The Free Amino Acid Pools of Two Aquatic Hyphomycetes

Though the literature on bacterial amino acid pools is extensive, relatively few studies have been made for fungi and no reference has been found to the use of cellulose thin-layer chromatography techniques in this respect (shown by JONES and HEATHCOTE[1] to resolve unambiguously up to 23 amino acids on 1 chromatogram).

Two aquatic hyphomycetes, *Heliscus submersus* and *Tetracladium setigerum* (about which nothing is known concerning their free amino acid pools), were grown in the presence of ammonium sulphate, L-glutamic acid (a 'key' amino acid), and L-tyrosine (an amino acid commonly found in leaf litter – their natural habitat) as sole nitrogen source.

The growth medium was as follows: glucose, 8 g; KH_2PO_4, 1.0 g; $MgSO_4$, $7H_2O$, 0.2 g; $FeCl_3$ (Tech), 0.02 g; biotin, 5 μg; pantothenic acid, 5 μg; distilled water, 1 l. The pH was adjusted to 6.0. Nitrogen sources used separately were: glutamic acid, 1.94 g; tyrosine, 2.40 g; or ammonium sulphate, 0.88 g.

Cultures were inoculated with 2 drops of homogenized mycelium and grown in 50 ml of medium in 250 ml conical flasks on a rotary shaker at 23 °C.

Amino acid analysis: According to BENT and MORTON[2] the amino acid pools of fungi differ with the age of the mycelium, therefore analysis of the free amino acid pools was carried out at 2 stages in growth, namely 6 and 13 days from the date of inoculation.

Mycelium was filtered from the medium and was found to dry readily at 60 °C to constant weight. Free amino acids were then extracted by grinding 0.5 g of dried mycelium with 10 ml of 70% ethanol/water, repeated twice, centrifuged and evaporated to 1 ml in a rotary evaporator.

The methods of analysis were: (1) Paper chromatography, solvents: (a) (first direction) isopropanol-water-0.88 ammonia (7:2:1); (b) *n*-butanol-glacial acetic acid-water (12:5:3). Sheets were loaded with a 25 μl sample and developed, after running and drying, by cadmium ninhydrin solution (ARTFIELD and MORRIS[3]). (2) Thin-layer chromatography: cellulose powder, 300 μ thick, on 20 × 20 cm glass plates were run in (a) (first direction) isopropanol-formic acid-water (40:2:10) (VON ARX and NEHER[4]); (b) tertiary butanol-methyl ethyl ketone-0.88 ammonia-water (50:30:10:10) (JONES and HEATHCOTE[1]). Plates were loaded with 25 μl samples, run and developed using ninhydrin-collidine reagent (BRENNER and NIEDERWIESER[5]). Analysis for sulphur amino acids was performed using nitro-prusside reagent (SMITH[6]).

Amino acid identification was carried out using Rf values compared with the tables of JONES and HEATHCOTE[1] and HEATHCOTE and WASHINGTON[7], the use of internal and external standards, specific staining reactions and comparison of T.L.C. with paper chromatography.

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Nitrogen source:	Ammonium sulphate				Glutamic acid				Tyrosine			
Organism	*T. setigerum*		*H. submersus*		*T. setigerum*		*H. submersus*		*T. setigerum*		*H. submersus*	
Incubation time (days)	6	13	6	13	6	13	6	13	6	13	6	13
Glutamic acid	–	a	a	b	a	b	a	c	a	c	b	c
Aspartic acid	–	–	a	a	a	b	a	c	–	a	a	b
Alanine	a	–	b	–	a	c	a	c	a	b	c	c
Serine	a	a	a	a	a	b	b	b	–	a	a	b
Tyrosine	–	a	b	b	a	c	a	b	d	b	c	b
Phenylalanine	–	–	a	–	–	–	a	b	–	a	a	a
Leucine	–	–	a	–	–	–	a	b	–	a	a	a
Isoleucine	–	–	a	–	–	–	a	b	–	a	a	a
Arginine	–	–	–	–	a	c	–	–	–	b	–	–
Lysine	–	–	–	–	–	–	a	a	–	–	–	–
Histidene	–	–	–	–	a	b	a	b	–	–	c	c
Proline	–	–	–	–	a	c	a	c	–	b	a	b
Hydroxyproline	–	–	–	–	–	b	–	–	–	–	–	–
Tryptophane	–	–	–	–	–	–	a	a	–	–	a	a
Cysteine	–	–	a	a	a	a	a	b	–	a	a	b
Cystine	a	–	–	a	a	a	a	a	–	–	–	–
Methionine	–	–	–	–	a	b	–	a	–	–	a	–
Glycine	–	–	–	–	–	–	–	–	–	–	b	b
Valine	–	–	a	a	a	a	a	a	–	a	a	a
Threonine	–	–	–	–	a	b	a	c	–	a	a	a
γ-Amino butyric acid	–	–	–	–	a	b	a	b	–	–	–	a
α-Amino butyric acid	–	–	–	–	–	–	–	–	–	–	–	a
Ethanolamine	–	–	–	–	a	a	a	a	–	–	a	a
Unknowns	2	none	2	3	2	3	3	3	none	2	1	1
Total detected	3 (+ 2)	3	10 (+ 2)	7 (+ 3)	15 (+ 2)	16 (+ 3)	18 (+ 3)	19 (+ 3)	3	13 (+ 2)	17 (+ 1)	18 (+ 1)

–, Not detected; a, present at less than 1 μmol amino acid/250 mg dry weight mycelium; b, present at approx. 1 μmol amino acid/250 mg dry weight mycelium; c, present at greater than 1 μmol amino acid/250 mg dry weight mycelium; d, an exceptionally high concentration of tyrosine.

The results can be expressed semi-quantitatively by comparing spot size and intensity of staining reactions with standard amino acids which were loaded using 1 μl at a concentration of 0.05 m, dissolved in aqueous propan-2-ol (10% v/v). The results are expressed as less than, equal to, or greater than 1 μmol amino acid/250 mg dry weight mycelium.

Results and discussion. The 2 organisms grew well on all nitrogen sources, except that *T. setigerum* grew poorly on the medium containing ammonium sulphate. This low yield may have been induced by the acidity of the medium which fell to pH 3. Amongst a range of ninhydrin reactive compounds normally found in fungi (see Table) were hydroxyproline, ethanolamine and α-amino butyric acid, which have been reported only rarely (e.g. SIEGEL and CROSSAN[8], MURRAY and ZSCHEILE[9], FÜRST and WAGNER[10]).

Hydroxyproline is normally thought to be formed from proline. VOGEL and BONNER[11] have shown that *Neurospora* derives proline from glutamate and it is therefore noteworthy that *T. setigerum* contains extractable proline when grown for 6 days with glutamic acid, but not with tyrosine, as nitrogen source. Otherwise tyrosine proved to be as good a nitrogen source as glutamic acid, and was found to saturate the pool of *T. setigerum* after 6 days.

Throughout the experiments tyrosine, normally found at low concentrations in fungi, was always present in pool quantities similar to glutamic acid and may be of metabolic importance. The similar quantities of tyrosine and glutamic acid recorded after only 6 days may suggest a glutamate/tyrosine transaminase system similar to that reported by FIELDMAN and GUNSALUS[12] in *Escherichia coli* and by AMES and HORECHER[13] in *Neurospora crassa*.

Alanine and serine occurred with great regularity. In fact *T. setigerum* in the presence of ammonium sulphate produced both alanine and serine *before* the recognized 'key' amino acids glutamate and aspartate. Evidence for the biosynthesis of serine from a carbohydrate precursor has been reported, but mostly in animal tissue (for references see MEISTER[14]).

The small number of amino acids detected using *T. setigerum* fed with ammonium ion may resemble the results of SIMONART and CHOW[15], who detected few amino acids at low pH but normal pool sizes at larger pH using *Aspergillus oryzae*[16].

Résumé. Les masses communes des amino-acides libres chez *Tetracladium setigerum* et *Heliscus submersus* ont été analysées par des méthodes chromatographiques utilisant une couche mince de cellulose. Elles sont typiques pour la plupart des mycètes. Les amino-acides libres n'apparaissant que rarement ont été: l'hydroxyproline chez *T. setigerum* cultivé sur de l'acide glutamique, l'éthanolamine chez *T. setigerum* et *H. submersus* cultivé sur l'acide glutamique et enfin l'acide α-aminobutyrique chez *H. submersus* avec comme nutrition la tyrosine.

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The Distribution, Navigation and Orientation by the Sun of *Delphinus delphis* L. in the Western Mediterranean[1]

The distribution of the Mediterranean dolphin, *Delphinus delphis* Lin. (Figure 1) has not yet been investigated. If a ship leaves the Gulf of Lyon with a course to the west along the Spanish coast, schools of dolphins are seen only occasionally. They are seldom seen between Capo de la Nao and Capo de Palos. However, in the Bay of Almeria off the Capo de Gata, the schools increase in number until in the waters off Malaga they are numerous. Between Malaga and Gibraltar they are most frequent. In the Straits of Gibraltar in August 1966 and July 1967 we saw numerous schools not only of *D. delphis* but also of *Stenella styx* Gray (Euphrosine Dolphin). We also encountered polyspecific schools of these 2 species. *D. delphis* are often seen along the coast of Morocco (ALONCLE[2]). We have proved by biometric investigations that the Atlantic animals are larger than those found in the Mediterranean, so that we are lead to believe that there are 2 forms of the same species which do not cross the Straits into each other's territory.

The consistency of the appearance of the schools of *D. delphis* always in the same region leads us to believe that they are confined in the western Mediterranean to a certain territory. Because the expeditions have always taken place in the summer months, we are unable to say whether or not the territory is the same throughout the whole year, or if the population of the territory varies from one season to another.

In July and August, most of the schools include calves and none of the harpooned females were pregnant. No pairing behaviour was seen in July 1967 but very often in August 1966, so that we presume that mating takes place in September or October.

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