
7·0	18	+++	6·5
8·0	38	+++	7·1
9·0	25	+++	7·6
10·0	12	+++	7·9
11·0	0	—	8·2

Twenty amino acids were tested as the sole source of nitrogen on the growth and sporulation of *M. hibiscifolia.* The data on dry weight, sporulation. and final pH are summarised in Table VI. *M. hibiscifolia* showed excellent growth on proline; good on serine, histidine mono HCl, histidine di HCl; fair on glutamic acid, lysine mono HCl, arginine mono HCl, threonine, nor-valine and glycine; poor on aspartic acid, phenylalaline, tryptophane, methionine, iso-leucine, tyrosine, leucine, valine and alanine; and no growth on cystine. The fungus showed poor sporulation on alanine and methionine and excellent or good sporulation on rest of the amino acids. The pH fell down slightly to considerably in all amino acids except arginine mono HCl and proline in which case it increased slightly to appreciably, respectively.

However, there was no change in pH in the case of control and cystine on which there was no growth.

TABLE VI

Effect of different amino acids used singly as the sole source of nitrogen on the growth and sporulation of M. hibiscifolia *after* 14 *days of incubation at* 28° *C. Initial pH adjusted to* 4·0

Nitrogen source	Dry wt. in mg	Sporulation	Final pH
Control (without carbon)	0	—	4·0
Glycine	52	+++	3·7
DL-iso-Leucine	20	++++	3·2
L-Leucine	15	++++	2·4
β-Alanine	12	+	3·8
DL-valine	15	++++	3·3
DL-nor-Valine	59	++++	3·3
DL-Threonine	61	++++	3·4
DL-Serine	139	++++	3·7
L-Proline	186	++++	5·8
L-Cystine	0	—	4·0
DL-Methionine	23	+	3·5
L-Arginine mono HCl	67	++++	4·7
L-Lysine mono HCl	81	++++	3·4
DL-Aspartic acid	38	+++	3·4
L-Glutamic acid	93	+++	4·2
L-Phenylalanine	28	++++	3·3
L-Tyrosine	20	++++	3·3
DL-Tryptophane	24	+++	3·6
L-Histidine mono HCl	123	++++	3·8
DL-Histidine di HCl	118	++++	3·7

In order to find out whether cystine is a poor source of nitrogen or it is inhibitory to the growth and sporulation of this pathogen, the following experiment was set up :

Equivalent amount of an amino acid so as to furnish 693 mg/l of nitrogen, which is present in 3·740 g/l of asparagine, was added to the basal medium containing cystine. The mixture of two amino acids, therefore, furnished twice the amount of nitrogen, *i.e.*, equivalent to 7·480 g/l of asparagine. Twenty-five millilitres of the basal medium were poured in each flask and three replicates were taken in each case. Rest of the procedure was as usual. The data on dry weight, sporulation and final pH are summarised in Table VII. It is apparent from the data that cystine inhibited the growth of *M. hibiscifolia* to some extent in the presence of other amino acids which were good supporters of its growth. Thus it is an inhibitory source of nitrogen for the growth of this pathogen.

TABLE VII

Effect of cystine used singly and in combination with other amino acids on the growth and sporulation of M. hibiscifolia *after* 14 *days of incubation at* 28° *C. Initial pH adjusted to* 4·0

Nitrogen source	Dry wt. in mg	Sporulation	Final pH
Cystine	0	—	4·0
Serine	144	++++	3·7
Serine + cystine	60	++++	4·0
Proline	188	++++	5·8
Proline + cystine	90	++++	5·2
Histidine mono HCl	125	++++	3·8
Histidine mono HCl + cystine	80	++++	3·9

DISCUSSION

Different carbon compounds affected the growth of *M. hibiscifolia* differently. Pentoses usually do not support good growth of fungi and, similarly, xylose, arabinose and ribose supported poor or no growth of *M. hibiscifolia* studied here.

Dextrose, sucrose, starch, raffinose and maltose supported excellent growth of *M. hibiscifolia*. Similarly, a very large number of fungi have been reported by various workers to make excellent or good growth on these carbon sources. Generally, inulin serves as a poor source of carbon for the growth of fungi, which is due to their failure to secrete the appropriate enzyme inulase rather than its inhibitory effect (Hawker, 1950). However, it supported good growth of *M. hibiscifolia*. Similalry, Prasad (1967) recorded good growth of *Pestalotia pauciseta* on inulin.

M. hibiscifolia made fair growth on melibiose and galactose. Similarly, fair growth on melibiose has been reported for *Linderina pennispora* and *L. macrospora* (Chan and Stephen, 1968).

M. hibiscifolia studied here made poor growth on lactose, pectin, fructose, sorbose and mannose. Out of these fructose, mannose and pectin are good while lactose and sorbose are poor sources of carbon for the growth of fungi, in general.

It is clear from Table II that sorbose markedly inhibited the growth of *M. hibiscifolia* in the presence of dextroes and sucrose and to some extent in the presence of maltose, raffinose, inulin and starch and thus it is inhibitory for the growth of this pathogen. Similarly, Murray and Andrian (1960) reported that sorbose severely inhibited the growth of *Neurospora crassa* (wild type) when mixed with galactose and fructose. Lilly and Barnett (1953) reported that the inhibitory effect of sorbose was more pronounced in the presence of maltose than glucose or sucrose. Thind and Madan (1967) reported that sorbose showed maximum growth inhibition of *Cephalothecium roseum* with fructose and minimum inhibition with dextrose, starch and maltose. Bilgrami (1963), on the other hand, showed that utilisation of sorbose by *Phyllosticta* spp. was considerably increased when used in combination with good carbon source like sucrose, maltose or glucose, etc.

In general, alcohols, oils and organic acids are poor sources of carbon for the growth of fungi. Similarly, *M. hibiscifolia* showed poor growth on mannitol, ethyl alcohol and succinic acid and no growth on rest of these carbon compounds.

M. hibiscifolia showed poor sporulation with arabinose and no sporulation with xylose and ribose. Similarly, pentoses, generally, are poor sources of carbon for the growth as well as for the sporulation of fungi.

Dextrose, fructose, galactose, sucrose, lactose, maltose, melibiose, raffinose, inulin, starch and pectin supported excellent sporulation of *M. hibiscifolia.* All these carbohydrates except lactose and inulin have been reported to show excellent or good sporulation for fungi, in general. Mannose which proved to be a poor source for the growth did not yield any sporulation of this pathogen. However, this sugar has been reported to be excellent or good source of carbon for the sporulation of many fungi, such as *Colletotrichum inamdarii* and *Alternaria citri* and *A. tenuis* (Hasija, 1965; 1970), *C. roseum* (Thind and Madan, 1967) and *Botryodiplodia ananassae* and *Macrophomina phaseoli* (Bhargava, 1971).

Sorbose is known to be a poor source of carbon for the growth as well as sporulation of a large number of fungi. Similarly, it induced poor growth and sporulation of the present fungus.

M. hibiscifolia showed good sporulation on mannitol and succinic acid; fair sporulation on ethyl alcohol; and no sporulation on rest of the alcohols, oils and organic acids. All these carbon compounds, in general, are poor sources for the growth as well as sporulation of fungi.

The pathogen studied here made excellent or good growth on nitrates of potassium and sodium and various ammonium salts. A majority of the fungi are known to utilise nitrate and ammonium nitrogen well. A great majority of the fungi are unable to utilise nitrite as the nitrogen source at their optimum pH values, which are generally below 7·0. Nitrites have been reported to be toxic on the acidic pH range (Cochrane, 1950, 1958; Cochrane and Conn, 1950; Lilly and Barnett, 1951). Likewise, *M. hibiscifolia* could not make any growth on acidic pH range of 2·0–6·0 while it made poor growth on pH 7·0–10·0 and no growth at pH 11·0. Similar results have also been reported for *Cercospora* spp. (Thind and Mandahar, 1965) *Pleospora indica* (Mandahar, 1965) and *C. roseum* (Thind and Madan, 1969).

Urea supported good growth of *M. hibiscifolia* as is true of *Cercospora hibiscina* (Thind and Mandahar, 1965) and *C. roseum* (Thind and Madan, 1967). Asparagine, in general, has been reported to be a good source of nitrogen for the growth of fungi investigated by various workers. Similarly, it yielded excellent growth of this pathogen. Peptone and yeast extract supported excellent growth while casein hydrolysate supported poor growth of *M. hibiscifolia.* All these nitrogen sources have been reported to support excellent or good growth of a large number of fungi. However, some fungi are known to make poor or no growth on casein hydrolysate, such as *Colleto-*

trichum capsici (Thind and Randhawa, 1957) and *Phallus revenelii* and *Crucibulum levis* (Howard and Howard, 1969).

M. hibiscifolia made excellent or good growth on proline, serine, histidine mono HCl and histidine di HCl. Out of these proline and serine are generally good sources of nitrogen for a great majority of fungi studied by various workers so far. Histidine mono HCl and histidine di HCl are well utilised by some fungi but poorly by others. Glutamic acid, lysine, arginine, threonine, nor-valine and glycine made fair growth of *M. hibiscifolia.* Similarly, lysine supported fair growth of *C. capsici* (Thind and Randhawa, 1957) and *P. indica* (Mandahar, 1965); nor-valine of *C. hibiscina* (Thind and Mandahar, 1965); and glycine of some members of Saprolegniaceae (Bhargava, 1945). However, glutamic acid, arginine and threonine have been reported to show excellent or good growth of many fungi, such as *C. hibiscina* (Thind and Mandahar, 1965), *P. indica* (Mandahar, 1965) and *C. roseum* (Thind and Madan, 1969). Aspartic acid, phenylalanine, tryptophane, methionine, iso-leucine, tyrosine, leucine, valine, alanine supported poor growth while cystine supported no growth of the present fungus. Out of these cystine and methionine are generally poor sources of nitrogen (Pelletier and Keitt, 1954). Likewise, tryptophane has been reported to support poor growth of *P. indica* (Mandahar, 1965), *P. ravenelii* and *C. levis* (Howard and Howard, 1969) and *Helminthosporium* spp. (Reddy, 1971); aspartic acid poor growth of *Zygorhynchus* spp. (Sarbhoy, 1965) and *Tieghemiomyces parasiticus* (Barnett, 1970); phenylalanine poor growth of *Zygorhynchus moelleri* and *Z. exponens* (Sarbhoy, 1965); tyrosine poor growth of *C. capsici* (Thind and Randhawa, 1957); leucine poor growth of *Z. exponens* (Sarbhoy, 1965); valine poor growth of *Helminthosporium rostratum* (Agarwal and Shinkhede, 1959); and alanine poor growth of *Tilletia caries* (Zscheile, 1951) and *Helminthosporium nodulosum* (Reddy, 1971). Cystine inhibited the growth of *M. hibiscifolia* to some extent in the presence of serine, proline and histidine mono HCl. Similarly, Lewis (1957) reported cystine a contsant inhibitor for the growth of *Alternaria solani* in the presence of other amino acids.

Potassium and sodium nitrates and all ammonium salts tested yielded excellent sporulation of *M. hibiscifolia.* These are known to be excellent or good sources of nitrogen for the growth as well as sporulation of fungi, in general.

Urea, asparagine, peptone, yeast extract and casein hydrolysate, in general, are excellent or good sources of nitrogen for the growth as well as sporu-

lation of fungi. All these nitrogen sources except casein hydrolysate supported excellent or good growth as well as sporulation of *M. hibiscifolia*. However, casein hydrolysate, which was a poor supporter of growth, yielded poor sporulation of this pathogen.

Amino acids, in general, are known to be good sources of nitrogen for the growth as well as sporulation of fungi. However, *M. hibiscifolia*, which showed varying growth on all the amino acids tested, yielded excellent sporulation on all of them except alanine and methionine.

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