Utilization of Amino Acids by two species of Mucor*.

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With 1 Fig. in the text.

Abstract

Utilization of 9 individual amino acids by two *Mucor* species was studied chromatographically. Growth and drift in pH was recorded. Among the amino acids tested, alanine, leucine, asparagine, and glutamic acid supported good growth of both the species. Glycine and valine were found to be good for *M. bainieri* only while for *M. genevensis* both were moderate sources for the growth. Leucine could stimulate moderate growth of both the species of *Mucor* and arginine supported moderate growth of *M. bainieri* and poor of *M. genevensis*. Methionine was found to be poor and moderate for *M. bainieri* and *M. genevensis* respectively.

Introduction

Amino acids serve as good sources of nitrogen. They are present in free form in the plants and are also obtained by the hydrolysis of the protein complex. Fungi are known to show diverse response towards different amino acids. Choice for a particular amino acid varies with the organisms involved and the experimental conditions used. Even biotypes of one organism can differ greatly in their amino acid nutrition. Such factors as pH (Foster, 1949), redox potential (Robbins, 1937), presence of trace elements (Steinberg, 1939), oxygen tension or aeration (Sarojini and Yogeshwari, 1947), osmotic concentration, lack of certain minerals, vitamins or other essential metabolites, all may play a part in determining the utilization of a particular amino acid. Steinberg (1941) is of the opinion that molecular structure of the amino acids is the main intrinsic factor, determining their assimilation. The concentration at which the amino acids are supplied and the presence of certain impurities in some commercial preparations were also shown to affect the results (Ajello, 1948; Barner and Cantino, 1952). The composition of the basal medium can also influence the growth responses induced by the amino acids. The factors involved in this case are, the presence or absence of other amino acids in the medium (Leonian and Lilly, 1938; Steinberg, 1942;

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Robbins and Ma, 1945); and different durations of incubation period (Ajello, 1948). Some of the other factors are the temperature of incubation, the amount and physiological condition of the inoculum.

Leonian and Lilly (1938), while studying the relative utility of 24 amino acids for 14 fungi reported that none of them was found best for all the species. Steinberg (1942), has made an extensive study on the utilization of 22 amino acids by *Aspergillus niger* van Tieghem and found that out of them seven were excellent nitrogen sources. He told that these seven amino acids were the acids first synthesised by the fungus and hence he called them as primary amino acids and from them the secondary amino acids were derived. Barnett and Lilly (1956), observed the effect of 8 amino acids on growth and zygospore formation of *Choanephora cucurbitarum* (Berk. & Rav.) Thaxter. They noted quite a good deal of variation in the response of the organism to different amino acids.

The present work deals with the utilization of a number of amino acids, when supplied singly, by two *Mucor* species.

Materials and Methods

Pure cultures of *Mucor bainieri* Mehrotra and Baijal and *Mucor genevensis* Lendner were taken. The synthetic *Mucor*-medium, commonly employed by Hesseltine (1954) for the studies of Mucorales and employed by the author for the earlier physiological studies, was used for the present study. This medium (KH_2PO_4 0.5 gm; $MgSO_4 . 7H_2O$ 0.25 gm; thiamine hydrochloride 0.5 mg; glucose 10 gm; and distilled water 1,000 ml.), without the nitrogen source served as the basal medium. To the basal medium different amino acids were added in equivalent amounts so as to supply 423 mgs. of nitrogen per litre. The pH of the medium was adjusted to 6 in each case before autoclaving because a pH range of 5—6.5 was earlier found to be ideal for the growth and sporulation of these organisms. 20 ml. of each of the media was poured in 150 ml. Erlenmeyer Pyrex flasks. They were sterilized at 15 lb pressure for 15 minutes. The flasks containing the media were inoculated daily at a fixed time ($\pm$ 15 minutes) for 15 days with the different species. Material for inoculation was taken from the margin of an actively growing young colony taking care that the size of the inoculum was equal (40 mm. in diam.) in all the cases. Care was also taken to carry the least amount of the medium along with the inoculum and, therefore, the medium for this purpose was poured in thin layers in Petri dishes on the sixteenth day of starting the experiment when one to fifteen days old cultures were available, the fungal mats were filtered separately. Only the mats of fifth, tenth and fifteenth day were dried and weighed to determine the amount of growth.

The filtrate of each day containing different amino acids was chromatographically analysed to detect the presence of various amino

acids. The circular paper chromatography method described by R a n j a n et al. (1955) was used. A circular piece of Whatman filter paper No. 1 having a diameter of 26 cm. was used. From the filtrate drops of 0.05 ml. were placed at the spot marked for this purpose (number 1, 2, 3—15). Equal volumes of drops of known solutions were also applied, when necessary, on the same chromatogram to facilitate the identification of bands. Drops of control solutions were also placed in each case. After placing the drop the chromatograms were run with n-butanol: acetic acid: water 4 : 1 : 5). The chromatograms had a single paper wick and were run for about 7 hours in the above solvent. They were dried at room temperature and sprayed with 0.1% ninhydrine in n-butanol. These chromatograms again were left at room temperature for 3 or 4 hours and were then placed at 110° C for 90 seconds. The average Rf values have been recorded at appropriate places in the text.

T a b l e 1. Showing dry weights (in mgs) of colonies of *Mucor bainieri* and *M. genevenis* developed on individual amino acids and the pH of medium on 5th, 10th and 15th day at 25° C (± 1).
Initial pH of the medium was 6.

Amino acids	Days of incubation	*Mucor bainieri* Dry weight	pH	*Mucor genevensis* Dry weight	pH
Glycine	5	25.3	6.2	28.0	6.4
	10	35.5	6.8	33.3	6.8
	15	40.0	7.6	38.0	7.4
Alanine	5	30.0	6.4	32.0	6.4
	10	45.3	7.0	45.3	7.4
	15	50.0	7.4	52.5	7.6
Valine	5	24.0	6.2	20.0	6.4
	10	35.0	6.4	30.5	6.8
	15	43.0	7.2	38.4	7.2
Leucine	5	22.0	6.4	19.1	6.8
	10	34.0	6.8	30.2	7.2
	15	38.0	7.2	35.2	7.6
Serine	5	28.0	6.8	29.3	6.8
	10	40.3	7.4	41.0	7.0
	15	45.5	7.6	49.3	7.4
Glutamic acid	5	32.0	7.0	34.0	6.8
	10	55.0	7.4	58.3	7.4
	15	60.3	7.6	65.0	7.6
Arginine	5	15.0	6.8	4.0	6.4
	10	25.0	7.4	15.4	7.0
	15	35.0	7.8	18.0	7.2
Methionine	5	4.0	6.4	18.0	6.6
	10	12.3	6.8	30.5	7.4
	15	17.0	7.2	38.0	7.6
Asparagine	5	35.0	6.4	25.0	6.2
	10	55.5	6.8	50.1	7.2
	15	69.0	7.4	59.4	7.4

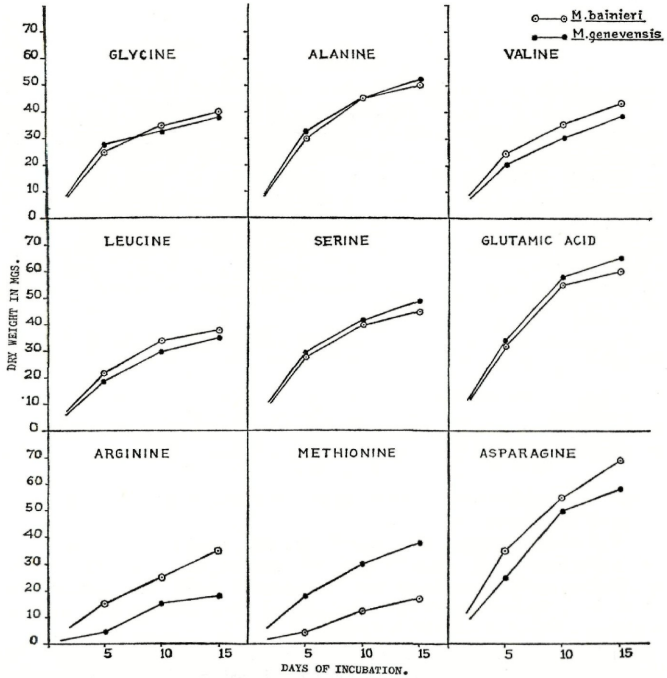

Fig. 1. Showing increase in dry weight on nine amino acids by *M. bainieri* O—O and *M. genevensis*.

Observations

The results are tabulated in tables 1 and 2 and graphically represented in Fig. 1.

It is apparent from the results that the amount of growth produced by the two *Mucor* species under study on four amino acids, viz., alanine, serine, glutamic acid and asparagine was good. Glycine, and valine, were found to be good for the growth of *Mucor bainieri* only while for *M. genevensis* both were moderate sources for the growth. Leucine supported moderate growth of both the species of *Mucor*. Arginine supported moderate growth of *Mucor bainieri*, and poor of *M. gene-*

Table 2.
Showing the utilization of individual amino acids by *Mucor bainieri* and and *M. genevensis*- (+ denotes the presence and — denotes the absence of amino acids)

Organismus	Amino acids	Days of incubation													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
M. bainieri	Glycine	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—
M. genevensis		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M. bainieri	Alanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M. genevensis		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M. bainieri	Valine	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—
M. genevensis		+	+	+	+	+	+	+	+	+	—	—	—	—	—	—
M. bainieri	Leucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M. genevensis		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M. bainieri	Serine	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
M. genevensis		+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
M. bainieri	Glutamic acid	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
M. genevensis		+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
M. bainieri	Arginine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M. genevensis		+	+	+	+	+	+	+	+	+	+	+	+	+	—	—
M. bainieri	Methionine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M. genevensis		+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
M. bainieri	Asparagine	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
M. genevensis		+	+	+	—	—	—	—	—	—	—	—	—	—	—	—

vensis. Methionine was found to be poor for the growth of *M. bainieri* and moderate for *M. genevensis*.

Out of the 9 amino acids tested, two viz., alanine and leucine were not completely assimilated by both the species till the end of the incubation period. Arginine and methionine were not utilized by *M. bainieri* till the end of incubation period while arginine was utilized within 13 days and methionine within 8 days by *M. genevensis*. Serine was utilized within 4 days by both the species.

Mucor bainieri and *M. genevensis* utilized glycine within 6 days and 8 days, valine within 11 days and 9 days, asparagine within 4 days and 3 days and glutamic acid within 3 days and 4 days respetively. Except in one or two cases good growth of the organisms was recorded on those amino acids which were rapidly utilized from the medium. Both the mucors could not consume alanine and leucine from the media but the growth produced by these fungi was good on these media.

pH in all the cases drifted towards alkaline side.

Discussion

Different amino acids vary greatly in their utilization by different fungi. The assessment of the value of individual amino acids for fungi is determined by the extent of growth produced on media containing the particular amino acid as the sole nitrogen source. The presence or absence of growth on a particular amino acid may be a reflection of

permeability, enzymatic capacities or nearly such secondary problems as acidity changes consequent upon utilization. It has already been mentioned, that assimilation of an amino acid is affected greatly by the environmental factors. There are two important views regarding the mode of utilization of amino acids. According to Raciborski (Lilly and Barnett, 1951, p. 107) the amino acids are deaminated before their utilization, while according to Czapek (Lilly and Barnett, 1951, p. 108) amino acids are utilized directly as such.

The former view point has been supported by Butkewitsch (1903), who concluded that utilization of amino acid nitrogen is preceded by deamination. Lilly and Barnett (1951, p. 108) have mentioned, that during the process of deamination, nitrogen in the form of ammonia is released, which is utilized by most fungi. The former idea seems to be more plausible because pH during the incubation period rose towards the alkaline side, and this rise in pH has been ascribed to the accumulation of ammonia which is released during the utilization of amino acid.

Alanine and leucine could support good growth of both the species, which corresponds with the results obtained by Lilly and Barnett (1956); Raizada, (1957); Sarbhoy, (1963); and Mehrotra (1963), for a number of Mucorales studied by them. Good growth of both the fungi in alanine and leucine was found but these were not consumed within the specified period. Mehrotra (1963) also found similar results in case of a number of *Choanephora* species in alanine and glycine. As in the present case good growth on glutamic acid as well as its rapid utilization have also been reported for other Mucorales, viz., *Phycomyces blakesleeanus* (Leonian and Lilly, 1940), *Absidia cylindrospora, Mucor hiemalis,* and *Mucor rouxii* (Raizada, 1957), *Helicostylum piriforme* (Mehrotra and Mehrotra, 1962), and for a number of *Choanephora* species (Mehrotra, 1963).

Asparagine is usually one of the best sources of nitrogen for fungi, as was found in the present case also. Poor growth of *Mucor genevensis* in arginine and also poor growth of *M. bainieri* in methionine, was found to be due to non-utilization of arginine and methionine from the media by these fungi within the specified time of fifteen days.

Results with glycine, serine, arginine, methionine and valine are in agreement with those of other workers, Raizada (1957); Sarbhoy (1963) and Mehrotra (1963).

It may be concluded that except one or two cases (alanine and leucine) good growth of the organisms was recorded on those amino acids which were rapidly utilized from the medium. All the amino acids do not show similarity in their utility for the same fungus and the structure of different amino acids may also play an important role in their utilization.

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