The Free and Protein Amino Acids of *Clavariopsis aquatica*, de Wild

The morphology of the aquatic Hyphomycetes has been described by INGOLD[1] and some of their physiology by THORNTON[2] and THORNTON and FOX[3]. They grow readily in pure culture, are not subject to dense sporulation or pigmentation, their physiology appears similar to most fungi and are consequently convenient organisms for the study of general fungal physiology.

Recently THORNTON and McEVOY[4] have developed improved techniques of free amino acid extraction whilst HEATHCOTE and HOWARTH[5] have perfected methods of quantitative analysis of these substances. A more accurate study of the distribution of free and protein amino acids of the Hyphomycetes and other fungi would fill a gap in existing knowledge and it is with this matter that this paper is concerned.

Materials and methods. The growth medium and cultural conditions for *Clavariopsis aquatica* were as described by THORNTON and FOX[3]. Three different nitrogen sources were used, a) L-glutamic acid, 1 g + aspartic acid, 1 g; b) yeast extract, 2 g; c) $(NH_4)_2SO_4$, 1 g; d) $(NH_4)_2SO_4$, 1 g + sodium acetate, 3 g.

The mycelium was harvested, washed, freeze-dried and stored over $CaCl_2$. Amino acids were extracted from the mycelium by a modification of THORNTON and McEVOY[4]: 500 g of mycelium were ground with 10 ml butanone/6*N* HCl (10:1) for 10 min and the supernatant collected after centrifugation. The residue was washed first with butanone/HCl and then with water. All washings were collected and combined. The residue was then added to 10 ml boiling water for 10 min, centrifuged and the residue washed with boiling water. All extracts and washings were combined and reduced to dryness. DAVIES[6] has since shown that this procedure removes virtually all ninhydrin-active compounds. Insoluble protein residue was hydrolysed by a modification of BENT and MORTON's[7] method and the hydrolysate reduced to dryness. All residues were redissolved in 1 or 2 ml 65% ethanol.

A modification of SMITH's[8] method of desalting was found the most successful. 1 ml ethanolic extract was reduced to dryness, using an air stream, and the crude residue was treated with butanone/6*N* HCl (10:1) in three 2 ml aliquots. This extract was reduced to dryness and redissolved in 1 ml 65% ethanol. No ninhydrin activity was detected in the remaining residue.

Quantitative chromatography was carried out on cellulose TLC, as described by HEATHCOTE and HAWORTH[5]. Free amino acid extracts were loaded at 30 λ and protein amino acids, which did not need desalting, at 25 λ.

Results and discussion. 1. Free amino acid pools. After 13 days growth in media a) and b) the average yields per flask were 84 mg and 114 mg respectively but only 27 mg in c). Such poor growth has been attributed to disruption of the cytoplasmic membrane by low pH by BENT and MORTON[9] and/or cytoplasmic binding sites[10].

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Table I. Free amino acid pools detected in *Clavariopsis aquatica* on 3 different media

Nitrogen source	Aspartate/ Glutamate	Yeast extract	$(NH_4)_4SO_4$
Total amino acid present µg/500 mg mycelium	404.33	614.55	329.43
Amino acid	Percentage		
Arginine	10.2	13.6	8.3
Lysine	5.4	6.9	3.2
Histidine	0.7	3.9	–
Glycine	2.5	2.0	2.2
Glutamate	24.7	13.6	19.4
Aspartate	5.6	5.9	6.9
Serine	3.6	2.4	6.5
Alanine	23.1	25.4	33.8
Proline	5.4	4.2	4.2
Threonine	2.4	5.3	4.2
Valine	2.0	1.6	1.3
Methionine	2.9	2.1	1.7
Tyrosine	0.4	0.5	–
Phenylalanine	3.2	2.9	2.9
Leucine	2.0	2.8	1.8
Isoleucine	1.7	2.1	1.5
α-Aminoaedipate	0.2	0.4	–
Cysteic acid	0.8	0.7	–
Cysteine	0.5	2.4	–
Glutamine	2.6	2.4	2.0
3 unknowns	present	present	present
Total	99.9%	101.1%	99.9%

Table II. % amino acid composition of mycelial hydrolysate from 3 different media

Nitrogen source	Aspartate/ Glutamate	Yeast extract	$(NH_4)_2SO_4$
Total amino acid µg/mg mycelium	164.06	248.28	136.15
Amino acid	Percentage		
Arginine	5.8	3.8	5.3
Lysine	7.8	13.6	8.3
Histidine	0.1	0.3	–
Glycine	1.5	1.8	2.6
Glutamate	14.7	14.1	11.9
Aspartate	22.2	16.2	17.1
Serine	5.7	3.9	6.0
Alanine	10.6	10.3	11.8
Proline	2.1	2.6	1.8
Threonine	2.6	1.0	2.8
Valine	5.6	8.1	10.4
Metheonine	1.5	1.2	2.2
Tyrosine	2.1	1.5	1.5
Phenylanine	5.2	6.3	6.1
Leucine	10.8	11.6	9.9
Isoleucine	1.8	3.6	2.2
α-Aminoaedipate	–	–	–
Cysteic acid	–	–	–
Cysteine	–	–	–
Glutamine	–	–	–
2 unknowns	present	present	present
Total	100.1%	99.9%	99.9%

The final acidity was pH 3, but when medium (d) was used, including a buffer, no increase in ammonium ion uptake occurred, did not significantly affect the free amino acid pool and no reduction was detected in the extracellular amino acids found in the medium. It appears as if this species at least can withstand low pH values without marked interference with its metabolism. When the percentage composition of the free amino acid pools (Table I) are compared they are found to only partly agree with BENT and MORTON[9] in that they change in size but not greatly in composition in response to downward pH drift. There is no obvious relationship between the free pools of *Clavariopsis*, *Penicillium*[7], and *Mucor*[11], but there may be a relationship between the high concentration of alanine and lysine in *Clavariopsis* and *Heliscus submersus*[4] (both are aquatic Hyphomycetes) though CLOSE[12] suggests little hope of classification of fungi by free amino acid pool composition.

The proportions of protein amino acids (Table II) to free amino acids shows little relationship, especially aspartic acid which frequently occurs in low concentrations in the free pool but high in protein whilst the reverse is frequently true of alanine. This is in agreement with results for yeasts[13] *Penicillium*[7] and *Mucor*[11].

Table III. Amino acid composition of bulk protein of *Clavariopsis aquatica* compared with 2 organisms of GC 25%

	Mycoplasma[a] *mycoides*	*Clavariopsis*[b] *aquatica*	*Tetrahymena*[c] *pyriformis*
% Guanine-cytosine content of DNA	24.8	?	25.0
Stable amino acids[d]	Percentage amino acid composition		
Arginine	4.0	3.8	6.1
Lysine	14.3	13.6	11.9
Histidine	2.8	0.3	2.8
Glutamate	15.7	14.1	17.6
Aspartate	16.7	16.2	16.5
Alanine	9.9	10.3	10.3
Proline	5.4	2.6	5.1
Valine	7.2	8.1	8.9
Tyrosine	4.6	1.5	4.3
Phenylalanine	5.6	6.3	6.0
Leucine	12.1	11.6	11.7

[a] CHELTON et al.[15]. [b] Results for medium b. [c] SUEOKA[14]. [d] For explanation of this term see CHELTON et al.[15].

2. Protein amino acids. Table II shows, with few exceptions, good agreement between all percentage values of amino acids of bulk protein of *Clavariopsis* regardless of nitrogen source supplied. This is in agreement with the findings of BENT and MORTON[7] for fungi and SUEOKA[14] for various bacteria.

SUEOKA[14] has shown a relationship between percentage composition of guanine-cytosine (%GC) in DNA and amino acid composition of bulk protein for bacteria. This was further verified by CHELTON et al.[15]. According to SUEOKA[14], a positive correlation exists between %GC and % alanine, arginine and proline and an inverse correlation between % lysine, aspartate, glutamate, tyrosine and phenylalanine. If the results of SUEOKA[14] and CHELTON et al.[15] are compared with *Clavariopsis* (Table III) it corresponds closely to organisms with a %GC of 25, e.g. *Mycoplasma mycoides* and *Tetrahymena pyriformis*. The exceptions are tyrosine and proline.

In *Penicillium griseofulvum*[7] arginine, alanine and proline correspond to a GC ratio of 25%, but lysine, aspartate, tyrosine and luecine correspond to GC 70%. It would clearly be of interest to investigate %GC for *Clavariopsis* and many more fungi, particularly *Penicillium griseofulvum*[7], to determine the possibility of applying SUEOKA's[14] suggestions to fungi.

Résumé. On a trouvé que *Clavariopsis aquatica* tolère une grande acidité. Le pourcentage des amino-acides qui composent la protéine fongique a été très peu affecté par la source d'azote et n'a aucun rapport avec la composition de la masse commune des amino-acides libres. La pourcentage des amino-acides qui composent la protéine correspond à un pourcentage de guanine/cytosine de 25.

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Amine Oxidative System in *Tetrahymena pyriformis* W

In 1966, JANAKIDEVI et al.[1] found that the ciliated protozoan, *Tetrahymena pyriformis* W, possesses noradrenaline (NA) and adrenaline (A), while the flagellated protozoan, *Crithidia fasciculata*, has only NA. Further, in these protozoa, isotopically labelled precursors were incorporated into these catecholamines as in mammals. They also suggested that the decomposition of NA was due to monoamine oxidase in *C. fasciculata*. Based on these findings, BLUM[2] studied the effect of reserpine on *Tetrahymena* and found that it inhibited the growth and decreased the catecholamine content.

Previously, we reported that low concentration of α-adrenergic blocking agents promoted the growth of *Tetrahymena*, while β-adrenergic blockers were inhibitory[3,4]. Thus it was of interest to investigate the nature of the enzyme involved in catecholamine metabolism in *Tetrahymena*. This paper is on the presence and nature of an oxidative deamination system for catecholamine in *T. pyriformis*.

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