Protein Content and Amino Acid Composition of Certain Fungi
Evaluated for Microbial Protein Production

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The protein and total amino acid contents of four mycelial fungal strains and one yeast were approximately the same for cultures harvested in the mid-log and early stationary growth phases. It was found that Fusarium oxysporum and Fusarium moniliforme contained approximately 30% more protein and total amino acids than Aspergillus niger. The amino acid composition of mycelial protein compares favorably with that of British Petroleum yeast protein Toprina produced commercially on hydrocarbon substrates. Fusarium spp. may be suitable for commercial production of microbial protein, especially when low-cost agricultural or industrial waste products are readily available as energy sources. Genetic manipulation of these fungi, such as induction of mutant strains through irradiation, may be desirable to obtain a mycelial product of improved yield and/or quality.

Yeast of the genus Candida are most commonly used for the production of microbial protein (1, 2, 10). There are, however, claims in the literature that mycelial fungi possess certain characteristics, such as texture, fast rate of growth, lower nuclear acid content, and ease of harvesting, which make them preferable in particular cases (12). Among mycelial fungi, species of Aspergillus and Fusarium are among those studied in this regard (9, 13). The suitability of an organism for commercial protein production should be based on its protein content, determined as accurately as possible, and its amino acid composition, in addition to the efficiency of converting substrate carbon and inorganic nitrogen into organic nitrogenous compounds. Moreover, the palatability of the product should be tested in animal trials. In this paper the protein content and amino acid composition of mycelia of two strains of Aspergillus niger and one strain each of Fusarium oxysporum and Fusarium moniliforme are compared with those of Candida tropicalis to obtain information on the possibility of these three mycelial species to compete with the yeast for protein production.

MATERIALS AND METHODS

Organisms. A. niger was isolated from air-borne conidia and maintained on slants containing Aspergillus complete medium (7). A. niger mutant 70 is one of 94 mutants obtained after ultraviolet irradiation. It is a purely mycelial mutant. It produces neither conidia nor pigment and is a very fast-growing organism. Under optimal growth conditions it attains a growth plateau within 20 h after inoculation, whereas A. niger wild type needs 33 h to reach the same growth phase. C. tropicalis, F. oxysporum, and F. moniliforme from our stock cultures were maintained on potato dextrose agar slants.

Growth medium. The organisms were grown in liquid Aspergillus complete medium containing (per liter): glucose 10 g; NaNO₃ 6 g; KH₂PO₄ 1.52 g; MgSO₄·7H₂O 0.52 g; KCl, 0.52 g; peptone (Difco), 2 g; yeast extract, 1 g; Casamino Acids, 3 g; and hydrolyzed nucleic acid, 0.5 g (7).

Inoculation and incubation. Aspergillus was grown in 1-liter Erlenmeyer flasks containing 250 ml of liquid complete medium. These flasks were inoculated with 20 5-mm agar disks cut with a cork borer from the periphery of young, actively growing agar cultures and incubated for 24 h at 30°C in a rotary shaker operating at 180 rpm. At the end of the incubation period, the liquid medium was strained away through cheese cloth and the spheres of mycelial growth were transferred into a Waring blender containing 50 ml of fresh Aspergillus complete medium and blended at medium speed for 6 min. In this way a uniform suspension at propagules was obtained. All operations were carried out under aseptic conditions. Ten milliliters of the resultant mycelial suspension was used to start new flask cultures by inoculating 1-liter Erlenmeyer flasks containing 250 ml of liquid Aspergillus complete medium. The flasks were then incubated in a rotary shaker at 30°C and 180 rpm. Fusarium spp. were grown in the same way, but with no blending. Under liquid shake culture conditions, Fusarium spp. produce no mycelial mats, but they produce a very fine suspension of small thalli, which was used directly for inoculation. In the case of C. tropicalis, 50 ml of the liquid medium, dispensed in 500-ml Erlenmeyer flasks, was inoculated with 0.1 ml of cell suspension (18 µg [dry weight]) taken from
a liquid shake culture at the mid-log phase of growth. After inoculation, the flasks were incubated at 30°C in a rotary shaker-incubator.

**Harvesting and drying of biomass.** Mid-log and early stationary phase mycelia were harvested by filtration through a Whatman no. 1 filter paper in a suction funnel. The mycelia were thoroughly washed and freeze-dried in a Virtis model 10-146MR-BA freeze drier for 24 h. C. tropicalis cells were harvested by centrifugation at 3,000 rpm for 10 min. The freeze-dried material was ground in a mortar to a very fine powder and kept desiccated to be used for analysis.

**Nitrogen and protein determination.** Total nitrogen was determined using a Coleman model 29 nitrogen analyzer. The protein content of the samples was measured by the modification of the Folin phenol method described by Lowry et al. (6), and also by the Biuret method (4).

**Extraction of free amino acids.** Freeze-dried mycelial powder (50 g) was extracted with 50 ml of distilled water with continuous shaking for 10 min at room temperature. This procedure was found in preliminary experiments to be just as efficient in extracting free amino acids as those using boiling water and hot 5% trichloroacetic acid. The suspension was centrifuged and the supernatant was collected. The pellet was washed twice with 50 ml of distilled water. The combined supernatant was evaporated to dryness under reduced pressure at 45°C. The residue was taken up in 10 ml of distilled water and passed through a Dowex-50 cation exchange column. After elution with 1 N NH₄OH, the eluate was evaporated to dryness and washed three times with small portions of distilled water to remove the remaining NH₄OH. Finally, it was taken up in 5 ml of 0.1 M sodium citrate buffer, pH 2.2. An aliquot of 1 ml was used for analysis.

**Hydrolysis of protein.** A 50-mg amount of the dried mycelial powder was placed into 10-ml am- poules. Five milliliters of 6 N HCl was added. Hydrolysis was carried out in vacuum-sealed am- poules at 110°C for 24 h. The hydrolyzed samples were then passed through a fritted-glass filter, and the filtrate was evaporated to dryness under reduced pressure at 45°C in a flash evaporator. The residue was washed twice with small portions of distilled water and evaporated to dryness. Finally, it was taken up in 10 ml of distilled water.

**Purification of amino acids.** Hydrolysate (4 ml) was passed through a Dowex-50 cation exchange column. After washing with 150 ml of distilled water, elution was accomplished with 1 N NH₄OH. The eluate was evaporated to dryness under reduced pressure at 45°C, washed with distilled water, and evaporated to dryness three times to remove the remaining NH₄OH. The amino acids were finally taken up in 5 ml of 0.1 M sodium citrate buffer, pH 2.2.

**Amino acid analysis.** Separation of amino acids was accomplished using a Beckman/Spinco, model 120 C, automatic amino acid analyzer. An aliquot of 0.25 ml was added to each chromatographic column. Qualitative and quantitative estimation of each amino acid was made by comparison with standard chromatograms, using samples of known amino acid concentrations.

**Determination of tryptophan and sulfur-contain- ing amino acids.** To prevent the high loss of sulfur-containing amino acids, which occurs during acid hydrolysis of proteins, cystine and cysteine were oxidized to cysteic acid, and methionine to methio- nine sulfone, by performic acid treatment before hydrolysis (5, 8). Tryptophan was determined colori- metrically after hydrolysis with Pronase, as described by Spies (14).

**RESULTS AND DISCUSSION**

In estimating the true protein content of biological material it is important that a method of protein determination is chosen which is accurate, yet convenient enough to use for routine testing. A comparison of the cur- rently used methods was made with protein determination, based on measurement of the quantities of individual protein-bound amino acids (Table 1).

It is known that the followed procedure of multiplying total nitrogen by the factor 6.25 provides an estimate of the crude protein content which may be misleading when reference is made to the true protein content. The over-estima- tion judged by the above procedure was 30 to 40% in all fungi studied in this work (Table 1). It should be pointed out, however, that N x 6.25 may be used conveniently in cases where total nitrogen and crude protein are the accepted parameters rather than the true protein value. The Folin method of protein determination, applied to hot alkali extracts of fungal biomass, is in good agreement with protein estimation based on amino acid analysis. These results suggest that the Folin method, once appropriately standardized, can be reliable and conven- ient for routine determinations of the true protein content. The data presented in this report do not seem to support Solomons' assertion that the Folin method is not reliable for protein determination in microfungi (11). Preliminary experiments have shown that approximately 80% of the total nitrogen is solubilized in hot alkali extracts. The close agreement of the Folin method with the results of amino acid analysis (Table 1) indicates that apparently all the protein-bound nitrogen is extracted. Certain difficulties were experienced when the biuret method was used. When applied to A. niger extracts, this method gave consistently higher values for protein content than the Folin method. Moreover, it could not be used with Fusarium extracts. The slight turbidity of these extracts resulted in very high values of protein content.
It is clear from Table 1 that C. tropicalis, F. oxysporum, and F. moniliforme have high protein content. A. niger wild type and mutant 70 have nearly the same protein content, but both are considerably inferior as compared with the other three fungi. Any advantage of mutant 70 should be sought in its faster rate of growth and possibly in cell wall alterations which may affect texture. There is no difference in protein content between the mid-log and early stationary growth phases for all fungi tested.

The amino acid composition of mycelia and yeast cells harvested at the mid-log phase of growth is shown in Table 2. In general, the amino acid composition of A. niger wild type and mutant 70 protein appears to be the same, except that mutant 70 has a higher cysteine and methionine content. Arginine and alanine are the predominant amino acids in the free amino acid pool of these two strains. Their total amino acid content is also nearly the same. The two *Fusarium* spp. have protein of comparable amino acid composition, except that *F. oxysporum* protein has almost twice as much serine as *F. moniliforme*. Alanine is the predominant amino acid in the free amino acid pool of the two *Fusarium* spp. It accounts for 43 and 33% of the pool of *F. oxysporum* and *F.

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**Table 1.** Protein content of fungal mycelia and yeast cells estimated by four different methods

| Fungus                | Time of harvest | Percent protein content estimated by |  |
|-----------------------|----------------|-------------------------------------|---|
|                        |                | Total N x 6.25 | Biuret method | Folin method | Protein-bound amino acid analysis |
| A. niger wild type    | Mid-log        | 41.6           | 28.7          | 24.0          | 23.8                               |
|                       | Stationary     | 39.1           | 28.1          | 25.1          | 24.6                               |
| A. niger mutant 70    | Mid-log        | 39.6           | 27.5          | 22.7          | 24.2                               |
|                       | Stationary     | 42.4           | 29.3          | 27.2          | 24.0                               |
| F. oxysporum          | Mid-log        | 54.9           | 34.4          | 35.4          |                                    |
|                       | Stationary     | 55.5           | 37.5          | 34.8          |                                    |
| F. moniliforme        | Mid-log        | 54.9           | 34.2          | 33.6          |                                    |
|                       | Stationary     | 58.2           | 35.6          | 33.2          |                                    |
| C. tropicalis         | Mid-log        | 59.4           | 39.0          | 36.0          |                                    |
|                       | Stationary     | 58.1           | 39.0          | 35.3          |                                    |

**Table 2.** Amino acid composition of protein and free amino acid pool of four mycelial fungi and one yeast harvested at the mid-log phase

| Amino acid | A. niger wild type | A. niger mutant 70 | F. oxysporum | F. moniliforme | C. tropicalis |
|------------|--------------------|--------------------|--------------|---------------|--------------|
|            | Protein | Pool | Total | Protein | Pool | Total | Protein | Pool | Total | Protein | Pool | Total |
| Lysine     | 11.51   | 4.34  | 15.85 | 12.72   | 2.38  | 15.10 | 16.94   | 4.04  | 20.98 | 12.81   | 7.24  | 20.05 |
| Histidine  | 4.10    | 0.90  | 5.00  | 4.08    | 0.57  | 4.65  | 4.13    | 0.84  | 4.97  | 4.39    | 1.53  | 5.92  |
| Tryptophan | 3.08    | Tr    | 3.08  | 3.08    | Tr    | 3.08  | 4.31    | Tr    | 4.31  | 4.06    | Tr    | 4.06  |
| Arginine   | 8.37    | 7.68  | 16.05 | 10.45   | 5.50  | 15.96 | 11.38   | 1.92  | 13.30 | 10.84   | 2.36  | 13.20 |
| Aspartic acid | 18.08  | 2.32  | 20.40 | 18.61   | 2.49  | 21.10 | 28.06   | 3.12  | 31.18 | 27.16   | 1.29  | 28.45 |
| Threonine  | 10.33   | 1.92  | 12.25 | 10.84   | 1.56  | 12.40 | 15.39   | 2.86  | 18.25 | 15.29   | 2.71  | 18.00 |
| Serine     | 11.84   | 3.46  | 15.30 | 10.90   | 4.50  | 15.40 | 18.02   | 2.88  | 20.90 | 9.87    | 7.05  | 16.92 |
| Glutamic acid | 25.30  | 3.90  | 29.20 | 20.92   | 4.33  | 25.25 | 35.10   | 3.40  | 38.50 | 38.06   | 3.69  | 41.75 |
| Proline    | 9.49    | 10.75 | 20.24 | 10.65   | Tr    | 10.65 | 13.68   | 1.20  | 14.88 | 15.24   | 1.01  | 16.25 |
| Glycine    | 18.88   | 2.12  | 21.00 | 19.99   | 1.36  | 21.35 | 26.24   | 2.08  | 28.32 | 26.86   | 1.94  | 28.80 |
| Alanine    | 16.91   | 8.24  | 25.15 | 17.43   | 9.27  | 26.70 | 35.48   | 23.40 | 57.88 | 27.49   | 17.26 | 44.75 |
| Half-cystine | 1.93  | Tr    | 1.93  | 2.35    | Tr    | 2.35  | 3.00    | Tr    | 3.00  | 1.91    | 1.04  | 2.95  |
| Valine     | 11.23   | 1.82  | 13.05 | 13.07   | 1.28  | 14.35 | 15.80   | 2.32  | 18.12 | 17.81   | 2.06  | 19.87 |
| Methionine | 2.04    | 0.52  | 2.56  | 2.99    | 0.56  | 3.55  | 3.21    | 1.04  | 4.25  | 3.97    | 0.38  | 4.35  |
| Isoleucine | 8.33    | 1.32  | 9.65  | 8.13    | 0.92  | 9.05  | 12.42   | 1.26  | 13.68 | 13.01   | 0.99  | 14.00 |
| Leucine    | 14.59   | 2.56  | 17.15 | 12.46   | 1.69  | 14.15 | 21.46   | 2.24  | 23.70 | 20.09   | 1.63  | 21.72 |
| Tyrosine   | 4.00    | 1.00  | 5.00  | 4.32    | 0.83  | 5.15  | 6.48    | 0.94  | 7.42  | 6.25    | 0.67  | 6.92  |
| Phenylalanine | 6.44  | 1.18  | 7.62  | 6.60    | 0.95  | 7.55  | 9.61    | 1.04  | 10.65 | 8.62    | 0.78  | 9.40  |

*Results are given in micromoles per 100 mg (dry weight); Tr, trace amounts.*
moniliforme, respectively. In all mycelial fungi tested, 16% of the total amino acids is in the pool, as compared with 24% for C. tropicalis. Based on total amino acid analysis, Fusarium mycelia have an average of 30% more amino acids than Aspergillus.

The amino acid content of mycelia and yeast cells harvested at the early stationary phase is comparable to that of the mid-log phase for all fungi tested (Table 3). This observation is significant and suggests that harvesting at the beginning of the stationary phase is to be preferred, since protein content remains high, yet the yield of biomass is increased considerably. Fusarium mycelia harvested at the early stationary phase have an average of 30% more total amino acids than Aspergillus mycelia. In Table 3 a comparison can be made of the amino acid composition of the fungi studied with the BP yeast protein Toprina. It is evident that the fungi do not suffer from any deficiencies in individual or total amino acid content. However, it should be kept in mind that the biological availability of a protein or bound amino acids depends on several factors which might differ in the case of filamentous fungi and the yeasts. Both F. oxysporum and F. moniliforme contained higher amounts of all essential amino acids than A. niger wild type, mutant 70, and Toprina. It should be pointed out, however, that this comparison is only tentative, since the medium used for the growth of these microorganisms and the substrate used for the production of the Toprina protein were different.

Based on protein and total amino acid analysis, it appears that, contrary to published reports (3,'9), Fusarium is a much better organism than Aspergillus to use for microbial protein production. Preliminary experiments in our laboratory have shown that Fusarium spp. grow well and produce satisfactory yields of biomass in liquid shake cultures utilizing inexpensive agricultural waste products, including hot-water extracts from carob bean pods (the fruit of Ceratonia silique) as an energy source. Ultraviolet irradiation for the induction of mycelial mutants may be a valuable means to select fungal strains with satisfactory yields of biomass, comparable to those of the wild parent strains and yet more palatable and acceptable to experimental animals. The nutritive value and non-toxicity of the final product, however, should be assessed by the appropriate animal bioassay procedures.

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Table 3. Total amino acid content of mycelia of four mycelial fungi as compared with that of yeast cells harvested at the early stationary growth phase*  

| Amino acid | A. niger wild type | A. niger mutant 70 | F. oxysporum | F. moniliforme | C. tropicalis | Toprina* |
|------------|--------------------|--------------------|-------------|---------------|--------------|---------|
| Lysine     | 13.90              | 15.58              | 20.55       | 21.05         | 19.90        | 16.66*  |
| Histidine  | 4.72               | 4.32               | 5.45        | 5.80          | 4.60         | 4.40    |
| Tryptophan | 3.18               | 2.89               | 3.92        | 4.60          | 4.36         | 1.65    |
| Arginine   | 14.75              | 13.38              | 12.85       | 11.30         | 12.85        | 10.07   |
| Aspartic acid | 20.88           | 20.25              | 32.35       | 30.10         | 35.50        | 23.18   |
| Threonine  | 12.48              | 12.12              | 18.48       | 17.90         | 20.10        | 12.86   |
| Serine     | 14.85              | 14.38              | 19.58       | 17.95         | 21.70        | 13.60   |
| Glutamic acid | 27.40           | 25.78              | 41.10       | 40.04         | 48.90        | 28.92   |
| Proline    | 12.88              | 11.92              | 15.55       | 16.85         | 14.80        | 9.66    |
| Glycine    | 23.25              | 22.28              | 29.72       | 29.20         | 30.30        | 23.36   |
| Alanine    | 23.15              | 24.00              | 40.42       | 40.50         | 35.20        | 26.99   |
| Valine     | 15.00              | 15.08              | 20.40       | 20.20         | 27.40        | 15.53   |
| Half-cystine| 1.92              | 1.75               | 2.10        | 3.87          | 3.20         | 2.65    |
| Methionine | 3.78               | 3.00               | 5.00        | 4.92          | 4.74         | 3.85    |
| Isoleucine | 9.90               | 9.85               | 15.02       | 13.70         | 26.10        | 11.88   |
| Leucine    | 15.68              | 15.68              | 24.65       | 22.20         | 31.50        | 18.32   |
| Tyrosine   | 4.85               | 5.52               | 7.72        | 7.25          | 8.70         | 6.27    |
| Phenylalanine | 7.95             | 8.30               | 10.60       | 9.70          | 12.90        | 8.45    |

* Results are given in micromoles per 100 mg (dry weight).
* BP yeast protein grown commercially on hydrocarbons.
* Values adapted from Woodham and Deans (15).
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