Pakistan J. Sci. Ind. Res., Vol. 26, No. 4, August 1983

# STUDIES ON NUTRITION OF FUNGI Part VII. Effect of Different Amino Acids and Their Combinations on the Growth of Different Fungi

## Mrs. Naheed Anwar and S. Shahid Hussain

PCSIR Laboratories, Karachi, Pakistan

### (Received June 25, 1981)

Effect of four basic amino acids, lysine, arginine, histidine asparagine and two acidic amino acids, glutamic and aspartic acid and their combinations has been studied on the growth of *Botryodiplodia* theobromae, Fusarium sulphureum, Curvularia lunata, Glomerella cingulata and Pleospora infectoria. It was observed that mixtures of some amino acids support better growth than others while some fungi grow more profusely in the presence of a single amino acid.

## INTRODUCTION

Considerable work has been done on the nutrition of fungi. Fothergill and Jones [1] studied the utilization of carbohydrates and different nitrogen sources by various species of *Zygorhynchus*. Bhargava [2] carried out investigations on sulphur and phosphorus requirements of some members of family Saprolegniaceae. Dayal [3] continued the work of Bhargava and studied the effect of various nitrogen sources on other members of the same family. Steinberg [4] studied effect of 22 amino-acids on the growth of *Aspergillus niger* and found seven of these amino-acids i.e. alanine, arginine, aspartic acid, glycine, glutamic acid, proline and hydroxyproline, suitable nitrogen sources for *A. niger*. Stock and Ward [5] observed considerable differences among amino acids requirements for *Rhizopus nigricans*.

Studies on the nutrition of fungi have been carried out by the authors. Husain *et al.* [6,7,8,9], reported the effect of various carbon and nitrogen sources in different concentrations on the growth of some fungi. They observed that all nitrogen sources may not be suitable for all fungi.

Present studies were carried out to determine the combined effect of different amino acids on the growth of certain fungi and also to compare them with the effect of single amino acids.

#### MATERIALS AND METHODS

Curvularia lunata, Pleospora infectoria, Botryodiplodia theobromae, Glomerella cingulata, Fusarium sulphureum were grown in Czapek's liquid medium. NaNO<sub>3</sub>-2)g, K<sub>2</sub>HpO<sub>4</sub>-1.00g, KCl<sub>2</sub>-0.50g, MgSO<sub>4</sub>-0.50 g, FeSO<sub>4</sub>-.01g, Sucrose-30g, Distilled water-1000 ml). Calculated amounts of mixtures of amino acids were used in the Czapak's medium in place of sodium nitrate. Basic amino acids viz., lysine, asparagine, arginine and histidine, as well as acidic amino acids viz asparatic acid and glutamic acid were used with 0.1% nitrogen in these studies.

The following combinations were used: Histidine + Arginine, Lysine + Arginine, Asparagine + Arginine, Asparagine + Lysine, Asparagine + Histidine, Glutamic Acid + Asparagine, Glutamic Acid + Arginine, Glutamic Acid + Lysine, Glutamic Acid + Histidine, Asparatic Acid + Asparagine, Aspartic Acid + Arginine, Asparatic Acid + Lysine, Aspartic Acid + Histidine and Glutamic Acid + Aspartic Acid.

Fungi were cultured on Czapek's solid medium. 250 ml flasks were used during the experiment containing 100 ml of liquid medium. Two flasks were used for each fungus. Flasks containing medium and mixture of amino acids were autoclaved at 15 lb pressure for 15 mins, allowed to cool and then inoculated with 4 mm. disc of four day old culture of fungi. Inoculated flasks were incubated at  $28^{\circ} \pm 1^{\circ}$  for eight days. After incubation flasks were taken out, mycelium filtered and dried at  $60^{\circ}$  for 24 hrs and then weighed.

#### RESULTS

Among the mixtures of basic amino acids Lysine + Asparagine yielded maximum growth of Fusarium sulphureum (1040 mg) followed by Curvularia lunata (529 mg), Botryodiplodia theobromae (404 mg), Glomerella cingulata (140 mg) and Pleospora infectoria (124 mg). On Asparagine + Histidine, Fusarium sulphureum yielded maximum growth (975 mg) followed by Curvularia lunata (484 mg), Botryodiplodia theobromae (295 mg), Pleospora infectoria (100 mg), Glomerella cingulata (85 mg), Mixture of Asparagine + Arginine produced maximum growth of Fusarium sulphureum (695 mg), followed by Botryodiplodia theobromae (550 mg), Curvularia lunata (509 mg), Glomerella cingulata (213 mg), and Pleospora infectoria (129 mg), Lysine + Arginine yielded maximum mycelium (685 mg) of Fusarium sulphureum followed by Curvularia lunata (341 mg), Glomerella cingulata (338 mg), Botryodiplodia theobromae (242 mg), and Pleospora infectoria (145 mg), Histidine + Arginie produced highest amount of Glomerella cingulata (430 mg), followed by Fusarium sulphureum (395 mg), Curvularia lunata (335 mg), Botryodiplodia theobromae (225 mg), and Pleospora infectoria (130 mg).

Among the mixtures of acidic and basic amino acids, Arginine + Aspartic acid produced maximum growth of Botryodiplodia theobromae (1180 mg), followed by Fusarium sulphureum (1136 mg), Curvularia lunata (1080 mg), Glomerella cingulata (336 mg), and Pleospora infectoria (145 mg), Asparagine + Aspartic acid yielded 1130 mg, of Botryodiplodia theobromae mycelium followed by Curvularia lunata (820 mg), Pleospora infectoria (186 mg), Glomerella cingulata (183 mg), and (Fusarium sulphureum (100 mg). Lysine + Aspartic acid produced maximum amount of Botryodiplodia theobromae (935 mg), followed by Curvularia lunata (860 mg), Glomerella cingulata (560 mg), Pleospora infectoria (125 mg), and Fusarium sulphureum (115 mg). Histidine + Aspartic acid vielded (400 mg), of Glomerella cingulata followed by Botryodiplodia theobromae (370 mg), Curvularia lunata (115 mg), Pleospora infectoria (70 mg), and Fusarium sulphureum (55 mg). Mixture of Histidine + Glutamic Acid yielded highest amount of Fusarium sulphureum (1160 mg), followed by Botryodiplodia theobromae (751 mg), Curvularia lunata (750 mg), Glomerella cingulata (145 mg), and Pleospora infectoria (100 mg). Lysine + Glutamic Acid yielded highest amount of Botryodiplodia theobromae (444 mg), followed by Curvularia lunata (158 mg), Glomerella cingulata (153) mg), Fusarium sulphureum (153 mg), and Pleospora infectoria (84 mg). Arginine + Glutamic Acid produced maximum amount of Botryodiplodia theobromae (851 mg), followed by Curvularia lunata (337 mg), Glomerella cingulata (175 mg), Fusarium sulphureum (131 mg), and Pleospora infectoria (80 mg). Asparagine + Glutamic Acid produced (546 mg) of Botryodiplodia theobromae followed by Curvularia lunata, 276 mg), Fusarium sulphureum (150 mg), Pleospora infectoria (135 mg), and Glomerella cingulata (128 mg).

Mixture of acidic amino acids Glutamic Acid+Aspartic Acid yielded highest amount of *Curvularia lunata* (810 mg), followed by *Botryodiplodia theobromae* 293 mg), *Pleospora infectoria* (140 mg), *Glomerella cingulata* (120 mg), and *Fusarium sulphureum* (105 mg),

Among the individual basic amono acids Asparagine produced highest amount of growth of *Fusarium slphu*reum (765 mg), followed by *Botryodiplodia theoromae* (640 mg), *Curvularia lunata* (405 mg), *Glomerella cingulata* (145 mg), and *Pleospora infectoria* 78 mg). Arginine yielded highest amount of Botryodiplodia theobromae (410 mg), followed by Fusarium sulphureum (360 mg), Curvularia lunata (300 mg), Glomerella cingulata (186 mg), and Pleospora infectoria (165 mg), Lysine produced (325 mg) of Botryodiplodia theobromae following by Fusarium sulphureum (210 mg), Curvularia lunata (210 mg), Glomerella cingulata (210 mg), and Pleospora infectoria (190 mg). Histidine individually yielded maximum amount of Fusarium sulphureum (380 mg) followed by Curvularia lunata (320 mg), Botryodiplodia theobromae (305 mg), Glomerella cingulata (290 mg), and Pleospora infectoria (145 mg).

Among the acidic amino acids Glutamic Acid yielded 665 mg of *Botryodiplodia theobromae* followed by *Pleospora infectoria* (190 mg), *Curvularia lunata* (130 mg), *Fusarium sulphureum* (120 mg) and *Glomerella cingulata* (100 mg). Aspartic Acid produced maximum amount of *Botryodiplodia theobromae* mycelium (430 mg) followed by *Pleospora infectoria* (100 mg), *Glomerella cingulata* (95 mg), *Curvularia lunata* (85 mg) and *Fusarium sulphureum* (70 mg).

#### DISCUSSION

Among mixtures of acidic and basic amino acids Arginine + Aspartic Acid is the best source of nitrogen for *Botryodiplodia theobromae. Fusarium sulphureum* utlized mitrogen most profitably from Arginine + Aspartic Acid, and *Glomerella cingulata* from Lysine + Aspartic Acid. Arginine + Aspartic Acid produced maximum growth of *Curvularia lunata*, while Asparagine + Aspartic Acid proved best source of nitrogen for *Pleospora infectoria*. Histidine + Glutamic Acid produced best growth of *Fusarium sulphureum*.

Among mixtures of basic amino acid Lysine + Asparagine proved best source of nitrogen for Fusarium sulphureum. Asparagine + Arginine was best, as a source of nitrogen, for Botryodiplodia theobromae. Curvalaria lunata utilized nitrogen in highest amount from Lysine + Asparagine, while Histidine + Arginine produced maximum growth of Glomerella cingulata. Lysine + Arginine was best source of nitrogen for Pleospora infectoria. With regard to the effect of mixture of acidic amino acids, Curvularia lunata yielded maximum mycelium on a mixture of Glutamic and Aspartic Acid than other fungi.

Among individual amino acids Asparagine and Histidine supported best growth of *Fusarium sulphureum*, while Aspartic Acid, Glutamic Acid, Arginine, and Lysine were the best source of nitrogen for *Botryodiplodia theobromae*. Basic and acidic amino-acids appear poor sources of nitrogen for fungi than neutral amino acids [8]. The present studies show that some fungi utilize better single amino acids as a source of nitrogen, other grow better in the mixture of acidic (acidic + acidic) and basic (Basic + Table 1. Effect of amino acids on the growth of various fungi.

AMINO ACIDS	F. sulphureum (mg)	C. lunata (mg)	B. theobromae (mg)	G. cingulata (mg)	P. infectoria (mg)
BASIC AMINO ACIDS (Individual)					
Asparagine	765	405	640	145	78
Arginine	360	300	410	186	165
Lysine	210	210	325	210	190
Histidine	380	320	305	290	145
ACIDIC AMINO ACIDS (Individual)					
Glutamic Acid	120	130	665	100	100
Asparatic Acid	70	85	430	95	100
BASIC AMINO ACID (Mixture)					
Lysine + Asparagine	1040	520	404	140	124
Asparagine + Histidine	975	484	295	85	100
Asparagine + Arginine	695	509	550	213	129
Lysine + Arginine	686	341	242	338	145
Histidine + Arginine	395	335	225	430	130
ACIDIC AMINO ACIDS (Mixture)					
Glutamic acid + Asparatic aci	d 105	810	293	120	140
ACIDIC & BASIC AMINO (A Mix ture)	CIDS)				
Arginine + Asparatic acid	1136	1080	1180	336	145
Asparagine + Asparatic acid	100	820	1130	183	186
Lysine + Asparatic acid	115	860	935	560	125
Histidine + Asparatic acid	55	115	370	400	70
Histidine + Glutamic acid	1160	750	751	145	100
Lysine + Glutemic acid	153	158	444	153	84
Arginine + Glutamic acid	131	337	851	175	80
Asparagine + Glumatic acid	150	276	546	128	135

basic) amino acids while still others utilize mixture of acidic and basic amino acids most profitably. Aspartic Acid was a poor source of nitrogen for fungi as compare to Glutamic Acid. This is in agreement with the work of others [7,8,10].

#### REFERENCES

- 1. P.G. Fothergill, and M.J. Jone, Gen. Microbiol, 298 (1958).
- 2. K.S. Bhargava, Indian Acad, Sci., B, 21, 344 (1945).
- 3. R Dayal, Proc. Natt. Acad. India, 31, 322 (1961).

- 4. R.A. Steinberg. Botan., Rev. 16, 208 (1950).
- 5. P.K. Stocks and B.Q. Ward, Can. J. Microbiol., 8, 761 (1962).
- S.S. Husain, S.A. Hasan and K. Zamir, Pak. J. Sci. Ind. Res., 10, 127 (1967).
- 7. S. S. Husain and K. Zamir, Sci. Ind., 5, 196 (1967).
- 8. K. Zamir, S. S. Husain Sci. & Ind., 7, 171 (1970).
- V.G. Lilly and H.L. Barnett. Physiology of fungi (McGraw Hill Book Company Inc., 1951), pp 97, 107, 117-120.
- 10. A.K. Sarbhooy, Can. J. Microbial., 11, 297 (1965).