High concentrations of mannitol in the shiitake mushroom *Lentinula edodes*

Y. H. Tan and David Moore*

Microbiology Research Group, School of Biological Sciences, 1.800 Stopford Building, The University of Manchester, Oxford Road, Manchester M13 9PT, Great Britain
(*Reprint address)

Key words: chemistry, GLC analysis, nutrients, expansion, differentiation, development

Abstract

A quantitative analysis of mannitol, using gas-liquid chromatography (GLC), was carried out in the various tissues of *Lentinula edodes* (Berk.) Pegler in comparison with *Agaricus bisporus* (Lge) Imb. and *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray. Mycelia of *L. edodes* had a low level of mannitol (*ca* 1% on a dry wt basis) compared with the fruit body stipe and pileus (20–30%). The highest level of mannitol was observed in the pileus of *A. bisporus* (close to 50%). These observations of similar levels of accumulation of mannitol suggest that glucose catabolism in *L. edodes* and *A. bisporus* are similar. No mannitol was detectable in the pileus of *C. cinereus*.

Introduction

Mannitol belongs to a group of compounds known as compatible solutes which are generally defined as being low molecular weight, neutral compounds which can be accumulated to high intracellular concentrations without causing enzyme inhibition (Brown and Simpson, 1972; Brown, 1978). One of the roles ascribed to mannitol is that of osmoregulation or turgoregulation, that is the maintenance of a high osmotic or suction pressure inside the cell to establish turgor and create an inflow of water, especially when the substrate is of high osmolality (Lewis and Smith, 1967; Ellis *et al.*, 1991).

Mannitol has been found to be accumulated in quantities up to 50% dry wt in *Agaricus bisporus* (Rast, 1965; Hammond and Nichols, 1976). As its turnover rate was low and the apparent cellular concentration remained at about 150 mM (Hammond and Nichols, 1977), mannitol was assigned an osmotic function, being thought to provide for support and expansion of the fruit body. In contrast, in *Coprinus cinereus*, a similar function was ascribed to urea on the grounds that its accumulation was paralleled by influx of water (content of urea increased during fruit body development when expressed on a dry wt basis but not on a fresh wt basis) (Moore, 1984).

The mannitol content of *Lentinula edodes* has not previously been determined. We have made an investigation of the mannitol content in different tissues of *L. edodes* as part of a broader study of the relation between metabolic pathways and fruit body development in this species. Comparisons were also made with samples of both *A. bisporus* and *C. cinereus*.

Materials and methods

The strain of *Lentinula edodes* used was Le-11; its origin and the routine cultivation techniques have been described by Tan and Moore (1992). The 'Meathop' wild type dikaryotic strain of *Coprinus cinereus* was used and cultivated as described by Moore and Pukkila (1985). Fresh fruit bodies of *Agaricus bisporus* were bought from local shops.

Analysis of the trimethylsilylated sugar alcohols was carried out with a Packard 437A gas chromatograph equipped with an SE 30, 1 m x 2 mm glass column packed with 10% SE 30 on WHP 80-100 mesh. Nitrogen (200 KPa, 20 ml min^{-1}) was used as the carrier gas. Hydrogen (200 KPa, 25 ml min^{-1}) and air (150 KPa, 250 ml min^{-1}) were used to supply the flame ionization detector. The chromatograph oven temperature was set to rise from 80°C at the time of sample injection to 280°C at a rate of 5°C min^{-1}. The temperature of injection and detection were 200°C and 270°C, respectively. The time of run was set for 40 min. Peak areas were determined and graphs plotted with an automated digital integrator (Shimadzu Chromatopac C-R3A data processor) indicating the retention time as the distance from the injection point to the respective peak tip, and quantification made using the internal standard method using authentic arabitol.

Extraction of the mannitol was carried out with a modification of the methods of Horikoshi *et al.* (1965) and Domelsmith *et al.* (1988). Fresh tissue samples were well macerated, finely ground and freeze-dried, then 34 mg of the freeze-dried tissue were suspended in 5 ml of water and thoroughly homogenized for 30 min. The suspension was boiled, under constant stirring, for 15 min. Following freeze-drying, the material was redissolved in 20 ml of pyridine (containing molecular sieves) and heated for 30 min. One ml of this mannitol solution in pyridine equivalent to an extraction from 1.7 mg dried tissue (34 mg/20 ml) was then withdrawn and thoroughly mixed with 1 ml of arabitol (10 μmol ml^{-1}). This solution (20 μl) was mixed with 20 μl of a derivatizing agent in an Eppendorf tube, the mixture vortexed and allowed to stand at room temperature for 15 min. Two μl were then withdrawn and injected into the GC. The derivatizing agent used was a mixture of N,O-bistrimethylsilyl trifluoroacetamide (BSTFA) plus trimethylchlorosilane (TMCS), purchased from Phase Separations Limited, Clwyd, Wales.

Results and discussion

Arabitol was used as the internal standard since it is chemically similar to mannitol, and gives rise to a single peak emerging in an unoccupied region of the chromatogram close to the mid-point (20.6 min emergence) of the run (40 min). Furthermore, among 27 species of fungi screened by Pfyffer and Rast (1980), arabitol was detected only occasionally and was restricted to particular systematically related groups which did not include the basidiomycetes *Coprinus friesii* and *Pleurotus ostreatus*. Even when found, arabitol

Table 1 Mannitol content (dry wt basis) of tissues of *Lentinula edodes*, *Coprinus cinereus* and *Agaricus bisporus* as determined by gas chromatography

Tissue	RRT	Mannitol content: (μmol mg^{-1})	(%, w/w)
L. edodes mycelium	1.20	0.07 ± 0.02	1.29 ± 0.28
L. edodes young pileus	1.23	1.16 ± 0.07	21.16 ± 1.21
L. edodes young stipe	1.22	1.68 ± 0.07	30.57 ± 1.22
C. cinereus young pileus	0	0	0
A. bisporus young pileus	1.22	2.54 ± 0.41	46.33 ± 7.46

RRT, relative retention time (mannitol/arabitol), RRTs for eight mixtures of authentic standards at various molar ratios ranged between 1.212 and 1.229 (mean 1.217 ± 0.007). Entries show mean ± SEM of two replicate samples.

occurred in a much lesser quantity than mannitol; only trace amounts of arabitol being found in *A. bisporus* (Pfyffer and Rast, 1988). In our chromatograms, mannitol emerged in a clear region about 4.5 min from the internal standard. The tissues did not contain many interfering compounds in these regions, thus allowing both arabitol and mannitol to be easily identified and quantified.

A summary of the mannitol content (on a dry wt basis) in the different tissues examined is shown in Table 1. Mycelia of *L. edodes* had a low level of mannitol (*ca* 1%) compared with the fruit body pileus and stipe (20—30%). The highest level of mannitol was observed in the pileus of *A. bisporus* (close to 50%), whilst no mannitol was detectable in the pileus of *C. cinereus*. The values obtained in these studies with *L. edodes* are thus quite similar to those obtained from *A. bisporus*.

Synthesis of mannitol in *A. bisporus* is mediated by an NADPH-dependent mannitol dehydrogenase using fructose as substrate (Edmundowicz and Wriston, 1963), the NADPH being obtained through the pentose phosphate pathway (PPP) (Dütsch and Rast, 1972); a greater proportion of glucose oxidation occurs *via* the PPP in the basidiome than in the mycelium in *A. bisporus* (Hammond and Nichols, 1976, 1977; Hammond, 1977). This contrasts with *C. cinereus*, in which the Embden-Meyerhof-Parnas pathway (EMP) represents the major route of sugar catabolism in both basidiome and mycelium, with the PPP playing only a minor role and being completely dispensed with in early basidiome development (Moore and Ewaze, 1976).

This seems to be reflected in the fact that the adjustable osmoticum in *C. cinereus* is urea. Indeed, the total polyol concentration of the *C. cinereus* basidiome did not exceed 6% of the dry wt and declined as the basidiome developed (Darbyshire, 1974), and neither mannitol (this analysis) nor NADP-linked mannitol dehydrogenase have been detected (Rao and Niederpruem, 1969).

Considering the large amount of mannitol which is accumulated by *L. edodes*, it is possible that glucose catabolism in this species resembles that of *A. bisporus*, with the PPP being the primary catabolic route in particular tissues of the fruit body. We intend to test this possibility by use of radiorespirometry. If mannitol does serve an osmoregulatory function in both *A. bisporus* and *L. edodes*, it is particularly noteworthy that the two species differ in the way in which the basidiome expands: in *A. bisporus* this occurs by hyphal inflation, whilst in *L. edodes* hyphal proliferation is responsible. Thus, the two very different basidiomycete strategies for tissue expansion can be facilitated by the same metabolite. If the intracellular volume is similar to the 2.1 μl mg^{-1} dry wt which has been measured for *Coprinus cinereus* (Moore and Devadatham, 1979), and the mannitol is assumed to be uniformly distributed within it, then the concentration of mannitol in the *L. edodes* stipe would be of the order of 500 mM, and in the cap 750 mM. These values can be considered only as gross approximations, but concentrations like this would provide a formidable driving force for water through the fruit body and protect the most exposed tissues against desiccation.

Acknowledgement

We thank the Universiti Pertanian Malaysia for a scholarship and study leave which enabled YHT to carry out this work.

References

BROWN A. D. 1978. Compatible solutes and extreme water stress in eukaryotic micro-organisms. *Adv. Microbial Physiol.* **17** 182–242.

BROWN A. D. and Simpson J. R. 1972. Water relations of sugar-tolerant yeasts: the role of intracellular polyols. *J. gen. Microbiol.* **72** 589–91.

DARBYSHIRE J. 1974. Developmental studies on *Coprinus lagopus*. PhD thesis, University of Manchester.

DOMELSMITH L. N., Klich M. A. and Goynes W. R. 1988. Production of mannitol by fungi from cotton dust. *Appl. Environ. Microbiol.* **54** 1784–90.

DÜTSCH G. A. and Rast D. 1972. Biochemische beziehung zwischen mannitbildung und hexose-monophosphatzyklus in *Agaricus bisporus*. *Phytochemistry* **11** 2677–81.

EDMUNDOWICZ J. M. and Wriston J. C. Jr. 1963. Mannitol dehydrogenase from *Agaricus campestris*. *J. biol. Chem.* **238** 3539–41.

ELLIS S. W., Grindle M. and Lewis D. H. 1991. Effect of osmotic stress on yield and polyol content of dicarboximide-sensitive and -resistant strains of *Neurospora crassa*. *Mycol. Res.* **95** 457–64.

HAMMOND J. B. W. 1977. Carbohydrate metabolism in *Agaricus bisporus*: oxidation pathways in mycelium and sporophore. *J. gen. Microbiol.* **102** 245–8.

HAMMOND J. B. W. and Nichols R. 1976. Carbohydrate metabolism in *Agaricus bisporus* (Lange) Sing.: changes in soluble carbohydrates during growth of mycelium and sporophore. *J. gen. Microbiol.* **93** 309–20.

HAMMOND J. B. W. and Nichols R. 1977. Carbohydrate metabolism in *Agaricus bisporus* (Lange) Imbach.: metabolism of [^{14}C] labelled sugars by sporophores and mycelium. *New Phytol.* **79** 315–25.

HORIKOSHI K., Uda S. and Ikeda Y. 1965. Mannitol and mannitol dehydrogenases in conidia of *Aspergillus oryzae. J. Bact.* **89** 326–30.

LEWIS D. H. and Smith D. C. 1967. Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. *New Phytol.* **66** 143–84.

MOORE D. 1984. Developmental biology of the *Coprinus cinereus* carpophore: metabolic regulation in relation to cap morphogenesis. *Expl Mycol.* **8** 283–97.

MOORE D. and Devadatham M. S. 1979. Sugar transport in *Coprinus cinereus. Biochim. biophys. Acta* **550** 515–26.

MOORE D. and Ewaze J. O. 1976. Activities of some enzymes involved in the metabolism of carbohydrate during sporophore development in *Coprinus cinereus. J. gen. Microbiol.* **97** 313–22.

MOORE D. and Pukkila P. J. 1985. *Coprinus cinereus*: an ideal organism for studies of genetics and developmental biology. *J. biol. Ed.* **19** 31–40.

PFYFFER G. E. and Rast D. M. 1980. The polyol pattern of some fungi not hitherto investigated for sugar alcohols. *Expl. Mycol.* **4** 160–70.

PFYFFER G. E. and Rast D. M. 1988. The polyol pattern of fungi as influenced by the carbohydrate nutrient source. *New Phytol.* **109** 321–6.

RAO P. S. and Niederpruem D. J. 1969. Carbohydrate metabolism during morphogenesis of *Coprinus lagopus sensu* Buller. *J. Bact.* **100** 1222–8.

RAST D. M. 1965. Zur stoffwechselphysiologischen Bedeutung von Mannit und Trehalose in *Agaricus bisporus* eine gaschromatographische Studie. *Planta* **64** 81–93.

TAN Y. H. and Moore D. 1992. Convenient and effective methods for *in vitro* cultivation of mycelium and fruiting bodies of *Lentinus edodes. Mycol. Res.* **96** 1077–84.

Accepted 15 May 1994