Fungal Growth on C\textsubscript{1} Compounds: a Study of the Amino Acid Composition of a Methanol-Utilizing Strain of *Trichoderma lignorum*

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The amino acid composition of the C\textsubscript{1}-utilizing fungus *Trichoderma lignorum*, growing at the expense of methanol as the sole source of carbon, was determined. With the exception of an insufficient content of methionine, the essential amino acid composition of this novel protein source appears adequate in terms of the Food and Agricultural Organization Reference Protein for both direct and indirect use in the human diet as a food or animal feed, respectively.

The exploitation of C\textsubscript{1}-utilizing bacteria as a potential source of dietary protein for animals is under active development (2, 3). As a consequence of this interest, the amino acid composition of the protein from several methanol-utilizing (2, 3) and methane-utilizing bacteria (4) has been determined: almost all of these prokaryotes contained an essential amino acid complement compatible with their successful adaption as a nutritionally beneficial dietary protein for animals. Particularly significant in this respect was the fact that several such prokaryotic protein sources contained a higher percentage of methionine than conventional dietary plant proteins, such as soya bean meal, or novel proteins, such as British Petroleum (BP) yeast concentrate.

The recent isolation of a methanol-utilizing strain of the fungus *Trichoderma lignorum* (10; R. J. Tye and A. J. Willetts, J. Appl. Bacteriol., in press) presents the possibility of exploiting mycelial protein produced by this eukaryotic microorganism as a source of dietary protein for animals. Fungi offer several advantages over bacteria in this respect, including a significantly lower nucleic acid-protein ratio and comparatively lower harvesting costs from spent media (6). The present investigation, conducted to examine the protein and amino acid composition of the methanol-utilizing fungus *T. lignorum*, was a pertinent contribution towards a rational assessment of this possibility.

**MATERIALS AND METHODS**

**Growth of the fungus.** The isolation, maintenance, and growth of the fungus, identified as a strain of *T. lignorum*, were as previously described (10; Tye and Willetts, in press).

**Analytical methods.** Amino acid analyses were made using a modification of the method described by D'Mello (4): 10 mg of methanol-grown fungal mycelium was hydrolyzed by autoclaving for 15 h at 115 C in 5 ml of 6 N HCl in a sealed glass ampoule under nitrogen. The hydrolysate was made up to 100 ml in a volumetric flask with deionized water and subsequently filtered twice through a sintered glass funnel. A 10-ml aliquot was evaporated to dryness in a rotary evaporator. The residue was taken up in 2 ml of 10% (wt/vol) sucrose in 0.1 N HCl and analyzed for amino acids using a Technicon autoanalyzer (Technicon Instruments Co. Ltd., Basingstoke, U.K.). 2,4,6-Trinitrobenzene sulphonic acid was used as the developing reagent (11).

Cystine was determined by the method of Moore (8), and tryptophan was analyzed in alkaline hydrolysates (5).

The nitrogen content of the fungal mycelium was established by the classical Kjeldahl method (7). Chitin (1) and nucleic acid (9) levels were assayed as previously described.

**RESULTS**

The nitrogen content of the mycelium of methanol-grown *T. lignorum* was 9.82\% of N, which was equivalent to a protein content of 61.4\% (N \times 6.25). Growth conditions which promote a high mycelial protein content are currently under investigation.

The content of all of the individual amino acids in the protein of the fungal mycelium after growth on methanol was remarkably consistent (Table 1). The essential amino acid content compared favorably with the 1957 Food and Agricultural Organisation (FAO) standard Reference Protein in most aspects; the one notable exception was a deficiency with respect to methionine (Table 2). The mycelial protein of...
methanol-grown *T. lignorum* also compared favorably with whole wheat protein, domestic fowl egg protein, and the proteins from several other microbial sources (Table 2). Over 80% of the total nitrogen compounds present in the mycelium of methanol-grown *T. lignorum* was recovered as amino acids during a quantitative analysis after acid hydrolysis.

The chitin and nucleic acid contents of the mycelium of methanol-grown *T. lignorum* were 3.8 and 5.1%, respectively.

**DISCUSSION**

The results indicate that the fungus *T. lignorum* growing at the expense of methanol as the sole source of utilizable carbon produces mycelial protein with a spectrum of essential amino acids superior in all but one respect to the FAO Reference Protein: the one major deficit of methanol-grown fungal protein is a subminimal methionine content, which shows a 15% shortfall with respect to the FAO standard. However, as indicated in Table 2, the methionine content of the mycelial protein of methanol-grown *T. lignorum* (1.85 g/16 g of N), although below the level in FAO Reference Protein, is nevertheless in excess of the content of many other sources of dietary protein including conventional plant proteins such as whole wheat protein (1.5 g/16 g of N) and soya bean meal (1.2 to 1.4 g/16 g of N), as well as other novel proteins such as BP yeast concentrate (1.4 to 1.6 g/16 g of N) and gas oil-grown Candida lipolytica (1.6 g/16 g of N).

Any dietary deficiency resulting from this subminimal content of methionine could be avoided by using mycelial protein from methanol-grown *T. lignorum* as the basis for a balanced food or feed incorporating compensatory amounts of either pure methionine or methionine-rich proteins such as domestic chicken egg protein (Table 2).

The mycelial protein of methanol-grown *T. lignorum* has an acceptable complement of all other essential amino acids, including lysine, as assessed against the FAO standard, as well as a full complement of non-essential amino acids (Table 1). The complement of arginine and histidine, two amino acids which are not catagorized as essential human dietary components, but supplementary sources of which are widely considered necessary for the normal growth of children, is similar to that of many conventional dietary proteins.

The amino acid composition of the mycelial protein of methanol-grown *T. lignorum* is similar to that of several methane-grown bacteria (4); the comparatively high content of tryptophan is particularly significant in this respect.

Both the dietary and commercial potential of

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**Table 1. Amino acid content of methanol-grown *T. lignorum***

| Amino acid     | Mean ± standard deviation (g/16 g of N) |
|----------------|----------------------------------------|
| Alaine         | 6.80 ± 0.20                            |
| Arginin        | 4.92 ± 0.16                            |
| Aspatic acid   | 8.63 ± 0.19                            |
| Cystine        | 0.90 ± 0.08                            |
| Glutamic acid  | 10.03 ± 0.22                           |
| Glycin         | 4.87 ± 0.09                            |
| Histidine      | 2.03 ± 0.12                            |
| Isoleucine     | 4.48 ± 0.09                            |
| Leucine        | 7.88 ± 0.11                            |
| Lysine         | 5.13 ± 0.09                            |
| Methionine     | 1.85 ± 0.08                            |
| Phenylalanine  | 4.38 ± 0.08                            |
| Serine         | 3.48 ± 0.09                            |
| Threonine      | 4.62 ± 0.11                            |
| Tryptophan     | 1.82 ± 0.08                            |
| Tyrosine       | 3.65 ± 0.08                            |
| Valine         | 5.72 ± 0.10                            |

*a Average of six experiments.

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**Table 2. Essential amino acid content of methanol-grown *T. lignorum*, FAO Reference Protein, wheat, egg, and some other established microbial protein sources (expressed as g/16 g of N)**

| Amino acid     | T. lignorum (methanol) | Scene-debuisobliquus (6) | Spiroplana maxima (6) | Saccharomyces cerevisiae (6) | C. lipolytica (gas oil) (6) | Pseudomonas (methanol) (6) | Whole wheat (6) | Whole egg (6) | FAO Reference Protein (6) |
|----------------|------------------------|--------------------------|----------------------|-----------------------------|----------------------------|--------------------------|----------------|--------------|--------------------------|
| Isoleucine     | 4.48                   | 3.8                      | 6.0                  | 4.6                         | 5.3                        | 3.9                      | 3.3            | 6.7          | 4.2                      |
| Leucine        | 7.88                   | 8.4                      | 8.0                  | 7.0                         | 7.8                        | 7.0                      | 6.7            | 8.9          | 4.8                      |
| Lysine         | 5.13                   | 5.7                      | 4.6                  | 1.4                         | 1.7                        | 1.8                      | 1.5            | 3.2          | 2.2                      |
| Methionine     | 1.85                   | 1.7                      | 1.4                  | 1.7                         | 1.6                        | 1.8                      | 1.5            | 3.2          | 2.2                      |
| Phenylalanine  | 4.38                   | 5.1                      | 5.0                  | 4.1                         | 4.8                        | 4.2                      | 4.5            | 5.8          | 2.8                      |
| Threonine      | 4.62                   | 5.1                      | 4.6                  | 4.8                         | 5.4                        | 4.5                      | 2.9            | 5.1          | 2.8                      |
| Tryptophan     | 1.82                   | 1.5                      | 1.4                  | 1.0                         | 1.3                        | —                        | 1.1            | 1.6          | 1.4                      |
| Valine         | 5.72                   | 5.7                      | 6.5                  | 5.3                         | 5.8                        | 5.9                      | 4.4            | 7.3          | 4.2                      |
mycelial protein from methanol-grown *T. lignorum* as a food or feed remain to be assessed directly.

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