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Protein patterns of edible fungi

DEZSÖ TÖRLEY

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Proteins of different cultivated and wild growing mushrooms were fractionated by polyacrylamide disc electrophoresis. The pherograms of young and mature fruiting bodies showed differences in the protein patterns. The number of protein fractions in the stipe and the pileus, and the intensity of the bands is also different and characteristic for different species. By the use of enzyme reactions the enzyme activities of different protein bands, and the presence of isoenzymes could be established in the fruiting bodies.

D. Törley, Department of Biochemistry and Food Technology, Technical University Budapest, Müegyetem rkp 3 H-1111 Budapest, Hungary.

With the development of polyacrylamide gel electrophoresis a new useful technique for the isolation and characterization of proteins has become widespread. Some years ago we began the investigation of the proteins of edible fungi grown in Hungary. The aim of this research work has been to get the pattern of the fungal proteins of different species and to study the distribution of the proteins in the different parts of the fruiting body, i.e. the pileus and the stipe. The investigations have been so directed that, simultaneously with the protein patterns we would obtain an insight into the distribution pattern of a few enzymes and isoenzymes.

Materials and methods

The cultivated fungi *Agaricus bisporus* and *Pleurotus ostreatus* were provided by the trade and the wild growing species were collected in the neighbourhood of Budapest.

The weight and the length of the mushroom and the diameter of the pileus were determined. The cleaned mushrooms were cut up into pileus and stipe, which were homogenized with quartz sand in mortars cooled with ice, with or without the addition of electrode buffer solution. The homogenates were centrifuged and the supernatants were applied to electrophoresis.

The electrophoretic technique employed in this study was a slight modification of the method described by Ornstein (1964) and Davies (1964). The whole gel consisted of two parts: spacer gel (upper gel) and separating gel (lower gel). For a run of 6 or 8 gels separating gels of 7.5% acrylamide were prepared by mixing 1 volume of buffer solution (containing 48 ml N HCl, 36.6 g Tris and 0.23 ml TEMED in a final volume of 100 ml, pH = 8.9), 2 volumes of acrylamide solution (containing 28.0 g acrylamide, 0.735 g methylene bisacrylamide in a final volume of 100 ml) and 1 volume of distilled water. This solution was mixed with the same volume of ammonium persulfate solution (0.14 g in a final volume of 100 ml).

The spacer gel was prepared by mixing 1 volume of buffer solution (containing 48 ml N HCl, 5.98 g Tris and 0.46 ml TEMED in a final volume of 100 ml, pH = 6.9), 2 volumes of acrylamide solution (containing 10.0 g acrylamide, 2.5 g methylene bisacrylamide in a final volume of 100 ml), 1 volume of riboflavin solution (4 mg in 100 ml) and 4 volumes of 40% saccharose (v/w) solution. After complete polymerization of the gels 0.2 ml of the prepared samples were applied on top of the gels.

The electrode buffer solution was prepared from the stock solution (Tris 6.0 g, glycine 28.8 g in a final volume of 1 000 ml, pH = 8.3) by diluting 1:10. Then 1 ml of bromophenolblue solution (0.001 g in 100 ml) was added as marker to 1 000 ml of diluted buffer solution.

Electrophoresis was carried out with a current of 2 mA per gel until the marker migrated to the top of the separating gel. The current was then changed to 5 mA per gel until the marker reached the bottom of the gel. After electrophoresis the gels were removed from the tubes and stained for 1 hour in a solution of amino black 10 B (1 g in 100 ml of 7% acetic acid). The gels were rinsed and destained with 7% acetic acid solution.

Enzyme reactions were carried out with special reagents (see below).

The pherograms of the disc electrophoresis show that the distrubution of the proteins within the fruiting body is very diverse. In some fungus species the same protein fractions occur in the pileus and in the stipe, but in most cases there is a difference in the number or in the intensity of the protein bands. In the pileus more bands are detectable for the most part, but

exceptional cases were also found.

In the course of development and maturation, changes take place in the protein content: some bands of the pherograms disappear, or their colour intensity is changed. In the young fruiting bodies 17, and in the mature ones only 13 bands could be detected. The distribution between pileus:stipe of some wild growing species was the following: *Suillus granulatus* 6:5, *Coprinus micaceus* 12:11, *Clitocybe nebularis* 15:17, *Tricholoma nudum* 17:11, *Lepiota procera* 16:17.

With the cultivated fungi i.e. *A. bisporus* and *P. ostreatus* systematic, repeated examinations were carried out. In all cases young and mature mushrooms were chosen and the fruiting bodies were grouped according to their size. Of the *A. bisporus* small, medium and large (length 2-3, 3-5, 5-8 cm, respectively),and of the *P. ostreatus* small (3-10 cm) and large (over 10 cm) fruiting bodies were separately examined.

In young fruiting bodies of *A. bisporus* 12 protein bands appeared constantly in the pileus in small, medium and large mushrooms; 3 other bands appeared at different parts of the gel. In the stipe 7 constant and 4 variable fractions were found. In mature fruiting bodies the number of the protein bands of the pileus decreased from 15 to 10-11, while the bands from the stipe remained constant.

In *P. ostreatus* the degree of maturity did not affect the number of protein bands, but the small fruiting bodies showed always more bands (18) than the large ones (13).

Some pherograms are shown in the figures.Fig. 1 shows the protein bands of pileus and stipe of *Limacium eburneum*. In Fig. 2 the protein bands of *L. eburneum* and *T. nudum* are seen. From left to right: pileus and stipe of *Limacium*, pileus and stipe of *Tricholoma*. Fig. 3 shows the protein pherograms of two *Lycoperdon* species. In Fig. 4 the protein bands of *C. nebularis* are shown. From left to right: the whole fruiting body, pileus, stipe. . The pherograms of *C. nebularis* (pileus and stipe) and *Lepiota procera* (pileus and stipe) are seen in Fig. 5. Fig. 6 shows the protein bands of the pileus of 7 species: *L. eburneum, S. granulatus, T. nudum, L. procera, C. nebularis* and *A. bisporus*.

The polyacrylamide gel electrophoresis technique was applied also to the study of some enzymes of fungi. To indicate the enzyme activity the developed gels were incubated with suitable substrates. In Figure 7 the colour reaction of O-diphenoloxydase in the pileus and stipe of *A. bisporus* is shown, substrate: catechol and proline. Fig. 8 shows tyrosinase activity of *A. bisporus*.

To indicate the presence of oxydoreductases the developed gels were incubated with iodo-nitro-tetrazolium chloride and the buffered solution of the substrate. Fig. 9 shows the lactate dehydrogenase activity of the pileus and stipe of young and mature fruiting bodies of the cultivated mushroom and the blue protein pattern. The enzyme activity of the young fungus was somewhat stronger.

In Fig. 10 mannitol dehydrogenase reactivity is seen in the cultivated mushroom. From left to right: pileus, stipe, pileus + stipe, protein pattern.

The zymograms show also the presence of isoenzymes. In young mushrooms of medium size the pileus contains three and the stipe two tyrosinase isoenzymes. In the case of large mushrooms and in mature fruiting bodies an additional and more intensive band appeared in both pileus and stipe.

In the case of lactate dehydrogenase the pileus of young and mature mushrooms (at left) contain two more isoenzymes than the stipes. Mannitol dehydrogenase reacts somewhat weaker, but it may be seen that more bands appear in the pileus. The number of dehydrogenase bands is changed sometimes. Fig. 11 shows the lactate dehydrogenase activity of two fungi which were gathered in different times. The chief bands are the same in both gels, but weaker bands may sometimes appear.

As it may be seen from the results, the protein patterns are characteristic for the different species. We have not yet tested a larger number of enzyme reactions on wild fungi, but we are continuing our work to complete the protein and enzyme patterns of the fungi that grow in Hungary.

References

Ornstein, L. 1964: - Ann. N.Y. Acad. Sci. 121: 321.
Davies, B. J. 1964: - Ann. N.Y. Acad Sci. 121: 404.

(Ed. note: The figures refered to in the text were not received in due time.)