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Amino Acid Composition of Wood-rotting Fungi (*Pleurotus*) and Total Amino Acid Balance of the Cultivating System

Anastázia Ginterová & Anna Lazarová

Feedstuffs Research Institute, 900 28 Ivanka pri Dunaji, Czechoslovakia

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ABSTRACT

Five strains of wood-rotting fungi of Pleurotus *sp. were studied to determine amino acid balance after 11 weeks of growing and fructification on barley straw. In two of the strains a positive balance was observed: in fruit bodies plus spent substrate, a considerably higher content of amino acids in comparison with initial substrate was found. The fruit bodies of these strains contain more proteins than the strains with balanced or negative amino acid balance. Atmospheric nitrogen is likely to be a source of amino acid nitrogen in the strains with a positive balance.*

INTRODUCTION

There has been a considerable endeavour to find new protein sources in recent years. One way is the cultivation of the wood-rotting fungi, *Pleurotus*, on lignocellulose materials. *Pleurotus ostreatus*, *P. pulmonarius*, *P. eryngii* and other fungi have been cultivated and investigated (Zadražil, 1979; Zadražil & Brunnert, 1981; Eger-Hummel, 1984).

Studies on the amino acid content of fruit bodies of different *Pleurotus* strains have been published (Bano *et al.*, 1963; Ginterová & Maxianová, 1975).

The ability of *Pleurotus* to fix atmospheric nitrogen has also been demonstrated (Ginterová, 1973; Ginterová & Maxianová, 1975; Ginterová & Gallon, 1979; Thayumanovan, 1980).

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The aim of the present study was to establish the composition and amino acid balance of some *Pleurotus* strains.

EXPERIMENTAL

Biological material

We used strains from the collection of our institute: No. 096, *P. ostreatus* from Florida, obtained in 1974, selected for yield; No. 133, *P. ostreatus* from Florida, obtained in 1975, selected for fructification at higher temperatures; No. 159, *P. ostreatus*, isolated from natural sources in 1970; No. 014, hybrid, *P. ostreatus* × *P. ostreatus* from Florida, obtained in 1976 from the Cathedra of Genetics, University of Alberta, by J. Weijer and No. 030, *P. sajor caju*, obtained in 1978.

Methods

Barley straw cut to particles smaller than 2 cm was dried to constant weight, 50 g was weighed into a polypropylene bag, wetted to 80% water content by tap water and sterilized twice in an autoclave for 60 min at 24 h intervals at a pressure of 120 kPa. Cooled straw, after the second sterilization, was inoculated with a mycelium. From each of the experimental variants, five parallel samples were prepared. The inoculated bags were closed by a cottonwool plug and grown in an incubator at 27°C for 3 weeks.

An 8-week fructification period under the natural thermal and humidity conditions (August–September) was used. Fruit bodies, gathered in three waves, as well as spent substrate, were dried at 105°C to constant weight, homogenized and hydrolyzed in 6M hydrochloric acid for 24 h at 105°C in sealed tubes (Davies & Thomas, 1973). The amino acids were estimated in fruit bodies, spent substrate and in the original straw by means of an automatic amino acid analyzer MIKROTECHNA T 339 (Czechoslovakia) according to Moore & Stein (1963). Sensory evaluation of the fruit bodies according to Kiermeyer & Haewecker (1972), by a 100 point system, was used.

RESULTS AND DISCUSSION

In Table 1 values of the average amino acid content of fruit bodies of separate strains are given. They are calculated on the basis of the fruit

TABLE 1
Average Amino Acid Contents of Fruit Bodies of Various *Pleurotus* Strains (grams/100 gram dry matter)

Amino acid	*Strain Nos.*				
	096	*133*	*159*	*014*	*030*
Aspartic acid	0·945	1·334	2·645	0·911	1·641
Threonine	0·431	0·595	1·240	0·434	0·750
Serine	0·432	0·591	1·082	0·413	0·730
Glutamic acid	1·135	1·823	3·233	0·889	2·137
Proline	0·497	0·671	3·641	0·684	0·832
Glycine	0·243	0·364	0·736	0·248	0·464
Alanine	0·605	0·848	1·347	0·707	1·001
Valine	0·436	0·601	1·268	0·475	0·775
Methionine	0·096	0·086	0·502	0·118	0·154
Isoleucine	0·334	0·467	0·922	0·397	0·578
Leucine	0·769	1·033	2·387	0·786	1·400
Tyrosine	0·414	0·646	1·580	0·394	0·751
Phenylalanine	0·346	0·535	1·209	0·447	0·680
Histidine	0·382	0·447	1·178	0·420	0·620
Lysine	0·537	0·642	1·885	0·616	1·079
Arginine	0·448	0·099	2·149	0·570	0·759
Total	8·050	10·782	27·004	8·509	14·351

bodies' weight and their amino acid content in individual waves:

$$SA = x_1 \times a_1 + x_2 \times a_2 + x_3 \times a_3$$

where: SA is average amino acid content; x_1 is the weight fraction of fruit bodies in the first wave; x_2 is the weight fraction of fruit bodies in the second wave; x_3 is the weight fraction of fruit bodies in the third wave; a_1 is the amino acid content in g/100 g dry matter in fruit bodies of the first wave; a_2 is the amino acid content in g/100 g dry matter in fruit bodies of the second wave and a_3 is the amino acid content in g/100 g dry matter in fruit bodies of the third wave.

The fruit bodies of the naturally isolated *P. ostreatus* strain (159) had a total amino acid content 2–3 times higher than other strains.

Table 2 compares the amino acid composition of the substrate (straw) and of *Pleurotus* fruit bodies (averages of all strains). In comparison with straw the fruit bodies had higher contents of proline, methionine, lysine and arginine. On the other hand, the contents of glutamic acid and glycine were lower.

Table 3 gives the total amino acid balance after 11 weeks of growing and

TABLE 2
Amino Acid Composition of Straw and Fruit Bodies (relative percentage)

Amino acid	Fruit bodies		Straw
	Average	Standard deviation	
Aspartic acid	11·2	0·99	13·0
Threonine	5·16	0·35	5·94
Serine	4·96	0·59	5·74
Glutamic acid	13·7	2·52	17·1
Proline	7·94	3·22	4·36
Glycine	3·05	0·26	4·45
Alanine	7·13	1·29	8·27
Valine	5·33	0·36	6·10
Methionine	1·26	0·40	0·25
Isoleucine	4·12	0·46	4·36
Leucine	9·39	0·36	11·1
Tyrosine	5·37	0·56	4·31
Phenylalanine	4·75	0·38	5·55
Histidine	4·50	0·33	3·56
Lysine	6·87	0·60	3·17
Arginine	5·29	2·66	2·73

fructification in individual *Pleurotus* strains (total amino acids in fruit bodies plus those in spent substrate). *P. ostreatus* 159 isolated from nature shows a distinct positive balance: its fruit bodies and spent substrate contain 68% more total amino acids than the initial substrate. *P. sajor caju* 030 has a slightly positive balance (16%). The other three strains show an almost balanced or negative total amino acid balance.

Table 4 shows the amino acid balance after 11 weeks of growing and fructification in individual *Pleurotus* strains, compared with the amino acid

TABLE 3
Amino Acid Balance in Growing and Fructification of *Pleurotus* Strains. The Initial Amount of Straw contained 969 mg of Amino Acids (100%)

Strain No.	Total amino acids (mg)		Amino acid balance	
	Fruit bodies	Spent substrate	(mg)	(%)
096	496	482	+9	+0·93
133	454	421	−94	−9·70
159	887	741	+659	+68·01
014	333	445	−191	−19·67
030	599	528	+158	+16·31

TABLE 4
Balance of Individual Amino Acids after the Fructification Process. The Amino Acid Changes are expressed in Per Cent Relative to the Amount of Each Amino Acid in Initial Substrate

Amino acid	*Strain No.*				
	096	*133*	*159*	*014*	*030*
Aspartic acid	−3·18	−2·95	+42·2	−12·2	+24·0
Threonine	−11·8	−9·96	+100·0	−16·7	−7·17
Serine	−12·8	−8·81	+20·3	−19·0	+17·1
Glutamic acid	−25·3	−16·6	+15·3	−35·1	+6·88
Proline	+31·6	+28·4	+319·0	+41·2	+99·8
Glycine	−21·6	−17·7	+16·5	−23·0	+7·87
Alanine	−16·2	−9·09	+17·6	−13·2	+11·8
Valine	−14·5	−7·99	+35·1	−16·2	+20·3
Methionine	+195·0	+116·0	+1 970·0	+242·0	+290·0
Isoleucine	−8·29	−4·47	+142·0	−9·60	+22·0
Leucine	−14·1	−14·4	−26·8	−15·3	+22·4
Tyrosine	−11·6	−0·21	+66·7	−18·4	+48·9
Phenylalanine	−26·4	−19·5	+29·4	−60·4	+21·6
Histidine	+20·6	+10·1	+112·0	+23·7	+0·00
Lysine	+47·6	+42·4	+156·0	+49·1	+134·0
Arginine	+43·6	−69·3	+193·0	+48·6	+90·7

$$X = \frac{\text{amino acids (fruit bodies + spent substrate)} - \text{amino acids (straw)}}{\text{amino acids (straw)}} \times 100$$

content in the initial substrate. All the *Pleurotus* strains synthesize proline, methionine, histidine, lysine and arginine. In 096, 133 and 014 strains these amino acids were synthesized from other amino acids of the initial substrate, because the total amino acid balance of these strains is balanced or slightly negative (Table 3). On the other hand, *P. ostreatus* 159 shows an increase of all amino acids with the exception of leucine. *P. sajor caju* 030 also shows increases of all amino acids except threonine and histidine.

The results show that the two strains from the naturally isolated *P. ostreatus* 159 and *P. sajor caju* 030 are significant producers of amino acids, including essential amino acids (histidine, isoleucine, lysine, methionine, phenylalanine, threonine and valine). The amino acid balance showed that the initial source of nitrogen for these amino acids, synthesized from straw, was insufficient; therefore, these strains probably use atmospheric nitrogen (Ginterová & Maxianová, 1975; Rangaswy *et al.*, 1975; Ginterová & Gallon, 1979; Thayumanovan, 1980).

The positive nitrogen balance is, in fact, even more emphatic because, in our balance studies, we did not consider glucosamine nitrogen.

Glucosamine constitutes almost one-third of the total *Pleurotus* fruit body nitrogen in which it forms a cellular membrane structure (O'Brien & Ralph, 1966).

A sensory evaluation of fruit bodies completed the study. The fruit bodies of the strains with a positive nitrogen balance (*P. ostreatus* 159 and *P. sajor caju* 030) have the best flavour, resembling meat. Fruit body flavour of the strains 014, 096 and 133 was less distinct. On the other hand, the texture of the fruit bodies showed a reverse trend. It seems that the fruit body texture reflects a nitrogen economy of the organism. The fruit bodies of the strains with positive nitrogen balance have a more solid, more consistent, structure.

From the genetic point of view it is interesting that the ability of atmospheric nitrogen fixation was not transferred from the winter strain of *P. ostreatus* to the hybrid strain 014.

CONCLUSIONS

Five production strains of *Pleurotus* synthesized proteins of almost equal quality but in different quantity. The quantity of the synthesized proteins is probably connected with an ability to fix atmospheric nitrogen. The strains not fixing atmospheric nitrogen synthesize some amino acids from other amino acids of the substrate (straw) to build up fruit body proteins of given qualitative composition. These strains have a high yield of fruit bodies (weight) but their protein content is lower than that of the strains with positive amino acid balance. From the sensory point of view the fruit bodies of the strains with positive amino acid balance are considered to be the better ones. The strains with balanced or negative amino acid balance have not such an expressive flavour but their texture is finer.

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