AMINO ACID COMPOSITION OF THE HETEROTHALLIC ASCOMYCETE, GELASINOSPORA AUTOSTEIRA

By

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AN ABSTRACT

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Abstract

The singly isolated ascospores of [redacted] were assayed microbiologically for ten amino acids. The spores were grown on a uniform medium and treated as nearly alike as possible throughout the experiment. Prior to assay, fat was extracted and nitrogen determined. [redacted] is heterothallic, consisting of two self-infertile strains, A and B. No conidia are produced and a culture containing only vegetative hyphae was obtained.

The results calculated to 16 percent nitrogen show no significant differences in the amino acid content of the cells.

Significant quantitative differences exist in the amino acid and protein content of the growth from singly isolated ascospores of [redacted] when expressed as percent of dry weight.

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INTRODUCTION

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Only in comparatively recent years has it been possible to make accurate and relatively easy quantitative determinations of the amino acids present in proteins. With tools such as microbiological assays at their disposal, research workers have attempted to define more exactly the nature of the cell protein of microorganisms. However, the attempts to determine the constancy of the amino acid content of bacteria and fungi under a given set of conditions have led to the appearance of a number of confusing and conflicting reports in the literature.

The first determination of amino acids in microorganisms dates back to the work of Abderhalden and Rona (1) in 1905. They isolated glycine, alanine, leucine, glutamic acid, and aspartic acid from Aspergillus niger irrespective of whether the nitrogen source in the growth media was potassium nitrate, glycine, or glutamic acid.

This observation was supported by the work of Tamura (14) in 1913 when he reported finding no significant difference in the amino acid composition of Mycobacterium laticola grown in nutrient broth, or as compared to the growth in a protein free medium consisting of mineral salts, ammonium lactate, asparagine, and glycerol.

The findings of these and of other early workers have not been taken too seriously by present day researchers, since the methods available to them for the determination of amino acids were neither highly

accurate nor specific. The introduction of more reliable quantitative methods has led several authors into conducting a more accurate and detailed investigation into microbial proteins. Camien, Salle, and Dunn (4) reviewed the work of previous analysts and conducted their own investigation on four lactobacilli and on Escherichia coli grown on two different media. Using a microbiological assay method, they were unable to find a significant difference in the amino acid composition of an organism, and concluded that the amino acid composition of the cell protein is nearly constant for defatted cells cultured on synthetic media consisting of widely varied constituents. This more accurate determination strongly supported the findings advanced by previous contributors.

Stokes and Gunness (13) provided comprehensive quantitative data on several microorganisms including bacteria, yeasts, and molds by assaying acid or alkali hydrolyzed cell material microbiologically. These studies were limited to the ten so-called essential amino acids and deal with the influence of cultural conditions on the quantitative content of these ten amino acids. Cultivation of a fungus under identical conditions yielded reproducible amino acid contents, furnishing evidence that the amino acid composition of an organism is, qualitatively and quantitatively, a stable and fixed characteristic property of the cell under fixed conditions of growth. By varying the growth conditions, quantitative differences greater than would be expected due to the inaccuracies of the method were noted. The evidence showed that the amino acid content of microorganisms was variable, and changed

with the nature of the medium, aeration conditions, and age of the cells.

The findings of Freeland and Gale (6) were directly contradictory to those of Stokes and Gunness (13) and tended to confirm the previous conclusions as to constancy of the amino acid content of microorganisms. Analyzing for arginine, tyrosine, lysine, histidine, and glutamic acid by means of a manometric specific decarboxylase method they found the amino acid composition of the protein of Escherichia coli and Aerobacter aerogenes to be unaffected by widely varying growth conditions.

Further confirmation of the conclusions of other investigators that the amino acid composition of microorganisms was constant for any one medium and one set of growth conditions was furnished by Dunlop (5) in an investigation of the synthesis of amino acids by Escherichia coli. In a second series of experiments, he found little difference in the amino acid composition of the cells of E. coli when grown under widely varying cultural conditions. However, the results of the latter experiments differed sufficiently from those of other investigators to suggest, to him, the possibility that the composition of the medium may affect the amino acid composition of the cells.

A microbiological assay of the amino acid content of selected Mycobacteria, done by Boniece (3), showed quantitative differences in the amounts present. The organisms, Mycobacterium phlei and Mycobacterium avium, were grown on media differing mainly in the source of nitrogen. The results obtained indicated a variation in the amino acid

and protein content of each test organism in response to changes in the substrate nitrogen.

Observations of the different cultural characteristics of the growth from singly isolated spores from a single ascus of Gelasinospora autosteira suggested the possibility that the chemical composition of the organism might not be the same for all the spores. This observation, coupled with the conflicting reports in the literature concerning the constancy of the amino acid composition of microorganisms led to the experiment presented in this thesis.

The Ascomycete, Gelasinospora autosteira, was isolated and described by Alexopoulos and Sung (2) in 1950. On corn meal agar, the mycelium consists of rapidly growing hyphae of various thicknesses. The colony is colorless at first, but in about a week a brown color begins to develop over the entire colony. Aerial mycelium when present abundantly takes on a faint pink color. Conidia and spermatia are unknown in the species.

The species consists of two self-incompatible strains, designated A and B. The cultures obtained from single ascospore isolations do not produce perithecia A cross between combinations of the same strain also fail to produce perithecia. Crosses of Strain A with Strain B develop perithecia along the line of contact between the two mycelia. The particular isolates used in this experiment formed perithecia when spores from Strain A, Nos. 1, 2, 5, and 6 were crossed with spores from Strain B, Nos. 3, 4, 7, and 8.

It was felt that the self-infertile strains might exhibit a variation in amino acid content which would parallel the strain differences; or that differences in the cultural characteristics would manifest themselves as changes in the composition of the cell. The isolated ascospores were therefore, grown under identical conditions, and treated as nearly alike as possible throughout the experiment. The defatted, hydrolyzed cell material was assayed microbiologically for ten amino acids.

A. PREPARATION FOR MICROBIOLOGICAL ASSAY

A. PREPARATION FOR MICROBIOLOGICAL ASSAY

The fungus selected for study was the Ascomycete, Gelasinospora autosteira. This organism is not known to produce conidia, and produces ascospores only after union with a different sex of the species. It was, therefore, possible to obtain a culture containing only vegetative hyphae. Single cell isolates of the eight spores from a mature ascus were germinated and used as stock cultures in the experiment. The spores were numbered according to the order of their appearance in the ascus, and the sexual strain of each was determined. Spores numbered 1, 2, 5, 6 were of Strain A, and spores numbered 3, 4, 7, 8 were of Strain B.

Filtered corn meal infusion broth, with 0.5 percent yeast extract, and 0.2 percent dextrose added, was used to grow a sufficient quantity of mycelium for assay. The medium was dispensed into 2000 ml Erlenmeyer flasks, 300 ml per flask. Inoculum was from a 48 hour culture grown on Difco corn meal agar plus the yeast extract and dextrose. Small discs were cut from the agar plates and floated on the surface of the broth. The flasks were incubated at room temperature for one week.

The cultures were harvested at the end of the seven day incubation-period. The mycelial mats were lifted undamaged from the Erlenmeyer flasks and dipped individually into distilled water to remove most of the adhering medium. The mycelia was then pooled in a liter beaker and washed several times with distilled water. The mats were then dried at 90 C. and subsequently finely ground for the following stages.

Fat extractions and nitrogen determinations were done on all isolates. Fat was extracted on continuous Soxhlet extractors with ether for eight hours. Micro-Kjeldahl methods were used in the determination of nitrogen.

B. MICROBIOLOGICAL ASSAYS

B. MICROBIOLOGICAL ASSAYS

The dried defatted hyphae were prepared for assay by hydrolysis to liberate the constituent amino acids. Acid hydrolysates for the determination of all acids except tryptophane were prepared by autoclaving one gm samples with 25 ml of 6N hydrochloric acid at 15 lbs pressure for eight hours. The tryptophane digest was prepared by autoclaving 500 mg samples with 16 ml of 4N sodium hydroxide (9). The samples were then neutralized and diluted to a final concentration of 10 mg of sample per milliliter of solution in volumetric flasks.

Each hydrolysate was assayed in duplicate at three levels of the standard curve. Preliminary assays were required to determine appropriate dilutions of the sample so that the values would fall on the nearly linear part of the standard curve. Levels of 1, 2, and 3 ml of the diluted sample were run in the final assay. The standard curve was determined in triplicate at each level. Increasing amounts of the standard solution of the amino acid in question were added to the series of tubes. Distilled water was added to all tubes to bring the volume of each up to five ml.

Stock solutions of the amino acids, nitrogen bases, salts and vitamins were used in the preparation of the basal medium. The composition of the basal medium and the organism used for the assay are given in Tables II-V. The basal medium, minus the amino acid being assayed, was added to the standard and to the unknown solutions, five ml of

medium per tube. The tubes, 18 x 150 mm, were capped and autoclaved at 121 C for 10 minutes.

The assay, as set up, was inoculated with the proper assay organism; and incubated at 37 C for 72 hours. The assay organisms, Lactobacillus arabinosus 17-5 (ATCC 1814), and Leuconostoc mesenteroides P-60 (ATCC 8042), were carried as stab cultures on a solid medium. Fresh stock cultures were prepared weekly. To prepare the organism as inoculum, broth subcultures were incubated for 12-18 hours, centrifuged, washed once with saline, and resuspended in saline. The saline suspension of the organism was added to the assay tubes by means of a burette, one drop of culture per tube. The broth subcultures were carried on a medium of the following composition.

Bacto peptone	0.8 %
Yeast extract	0.1
Na acetate (anhyd.)	0.1
Glucose	1.0
K_2HPO_4	0.05
KH_2PO_4	0.05
$MgSO_4.7HOH$	0.02
NaCl	0.001
$FeSO_4.7HOH$	0.001
$MnSO_4.HOH$	0.001

Stock cultures were carried on a medium of the same composition plus one percent agar.

After incubation the relative amounts of acid produced were determined. The contents of the tubes were rinsed into beakers with distilled water and titrated to pH 7.0 electrometrically using N/10 sodium hydroxide. Plotting milliliters of sodium hydroxide as ordinates and micrograms of the standard amino acid as abscissas the standard

curve was drawn. The amino acid content of the samples was determined by averaging the results obtained from each hydrolysate and at each level of the standard curve. The amounts of amino acids present were calculated to 16 percent nitrogen and recorded in Tables VI and VII. Recalculated to percent of dry weight the values are presented in Tables VIII and IX.

TABLE I

STOCK SOLUTIONS USED IN PREPARATION OF BASAL MEDIA

Solution	Conc. mg/ml	Solution	Conc. mg/ml
H_2O_2 treated peptone	50	Salts A	
		K_2HPO_4	100
Casein Hydrolysate	100	KH_2PO_4	100
DL- - Alanine	10	Salts B	
		$MgSO_4.7HOH$	40
L(+) Arginine. HCl	10	$FeSO_4.7HOH$	2
		$MnSO_4.4HOH$	2
L-Asparagine	20	NaCl	2
L(-)-Cystine	5	Adenine, Guanine, Uracil	
		Uracil	1
L(+)-Glutamic Acid	20	Adenine sulfate.2HOH	1
		Guanine HCl.2HOH	1
Glycine	10		
		Xanthine	5
L(+)-Histidine.HCl.HOH	10		
		Vitamin Solution	
DL-Isoleucine	10	Thiamin Hydrochloride	50
		Pyridoxine Hydrochloride	100
DL-Leucine	5	DL-Calcium Pentothenate	50
		Riboflavin	50
L(+)-Lysine.HCl.HOH	10	Nicotinic Acid	100
DL-Methionine	10	Para-Amino-Benzoic-Acid	10
DL-Phenylalanine	10	Biotin	0.5
L(-)-Proline	10	Folic Acid	100
DL -Serine	10		
DL-Threonine	10		
DL-Tryptophane	10		
L(-)-Tyrosine	10		
DL-Valine	10		

TABLE II

BASAL MEDIUM FOR ASSAY OF METHIONINE AND CYSTINE (10)
MEDIUM (PER 500 ML)

Component	Wt.		Component	Wt.	
H_2O_2 treated peptone	7.5	gm	NaCl	10	mg
L (-)-Cystine	100	mg	Adenine Sulfate.2HOH	10	
DL-Methionine	100		Guanine.HCl.2HOH	10	
DL-Tryptophane	100		Uracil	10	
L(-)-Tyrosine	100		Thiamine.HCl	1.0	
Glucose	20	gm	Pyridoxine.HCl	2.0	
Na acetate (anhyd.)	12		DL-Ca Pantothenate	2.0	
NH_4Cl	6		Riboflavin	2.0	
KH_2PO_4	500	mg	Nicotinic Acid	2.0	
K_2HPO_4	500		PABA	0.01	
$MgSO_4.7HOH$	200		Biotin	0.005	
$FeSO_4.7HOH$	10		Folic Acid	0.0015	
$MnSO_4.4HOH$	10				

Assay organism: Leuconostoc mesenteroides P-60 (ATCC 8042)

pH 6.9-7.0

TABLE III

BASAL MEDIUM FOR ASSAY OF GLYCINE, LYSINE, AND PROLINE (11)
MEDIUM (PER 500 ML)

Component	Wt.	Component	Wt.
DL- -Alanine	200 mg	Na acetate (anhyd.)	20 gm
L(+)-Arginine.HCl	100	KH_2PO_4	500 mg
L-Asparagine	200	K_2HPO_4	500
L(-)Cystine	200	$MgSO_4.7HOH$	200
L(+)-Glutamic Acid	400	$FeSO_4.7HOH$	10
Glycine	100	$MnSO_4.4HOH$	10
L(+)-Histidine.HCl.HOH	100	NaCl	10
DL-Isoleucine	200	Adenine Sulfate.2HOH	10
DL-Leucine	200	Guanine.HCl.2HOH	10
L(+)-Lysine.HCl.HOH	200	Uracil	10
DL-Methionine	200	Xanthine	10
DL-Phenylalanine	100	Thiamine.HCl	0.05
L(-)-Proline	50	Pyridoxine.HCl	1.0
DL-Serine	200	DL-Ca Pantothenate	0.50
DL-Threonine	200	Riboflavin	0.50
DL-Tryptophane	100	Nicotinic Acid	1.0
L(-)-Tyrosine	100	PABA	0.10
DL-Valine	200	Biotin	0.001
Glucose	20 gm	Folic Acid	0.01

Assay Organism: Leuconostoc mesenteroides P60 (ATCC 8042)

pH 6.8-7.0

TABLE IV

BASAL MEDIUM FOR ASSAY OF LEUCINE, ISOLEUCINE,* PHENYLALANINE, AND VALINE* (12) MEDIUM (PER 500 ML)

Component	Wt.	Component	Wt.
DL- -Alanine	200 mg	Na acetate (anhyd.)	20 gm
L(+)-Arginine.HCl	50	KH_2PO_4	500 mg
L-Asparagine	200	K_2HPO_4	500
L(-)-Cystine	100	$MgSO_4.7HOH$	200
L(+)-Glutamic Acid	400	$FeSO_4.7HOH$	10
Glycine	20	$MnSO_4.4HOH$	10
L(+)-Histidine.HCl.HOH	50	NaCl	10
DL-Isoleucine	200	Adenine Sulfate.2HOH	10
DL-Leucine	200	Guanine.HCl.2HOH	10
L(+)Lysine.HCl.HOH	200	Uracil	10
DL-Methionine	100	Xanthine	10
DL-Phenylalanine	100	Thiamine.HCl	0.50
L(-)-Proline	50	Pyridoxine.HCl	1.0
DL-Serine	50	DL-Ca Pantothenate	0.50
DL-Threonine	200	Riboflavin	0.50
DL-Tryptophane	50	Nicotinic Acid	1.0
L-Tyrosine	50	PABA	0.10
DL-Valine	200	Biotin	0.001
Glucose	20 gm	Folic Acid	0.01

Assay Organism: Lactobacillus arabinosus 17-5 (ATCC 1814)

pH 6.6-6.8

* plus 10 ml tomato eluate (8)

TABLE V

BASAL MEDIUM FOR ASSAY OF TRYPTOPHANE (7)
MEDIUM (PER 500 ML)

Component	Wt.	Component	Wt.
Casein hydrolysate	5.0 gm	Adenine sulfate.2HOH	10 mg
L(-)-Cystine	200 mg	Guanine	10
Glucose	20 gm	Uracil	10
Na acetate (anhyd.)	20	Thiamine.HCl	0.10
KH_2PO_4	500 mg	Pyridoxine.HCl	0.10
K_2HPO_4	500	DL-Ca Pantothenate	0.10
$MgSO_4.7HOH$	200	Riboflavin	2.0
$FeSO_4.7HOH$	10	Nicotinic Acid	0.40
$MnSO_4.4HOH$	10	PABA	0.10
NaCl	10	Biotin	0.0002

Assay Organism: Lactobacillus arabinosus 17-5 (ATCC 1814)

pH 6.6-6.8

TABLE VI

AMINO ACID CONTENT OF STRAIN A
CALCULATED TO 16% NITROGEN

Component	Spore Number			
	1	2	5	6
Nitrogen	6.43	6.57	4.87	6.89
Protein (Nx6.25)	40.21	41.06	30.45	43.05
Cystine	0.48	0.49	0.47	0.46
Glycine	3.6	3.8	3.3	3.6
Isoleucine	4.9	4.9	4.8	4.8
Leucine	6.7	6.7	6.1	6.4
Lysine	6.3	6.5	6.0	6.4
Methionine	1.8	1.9	1.8	1.9
Phenylalanine	3.6	3.7	3.4	3.8
Proline	4.2	4.3	4.3	4.2
Tryptophane	0.45	0.44	0.36	0.45
Valine	6.0	6.1	5.9	5.6

TABLE VII

AMINO ACID CONTENT OF STRAIN B
CALCULATED TO 16% NITROGEN

Component	Spore Number			
	3	4	7	8
Nitrogen	7.20	7.16	5.30	7.07
Protein (Nx6.25)	45.00	44.75	33.13	44.19
Cystine	0.44	0.45	0.46	0.44
Glycine	3.8	3.7	3.3	3.5
Isoleucine	4.4	4.4	4.5	4.6
Leucine	6.6	6.1	6.1	6.1
Lysine	6.3	6.1	6.2	6.3
Methionine	1.9	1.8	1.8	1.8
Phenylalanine	3.5	3.3	3.4	3.7
Proline	4.0	4.0	4.4	4.1
Tryptophane	0.41	0.38	0.36	0.41
Valine	5.8	5.6	5.5	5.5

TABLE VIII

AMINO ACID CONTENT OF STRAIN A
CALCULATED TO PERCENT DRY WEIGHT

Component	Spore Number			
	1	2	5	6
Nitrogen	6.43	6.57	4.87	6.89
Cystine	0.19	0.20	0.15	0.20
Glycine	1.5	1.6	1.0	1.6
Isoleucine	2.0	2.0	1.5	2.1
Leucine	2.7	2.7	1.9	2.8
Lysine	2.5	2.7	1.8	2.8
Methionine	0.74	0.76	0.54	0.82
Phenylalanine	1.4	1.5	1.0	1.6
Proline	1.7	1.8	1.3	1.8
Tryptophane	0.18	0.18	0.11	0.19
Valine	2.4	2.5	1.8	2.4

TABLE IX

AMINO ACID CONTENT OF STRAIN B
CALCULATED TO PERCENT DRY WEIGHT

Component	Spore Number			
	3	4	7	8
Nitrogen	7.20	7.16	5.30	7.07
Cystine	0.20	0.20	0.15	0.19
Glycine	1.7	1.7	1.1	1.5
Isoleucine	2.0	2.0	1.5	2.0
Leucine	3.0	2.7	2.0	2.7
Lysine	2.9	2.7	2.0	2.8
Methionine	0.81	0.78	0.58	0.81
Phenylalanine	1.6	1.5	1.2	1.6
Proline	1.8	1.8	1.5	1.8
Tryptophane	0.19	0.17	0.12	0.18
Valine	2.6	2.5	1.8	2.5

DISCUSSION

DISCUSSION

The data obtained in the experiment, calculated to percent protein, support the statements of previous investigators that the same organism grown under the same conditions will have the same amino acid content. Considering the value of each spore as a separate determination of the same organism, the average value of the amino acid content falls within the experimental error of the microbiological assay method. This is also true when a mean value is determined for the four spores of each strain, A and B. Strain A has a slightly higher content of each amino acid, with the exception of glycine, than has Strain B. The differences between the two strains are not significant, however.

Those spores which have a low nitrogen content, as would be expected, also have a low amino acid content. The lowered nitrogen content is closely paralleled by the lowered content of the amino acids so that the ratio of the constituent amino acids to protein is essentially the same. This can be more readily noted when the amino acid content is expressed as percent of the dry weight of defatted mycelium.

Recalculated to percent dry weight the similarity in amino acid content is no longer obvious. Although there is again no significant difference between the average values of the two self infertile strains of the organism, differences among the individual spores are apparent. All spores but numbers 5 and 7 are of essentially the same composition. These spores with their very low nitrogen content, differ widely from

the others. Spore 7 ranges from approximately a 20 percent lower proline content to a 30 percent deficit in glycine. The mycelia from the fifth spore are still lower, ranging from 25 percent lower in cystine to 39 percent lower in tryptophane than the average figures for the other spores, 5 and 7 being excluded from the average.

The low content of the amino acids cannot be ascribed to differences in the strain. Since there are no significant differences between spores 1, 2, and 6 of Strain A and spores 3, 4, and 8 of Strain B, the variance between spores 5 and 7 must be due to another factor beside strain difference.

The slow rate of growth may offer a partial explanation of the low nitrogen and amino acid content of the spores. At the end of the 7 day incubation period the growth was very scant covering less than one-third of the diameter of the culture flask. The mycelial mats exhibited all the normal, cultural, and morphological characteristics as the growth from the other spores except for rate of growth. All other cultures completely covered the surface of the corn meal infusion broth in 5 days or less. The faster growing cultures are typical of the species, and were also typical of spores 5 and 7 upon primary isolation. Dissociation of these spores occurred to such an extent, however, that the colonies were no longer typical.

In the process of dissociation the organism may have lost some of its ability to metabolize the nitrogenous constituents of the medium resulting in a lower protein content. A lack of certain enzymes which would result in the failure to synthesize cellular proteins would be a

two way mechanism leading to the observed discrepancies. The intracellular enzymes of the organism add to the sum total of amino acids present, and their lack would contribute to an unknown, and probably very small, portion of the deficiencies. The accompanying lessening of nitrogen metabolism would account for a much larger fraction.

SUMMARY

SUMMARY

1. No changes in the amino acid composition of cellular proteins are noted in the mycelium from singly isolated ascospores when calculated to percent protein.

2. Significant quantitative differences exist in the amino acid and protein content of the growth from singly isolated ascospores of Gelasinospora autosteira when expressed as percent of dry weight.

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