JOURNAL OF BACTERIOLOGY, Apr., 1965
Vol. 89, No. 4

Printed in U.S.A.

Polyol Metabolism in the Basidiomycete *Schizophyllum commune*

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Received for publication 4 November 1964

ABSTRACT

NIEDERPRUEM, DONALD J. (Indiana University Medical Center, Indianapolis), AMTUL HAFIZ, AND LYLE HENRY. Polyol metabolism in the basidiomycete *Schizophyllum commune*. J. Bacteriol. **89**:954–959. 1965.—The polyol metabolism of intact cells and extracts of the wood-rotting mushroom *Schizophyllum commune* was investigated during the developmental cycle. Exogenous polyols stimulated the cellular respiration of germlings, but were without effect on that of ungerminated basidiospores. Requisite enzymes of polyol metabolism were demonstrated in extracts of spores and of subsequent stages of development. Oxidation of mannitol and sorbitol appeared to be coupled to nicotinamide adenine dinucleotide (NAD) reduction and was favored in alkaline medium. Ketohexose formation was shown during mannitol oxidation, and the NAD-dependent oxidation of xylitol yielded ketopentose. Xylitol oxidation with nicotinamide adenine dinucleotide phosphate (NADP) as hydrogen acceptor led to pentose formation. Oxidation of reduced NAD was enhanced by fructose but not by sorbose. Reduction of aldohexose and pentose was dependent upon reduced NADP, and pentose reductase was maximal at *p*H 6.8. The specific activity of mannitol dehydrogenase was highest in extracts of vegetative mycelium. Growth in either glucose or xylose media had no significant effect on enzymes of polyol metabolism.

Recent studies dealing with the cellular respiration of basidiospores of the wood-rotting mushroom *Schizophyllum commune* have indicated that these propagative units are capable of significant aerobic metabolism when supplied with certain carbohydrates (Niederpruem, 1964). Polyols are conspicuously inactive as respiratory substrates, although glycerol and mannitol sustain growth and fruiting of *S. commune* (Niederpruem, Hobbs, and Henry, 1964). In contrast, the oxygen consumption of germlings is stimulated markedly by certain hexitols and pentitols (Niederpruem and Hafiz, 1964). This situation appears to be similar to mannitol metabolism in *Coccidioides immitis*, in that polyol utilization for growth occurs with mycelium but not with the spherule form of this dimorphic fungus; mannitol-1-phosphate oxidation was demonstrated in cell-free extracts of both forms (Lones and Peacock, 1964).

In contrast with the amount of information available concerning polyol metabolism in bacteria, yeast, certain filamentous fungi, and animal systems (Touster and Shaw, 1962; Hollmann, 1964), very little is known about the occurrence and role of these enzyme systems in the basidiomycetes. The finding that the cultivated mushroom *Agaricus campestris* contains a high level of mannitol relative to other carbohydrates (Hughes, Lynch, and Somers, 1958) suggests an important role for polyols during morphogenesis of these forms. More recently, Edmundowicz and Wriston (1963) described the purification of nicotinamide adenine dinucleotide phosphate (NADP)-dependent mannitol dehydrogenase from mushrooms of *A. campestris*.

The present report describes the occurrence and properties of enzymes of polyol metabolism in cell-free extracts of ungerminated basidiospores of *S. commune*, together with subsequent enzyme patterns during spore germination, mycelial growth, and fruiting of this mushroom.

MATERIALS AND METHODS

Culture conditions. S. commune Fr. was cultured and mated on a minimal medium which contained (per liter of distilled water): D-glucose, 20 g; L-asparagine (Cyclo Chemical Corp., Los Angeles, Calif.), 1 g; thiamine hydrochloride, 100 μg; KH_2PO_4, 0.46 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; and agar (Difco), 20 g. The ingredients were autoclaved together.

Basidiospores were collected from sporulating fruits which resulted from the cross 699 $A^{41}B^{41} \times$ 845 $A^{51}B^{51}$ as described previously (Niederpruem, 1964). Germinated basidiospores (germlings) were obtained after aseptic collection and inoculation

of spores into minimal broth medium and subsequent incubation for 12 hr at 25 C (±0.5 C) on a shaker-incubator (model G 27, New Brunswick Scientific Co., New Brunswick, N.J.) at 180 oscillations per minute.

Macerated mycelium served as inocula for broth cultures of homokaryotic mycelia of strains 699 and 845 of *S. commune*. Nutritional studies were performed on mycelium cultured in the above minimal medium, after the medium was sterilized by filtration (Millipore, 0.45-μ pore size).

Respiration studies. Storage experiments with germinated basidiospores of *S. commune* indicated that these cells could not be held at either 4 or −20 C without considerable loss of respiratory activity. Consequently, all work related to germling metabolism was performed with freshly harvested material. Respiratory rates of basidiospores and germlings of *S. commune* were measured at 10-min intervals for 2 hr at 30 C by conventional manometric techniques. Dry weights were determined by placing samples of cells in tared weighing cups at 100 C for 18 hr.

Preparation of cell-free extracts. Basidiospores and germlings were harvested by centrifugation (2,000 × *g*, 15 min), and were washed by suspending the cells in phosphate buffer (*p*H 6.8, 0.08 M) and centrifuging. Vegetative mycelium was harvested from culture broth by filtration on a Büchner funnel and extensive washing in phosphate buffer. Basidiospores and germlings were extracted directly in a chilled French pressure cell (Aminco, Silver Spring, Md.) at 24,000 psi; mycelium and dikaryotic fruits were treated for 10 sec at 4 C in a Waring Blendor before transfer to the French pressure cell. After extraction, the homogenate was centrifuged at 2,000 × *g* (10 min) and 105,000 × *g* (120 min) at 4 C in a Spinco preparative centrifuge; the final supernatant fluid served as the source of enzymes.

Since the NADP-coupled xylitol dehydrogenase system, as well as the reduced NADP ($NADPH_2$)-dependent aldohexose reductase activity, decayed rapidly after dialysis for 18 hr at 4 C in phosphate buffer (*p*H 6.8, 0.08 M) or after storage for several days at −20 C, all subsequent assays of quantitative enzyme patterns during morphogenesis of *S. commune* were carried out on extracts prepared the same day of culture harvest. Appropriate control experiments were performed to determine endogenous oxidation and reduction of pyridine nucleotides. Enzyme extracts dialyzed as above were used to determine the products formed during polyol oxidation.

Enzyme determinations. Polyol oxidation was coupled to the reduction of either nicotinamide adenine dinucleotide (NAD) or NADP, which show absorbance at 340 mμ in the reduced form. The kinetics of pyridine nucleotide reduction were followed on a Zeiss PMQII spectrophotometer at 30 C under the following conditions: polyol, 100 μmoles; tris(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer (*p*H 9), 74 μmoles; NAD or NADP, 0.4 μmole; enzyme extract; and water (glass-distilled), to 1.0 ml.

Ketohexose reduction was measured by the oxidation of reduced NAD ($NADH_2$) at 340 mμ, with the following reaction mixture: ketohexose, 100 μmoles; $NADH_2$, 0.10 μmole; Tris-HCl (*p*H 8), 74 μmoles; enzyme extract; and water, to 1.0 ml. Reduction of aldohexose and pentose was assayed as for ketohexose, with the following modifications: aldohexose or pentose, 100 μmoles; phosphate buffer (*p*H 6.8), 40 μmoles; $NADPH_2$, 0.10 μmole; enzyme; and water, to 1.0 ml.

Determination of enzyme products. Estimation of the products of polyol oxidation was performed by use of dialyzed enzyme extracts and assay mixtures as indicated above. The reaction was allowed to proceed for 60 min at 30 C, and was stopped by the addition of trichloroacetic acid (6%, final). Appropriate control experiments were performed without substrate and coenzymes. Nucleotides were removed with charcoal, and the solution was clarified by centrifugation. Ketohexose and ketopentose determinations were performed by the cysteine-carbazole reaction (Dische and Borenfreund, 1951); ketohexose was further substantiated by the procedure of Roe (1934). Pentose was identified by the orcinol test (Militzer, 1946), and mannitol by the chromotropic acid procedure of West and Rapoport (1949). Protein was determined by the method of Lowry et al. (1951), with bovine serum albumin as the standard.

RESULTS

Cellular respiration during basidiospore germination. Oxygen consumption by intact spores and germlings of *S. commune* was stimulated by sucrose, glucose, and acetate, whereas certain polyols were active as respiratory substrates only for germlings (Fig. 1). Erythritol and inositol were inactive at either stage of development. Permeability barriers to polyols in ungerminated spores appeared unlikely, since uptake of C^{14}-mannitol (3,500 counts per min per mg of protein) was readily demonstrated and was 73% of the rate obtained with C^{14}-mannose (4,750 counts per min per mg of protein). Moreover, the uptake of both carbohydrates increased in a similar fashion during spore germination; these results did not support the idea of selective permeability to polyols in germinated basidiospores. Consideration was also given to the possibility that a high intracellular polyol content in fresh spores may saturate the constitutive enzyme systems. However, no striking differences in polyol content of hog-water extracts of basidiospores and mycelium were detected by the chromotropic acid procedure of West and Rapoport (1949).

Polyol oxidation by extracts of basidiospores. The failure to promote spore respiration by

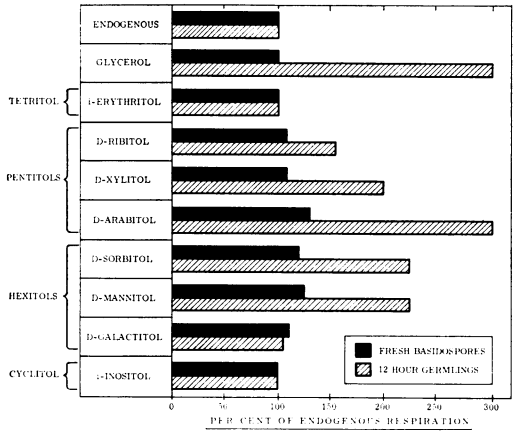

FIG. 1. *Effect of polyols on cellular respiration of basidiospores and germlings (12-hr) of Schizophyllum commune.*

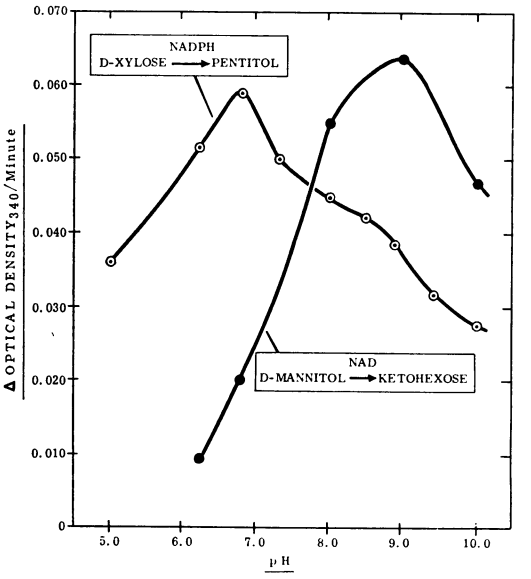

FIG. 2. *Effect of pH on mannitol dehydrogenase and xylose reductase in basidiospore extracts of Schizophyllum commune. Buffer systems for mannitol dehydrogenase (75 μmoles in 1 ml): phosphate buffer, pH 6.2 to 6.8; Tris-HCl buffer, pH 8 to 10. Buffers for xylose reductase (50 μmoles in 1 ml): acetate buffer (pH 5); phosphate buffer (pH 6.2 to 6.8); Tris-HCl buffer (pH 8.0 to 10.0). Mannitol dehydrogenase conditions (in 1 ml): D-mannitol, 100 μmoles; NAD, 0.4 μmole; enzyme extract, 0.35 mg of protein. Xylose reductase (in 1 ml): D-xylose, 100 μmoles; $NADPH_2$, 0.10 μmole; enzyme extract, 0.25 mg of protein. Temperature, 30 C.*

polyols could not be attributed to a lack of enzymes of polyol metabolism. Cell-free extracts of spores catalyzed an NAD-dependent oxidation of D-mannitol. Significant activity was also seen with D-sorbitol, but no reaction was observed with *i*-inositol or *i*-erythritol. Polyol oxidation was favored in alkaline medium; data obtained for mannitol oxidation are shown in Fig. 2. The K_s calculated from a Lineweaver and Burk plot of experiments performed at *p*H 9 was 5.6×10^{-3} M for mannitol and 2.4×10^{-4} M for NAD. The enzymatic product of mannitol oxidation gave a purple color with the cysteine-carbazole test, and showed an absorption maximum between 555 and 560 mμ, which is characteristic of ketohexose. In addition, the product of mannitol oxidation gave a cherry-red color with the Roe test. These data, taken in conjunction with the finding that $NADH_2$ oxidation was enhanced by D-fructose but not by L-sorbose, indicated that fructose is the likely product of mannitol oxidation by extracts of *S. commune*.

Pentitol oxidation was also demonstrated in alkaline medium with cell-free extracts of ungerminated basidiospores. Specifically, D-xylitol oxidation was shown with either NAD or NADP. Dialysis of enzyme extracts eliminated the endogenous reduction of both pyridine nucleotides, although considerable loss in activity of the NADP-dependent xylitol oxidation was observed (Fig. 3). The NADP-coupled oxidation of xylitol by dialyzed extracts yielded a product which reacted with orcinol and showed maximal absorbance at 665 to 670 mμ and suggested pentose. Reduction of NADP was also observed with L-arabitol as substrate, but no significant reaction occurred with D-arabitol and D-ribitol. The NAD-coupled oxidation of xylitol by spore extracts gave a product which was also positive with the cysteine-carbazole test. However, maximal absorbance at 540 to 545 mμ suggested the presence of a ketopentose, possibly xylulose.

Reduction of ketohexose, hexose, and pentose by spore extracts. The reverse reaction of mannitol oxidation, the oxidation of $NADH_2$ by fructose, was also demonstrated with extracts of basidiospores. No reduction of fructose occurred with $NADPH_2$ nor with L-sorbose or fructose-6-phosphate in the presence of $NADH_2$ (Fig. 4). In contrast with the K_s calculated for mannitol (5.6×10^{-3} M), the corresponding value obtained for fructose reduction was 5×10^{-2} M for fructose; in the case of pentose reduction, a value of 7.5×10^{-2} M was found for D-xylose (see below). Low-affinity constants for polyol dehydrogenases have also been reported in *Candida utilis* (Horecker, 1962).

Crude extracts of spores also catalyzed the oxidation of $NADPH_2$ by aldohexoses, including D-mannose, D-glucose, and D-galactose; the last

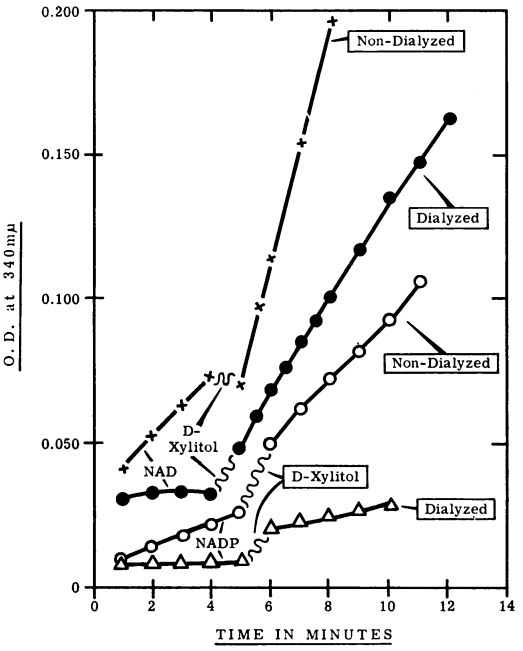

FIG. 3. *Xylitol oxidation by basidiospore extracts of Schizophyllum commune. Cuvette contained (in 1 ml): D-xylitol, 100 μmoles; NAD or NADP, 0.4 μmole; Tris-HCl buffer (pH 9), 74 μmoles; enzyme extract, 0.3 mg of protein. Temperature, 30 C.*

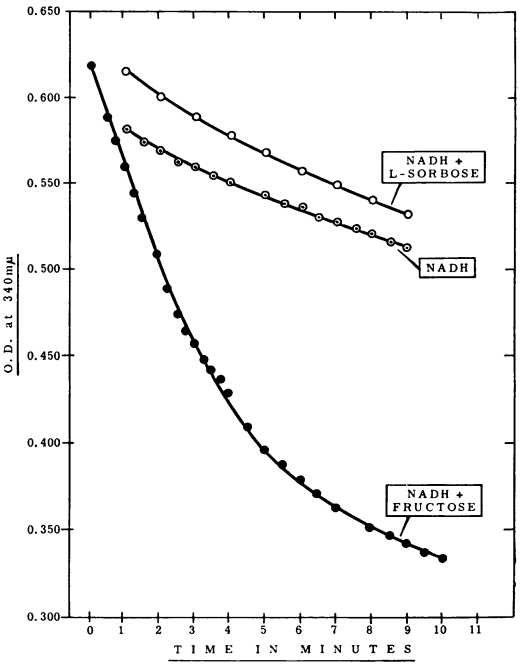

FIG. 4. *Enzymatic reduction of D-fructose by spore extracts of Schizophyllum commune. Cuvette contained (in 1 ml): ketohexose (where indicated), 100 μmoles; $NADH_2$, 0.10 μmole; Tris-HCl buffer (pH 8), 74 μmoles; enzyme extract, 0.25 mg of protein. Temperature, 30 C.*

two substrates are compared in Fig. 5. No significant enhancement of $NADPH_2$ oxidation was evident with L-sorbose or L-rhamnose. Aldohexose reductase activity was specific for $NADPH_2$, and no increased oxidation of $NADH_2$ was observed with either glucose or galactose. Pentose reduction likewise appeared dependent upon $NADPH_2$ oxidation, and optimal activity for D-xylose reduction was at *p*H 6.8 (Fig. 2). Oxidation of $NADPH_2$ was also enhanced by L-arabinose, whereas D-arabinose, D-ribose, and D-lyxose were inactive as substrates.

Polyol enzyme patterns during morphogenesis. Additional information concerning the role of polyols in growth and development of *S. commune* came from an examination of the specific activities of relevant enzymes during basidiospore germination, mycelial growth, and fruiting (Table 1). Whereas the NAD-coupled polyol dehydrogenase system showed an increase in specific activity in germling extracts, the highest activity of mannitol dehydrogenase was observed in extracts of vegetative mycelium. Moreover, all of the enzyme activities of polyol metabolism appeared to be reduced considerably in extracts of dikaryotic fruit bodies, compared with homokaryotic mycelia.

Effect of carbon sources on enzymes of polyol metabolism. The xylose reductase of *Penicillium chrysogenum* has been detected only in extracts of mycelium cultured in a medium containing D-xylose as the carbon source, and it therefore appears to be an inducible enzyme in this filamentous fungus (Chiang and Knight, 1959; Kornfeld and Knight, 1962). Since the present investigation provided evidence for xylose reductase and several other enzyme activities of polyol metabolism in glucose-grown cells of *S. commune*, it appeared of interest to examine the levels of pertinent enzymes in xylose-culture mycelium. No significant elevation in enzyme activities, including xylose reductase and xylitol dehydrogenase, was evident, nor were the ratios of various enzymes appreciably influenced by the nature of the carbon source in the culture medium. Enzyme activities for the oxidation of mannitol and xylitol, as well as the reduction of fructose, galactose, and xylose, were also demonstrated in extracts of mycelium cultured on a glucose medium in which the labile ingredients were sterilized by filtration.

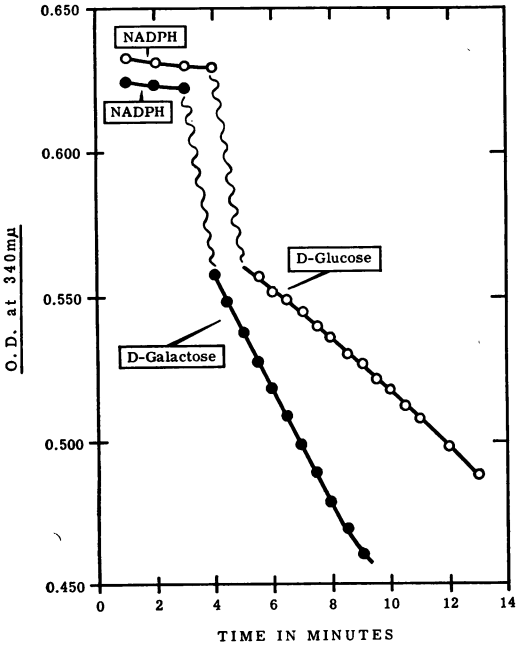

FIG. 5. *Aldohexose reduction by enzyme extracts of basidiospores of Schizophyllum commune. Reaction mixture (in 1 ml): aldohexose (as indicated), 100 μmoles; $NADPH_2$, 0.10 μmole; phosphate buffer (pH 6.8), 40 μmoles; enzyme extract, 0.3 mg of protein. Temperature, 30 C.*

TABLE 1. *Enzyme activities of polyol metabolism during morphogenesis of Schizophyllum commune on glucose medium*

Enzyme reaction	Change in optical density at 340 mμ per min per mg of protein			
	Spore	Germling	Mycelium	Fruit
Mannitol oxidation (NAD)	0.183	0.420	1.833	0.258
Xylitol oxidation (NAD)	0.090	0.138	0.450	0.102
Xylitol oxidation (NADP)	0.060	0.040	0.060	0.010
Fructose reduction ($NADH_2$)	0.200	0.181	0.670	0.155
Galactose reduction ($NADPH_2$)	0.066	0.127	0.146	0.010
Xylose reduction ($NADPH_2$)	0.266	0.100	0.350	0.032

DISCUSSION

The present study draws attention to the intermediary metabolism of polyols by the wood-rotting basidiomycete *S. commune*. Exogenous polyols, including mannitol and glycerol, stimulate the cellular respiration of germlings and sustain vegetative growth of this mushroom, even though these carbohydrates appear to be inactive as respiratory substrates for the basidiospore. In addition, polyols (including xylitol and mannitol) do not support spore germination in *S. commune* (Niederpruem, *unpublished data*). The inability to utilize exogenous polyols at this stage of the developmental cycle cannot be explained by a lack of enzymes involved in polyol metabolism, for significant levels of mannitol dehydrogenase and xylose reductase are readily demonstrated in basidiospore extracts. However, cell-free preparations of germlings and especially vegetative mycelium of *S. commune* show increases in the specific activity of mannitol dehydrogenase. Whether this apparent increase in enzyme activity is a reflection of the activation of pre-existing protein, or rather the synthesis of additional enzyme, remains a matter of conjecture. On the other hand, the possibility that specific changes in the intracellular ratios of NADP to $NADPH_2$ and NAD to $NADH_2$ could play an important role in the regulation of polyol metabolism has not been excluded, nor has the occurrence of an inhibitor of polyol metabolism in the basidiospore been ruled out.

The NAD-coupled polyol dehydrogenase system of *S. commune* catalyzes the oxidation of xylitol, mannitol, and sorbitol, and is favored in alkaline medium. Similar systems have been described in *Candida utilis* (Arcus and Edson, 1956; Chakravorty et al., 1962), *C. albicans* (Veiga, Bacila, and Horecker, 1960), and *Penicillium chrysogenum* (Chiang and Knight, 1959). In comparison, the NAD-dependent polyol dehydrogenase of *Piricularia oryzae* (Yamada et al., 1961) and *Coccidioides immitis* (Lones and Peacock, 1964) functions at the level of phosphorylated intermediates in the conversion of fructose-6-phosphate to mannitol-1-phosphate, whereas the reduction of fructose to mannitol in *A. campestris* (Edmundowicz and Wriston, 1963) and *Geotrichum candidum* (Shu-Cheng and Kao-Hsiang, 1964) requires $NADPH_2$.

The presence of an $NADPH_2$-dependent xylose reductase and an NAD-coupled oxidation of xylitol in *S. commune* is in keeping with the occurrence of this biochemical pathway in yeast and filamentous fungi; the conversion of aldopentose to ketopentose apparently proceeds via polyol rather than by direct isomerization as in bacteria (Chiang and Knight, 1960; Veiga et al., 1960). The K_s for D-xylose is 0.075 M in *S. commune*, compared with 0.09 M in *P. chrysogenum* (Chiang and Knight, 1959); maximal activity for both enzymes is near neutrality. Unlike the xylose reductases of *C. albicans* (Veiga et al.,

1960) and *P. chrysogenum* (Chiang and Knight, 1959) which require prior growth in xylose medium for maximal activity, and hence appear to be inducible enzymes, no differences in levels of xylose reductase and other enzymes of polyol metabolism were detected in extracts of *S. commune* cultured on glucose or xylose media. Xylose reduction with $NADPH_2$ has also been reported in *Oospora lactis* cultured on barley-malt medium (Moret and Sperti, 1962). Aldose- and keto-hexose-reductase activities may complement the usual pathways of carbohydrate utilization in *S. commune*, and thereby facilitate the interconversion of several sugars and polyalcohols during growth and morphogenesis. In addition, aldohexoses and pentoses may play an important role as hydrogen acceptors for $NADPH_2$ which arises through the oxidation of glucose-6-phosphate and 6-phosphogluconate in *S. commune* (Niederpruem, Updike, and Henry, 1965). The consideration of these possibilities illustrates the complexity of the cellular control mechanisms which could regulate polyol metabolism in this mushroom.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-04603 from the National Institute of Allergy and Infectious Diseases.

The authors gratefully acknowledge the technical assistance of Dolores Gutwein.

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