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Kinetics, Nutrition and Inhibitor Properties of Basidiospore Germination in Schizophyllum commune

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The delineation of respiratory characteristics of ungerminated basidiospores of the wood-rotting mushroom *Schizophyllum commune* has shown that a variety of carbohydrates including sucrose, glucose, fructose and xylose evoke considerable oxygen consumption while none of the polyhydric alcohols (polyols) are active at this particular stage of development (Niederpruem, 1964). On the other hand, after germination of these propagules on glucose-asparagine medium certain polyols, such as mannitol and arabitol, are active as respiratory substrates. In addition, mannitol supports mycelium growth and fruitbody formation in *S. commune* (Niederpruem, Hobbs and Henry, 1964). Furthermore, mannitol dehydrogenase and aldose reductase are present in ungerminated basidiospores and subsequent stages of the developmental cycle (Niederpruem, Hafiz and Henry, 1965). When viewed as a whole, these studies suggest that some important and as yet undisclosed physiological change must accompany polyol utilization during basidiospore germination in *S. commune*.

Basidiospore germination in *S. commune* primarily involves cell elongation and vacuolization prior to nuclear division and delimitation of presumptive vegetative hyphae by complex dolipore septa and associated parenthesomes (Bakerspigel, 1959; Voelz and Niederpruem, 1964). Although exogenous sugar alcohols do not enhance basidiospore respiration, the possibility remained that polyols may support spore germination in *S. commune*. Moreover, there is no specific information available concerning the overall nutritional requirements of the germination process in this mushroom. The present investigation offers data dealing with the kinetics, nutritional requirements and inhibitor properties of basidiospore germination in *S. commune*.

Materials and Methods

Culture Conditions. Basidiospores shed from dikaryotic fruit-bodies of *S. commune* Fr. were obtained solely from the cross *699 A41B41* × *845 A51B51*. Spore depositions which accumulated below sporulating fruits in sealed Petri dishes for 15 hrs at room temperature were suspended in sterile distilled water and served as

inocula for germination experiments. Basidiospore germination was studied by inoculating 75 ml of liquid minimal medium (NIEDERPRUEM, HAFIZ, and HENRY, 1965) with 25 ml of spore suspension (0.25—0.40 mg dry wt cells) contained in a DeLong culture flask (250 ml) equipped with a Morton stainless steel closure (Bellco Glass, Inc., Vineland, N. J.). Incubation was performed at 25° C (± 0.5) with aeration (180 oscillation/min) in a shaker incubator (Model G 27, New Brunswick Scientific Co.).

Measurements of Basidiospore Germination. Samples (e.g. 5 ml) were removed aseptically at various intervals and the absorbancy measured in a Klett-Summerson Photoelectric Colorimeter equipped with a red filter (No. 66). Dry weight determinations were made by harvesting cells with centrifugation (2,000 × g, 15 min),

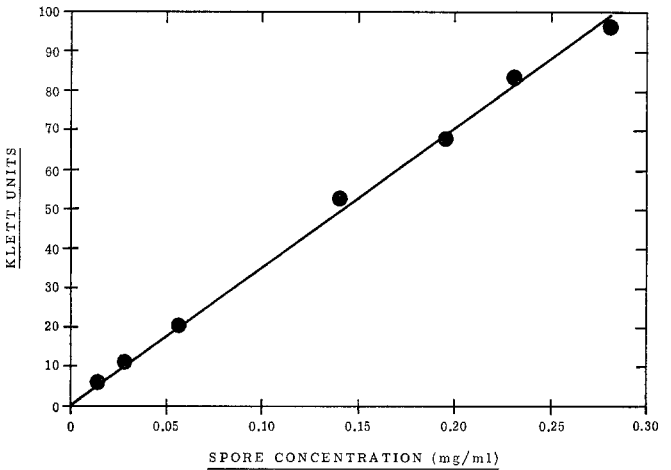

Fig. 1. Relationship between basidiospore dry weight of *Schizophyllum commune* and Klett units

washing the sedimented cells with distilled water and recentrifugation two additional times; the final cell sediment was placed in tared weighing cups at 100° C until constant weight was obtained. The linear relationship between basidiospore dry weight and absorbancy is shown in Fig. 1.

Measurements of increase in basidiospore length were performed by periodic sample removal and microscopic examination of cell populations with a Zeiss phase contrast microscope equipped with an ocular micrometer while continual microscopic observation of individual basidiospore germination was performed on minimal medium + gelatin (20%)-coated slides at room temperature.

Evaluation of Nutrition. Since basidiospore germination in *S. commune* primarily involves cell elongation rather than a conspicuous morphological stage equivalent to germ-tube emergence seen in other fungi, germlings are regarded in the present work as elongated basidiospores devoid of complex septations while vegetative hyphae are defined as those cells delimited by one or more cross-walls. The nutritional requirements for basidiospore germination were ascertained using single carbon sources supplied at a level equivalent to glucose-carbon while individual nitrogen sources were evaluated using an amount equal to asparagine-nitrogen, respectively. Complex organic nitrogen sources were supplied at 0.1% (w/v). All of the medium ingredients were autoclaved together. Sugars and related substances were purchased from Pfanstiehl Laboratories, Inc. (Waukegan, Illinois); all amino acids were obtained from Cyclo Chemical Corporation (Los Angeles, California).

Results

A. Morphological Sequence of Events in Basidiospore Germination

Basidiospore germination was previously described in *S. commune* on Sabouraud dextrose agar medium plus yeast extract (BAKERSPIGEL, 1959). However, there is no information concerning germination and direct measurements of cell elongation of *S. commune* in a chemically defined glucose-asparagine minimal medium utilized throughout most of

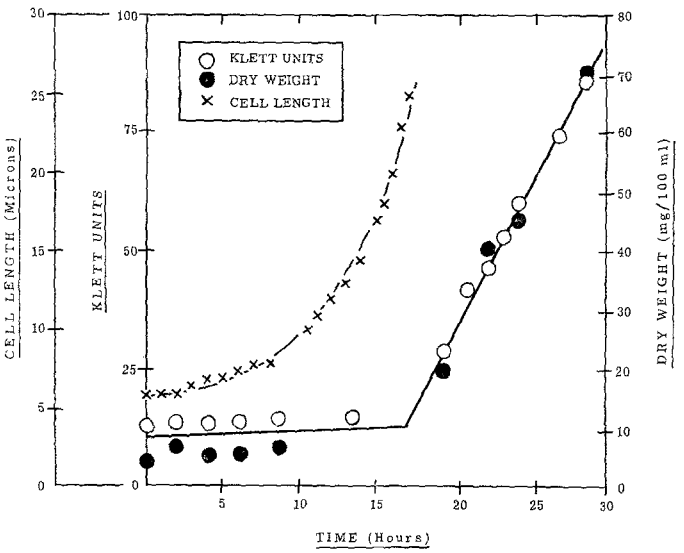

Fig. 2. Kinetics of basidiospore germination in *Schizophyllum commune*

the present investigation. Consequently, continuous microscopic observations were performed on the same individual basidiospore in minimal medium over a period of 30 hrs and this entire process was repeated on two separate occasions. The following description represents cytological features consistent upon repetition.

Freshly shed basidiospores each contained two nuclei and were devoid of cross-walls (septa). The faint, centrally located nuclei showed no signs of dark inner bodies (nucleoli ?) prominent later in germination. Cell elongation was not immediate and commenced between 5 and 8 hrs later (see Fig. 2). During this initial 8 hrs, intracellular granules (mitochondria ?) moved irregularly with occasional aggregations terminally and along the periphery of the cell while both nuclei became increasingly difficult to distinguish. Following this period, both nuclei were again discernable and one appeared at each end of the young germling. At this point, each nucleus appeared to contain a faint central body. Occasionally, short chains of intracellular particles were seen to move, almost as units. By

11 hrs, the original form of the basidiospore was clearly changed and slightly sinusoid and a single prominent dark central body was seen in each nucleus. Polar vacuolization was obvious at about 12 hrs while small peripheral vacuoles were noted later as well as a large central vacuole between the two nuclei. Intracellular filaments often showing movement were also common as were changes in the position of individual nuclei after 18 hrs. Often vigorous filament action was observed near large polar vacuoles and occasionally droplets seemed to actually leave the main vacuolar body and coalesce towards the center of the germling along the cell periphery.

Nuclear division and septum formation usually occurred in cells 5—7 times their original length (BAKERSPIGEL, 1959) on solid medium but considerably greater elongation preceded cross-wall formation in liquid medium (see Fig. 5). In glucose-asparagine minimal medium, the first indication of nuclear division was a marked granularity of the dark central nuclear body which culminated in the appearance of a prominent clear spot within this area. The nuclei gradually faded from view and intense filament action occurred near the ends of the germling. Finally septations appeared at these positions and the hypha, now 88 microns in total length, was composed of a short uninucleate cell (23 μ), a middle binucleate cell (40 μ) and a terminal uninucleate cell (25 μ). The entire process of nuclear division and septa formation required approximately 15—20 min.

It is important to note here that this particular sequence of events is not invariant, and often young hyphae are observed to contain only two cells. The shorter cell is uninucleate and the longer cell may contain two or three nuclei, before additional septations are formed. The regulatory devices which govern these changes are not currently understood.

B. Kinetics of Basidiospore Germination

The study of basidiospore elongation, as evaluated by absorbancy, dry weight increase as well as change in cell length, revealed a lag period of approximately 15—20 hrs for the former two events and approximately 8—10 hrs for individual basidiospore elongation to commence. These findings are shown in Fig. 2. If either the carbohydrate or nitrogen source was omitted from the culture medium, the transition from the lag period to the phase of maximum absorbancy did not occur, nor did any increase in absorbancy result if the basidiospores were incubated in only distilled water. The absorbance during the lag period was essentially independent of the inoculum size as well as the initial concentration of either glucose or $(NH_4)_2SO_4$. On the other hand, the phase of maximum absorbancy change was directly proportional to the amount of inoculum and to initial limiting levels of either carbohydrate or nitrogen source. The relation-

ship between absorbancy change and inoculum size is shown in Fig. 3 while the dependency of maximum absorbancy change on initial concentration of glucose and either L-asparagine or $(NH_4)_2SO_4$ as sole nitrogen sources is summarized in Table 1. All subsequent studies were performed with saturation-levels of D-glucose (15 mg/ml) and either L-asparagine (1 mg/ml) or $(NH_4)_2SO_4$ (1 mg/ml).

The lag phase in basidiospore germination recognized by absorbancy measurements was not eliminated by various levels of either the primary

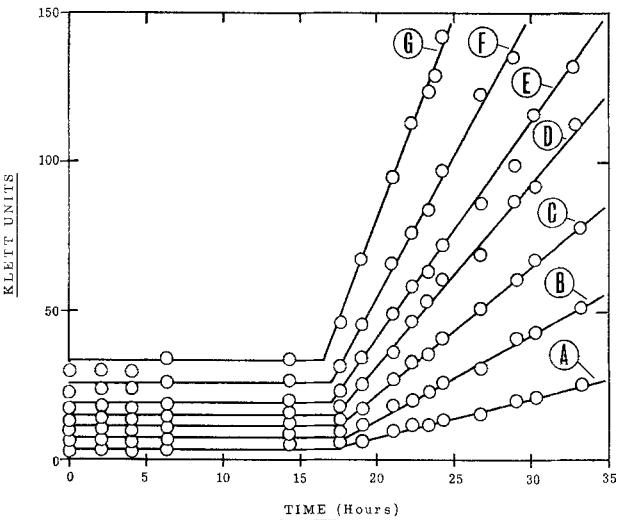

Fig. 3. Effect of inoculum size on kinetics of basidiospore germination in *Schizophyllum commune*. Initial spore concentration (µg/ml): A 1.6; B 4.0; C 14.0; D 21.0; E 26.0; F 47.0; G 68.0

carbohydrate or nitrogen sources within the limits outlined in Table 1 nor by the qualitative nature of the individual growth substrates (see later). In addition, the lag period was not removed by various treatments including incubation in the light (cool white GE fluorescent lamp, 250 ft-c $\pm$ 50) or dark, inoculum size, prior incubation for 1 hr at either 4° or 37°C, incubation in tightly stoppered culture flasks or in baffled bottom shake flasks, the employment of either filter-sterilized minimal medium or "spent medium" (e.g. 26-hr germling culture filtrate), the presence of furfural (10^{-5} M or 10^{-8} M, final conc.), or the inclusion of various individual trapping agents in specially constructed center-well flasks. The latter agents included KOH (2 N), H_2SO_4 (1 M), acetone (1%), $AgNO_3$ (20%), $KMnO_4$ (2%) and NH_4OH ($7 \cdot 10^{-2}$ M). The presence of alkali as a trapping agent strongly inhibited basidiospore germination in *S. commune* (HAFIZ and NIEDERPRUEM, 1963) although the effect was clearly dependent upon the nature of the primary nitrogen source (see later).

Table 1. *Effects of initial concentration of* D-*glucose and either* L-*asparagine or* $(NH_4)_2SO_4$ *on the phase of maximum absorbancy change during basidiospore germination in Schizophyllum commune*

Compound	mg/ml	Klett units (30-hour)
D-glucose*	15.00	150
	3.70	130
	0.94	105
	0.47	85
	0.23	50
	0.00	15
L-asparagine**	3.00	230
	1.50	220
	0.75	218
	0.37	170
	0.00	25
$(NH_4)_2SO_4$**	1.00	300
	0.50	272
	0.30	162
	0.10	54
	0.00	37

Initial Klett Reading: D-glucose culture, 12; L-asparagine culture, 18; $(NH_4)_2SO_4$-culture, 27. Each compound evaluated separately in minimal medium.

* D-glucose (15 mg/nl) constant; ** L-asparagine (1 mg/ml) constant.

C. Effects of Carbon Sources

It is evident from the foregoing kinetic studies that the degree of overall basidiospore germination can be conveniently evaluated by the magnitude of the absorbancy change subsequent to the lag period and advantage was taken of this in initially defining the nature of the individual carbon and nitrogen sources which support spore germination in *S. commune* over a 30-hr incubation period. A summary of the carbon sources examined in this regard is shown in Fig. 4. Glycogen, starch, as evaluated by microscopic observations (data not shown), and certain disaccharides containing glucose moieties or glucose plus fructose and having specific α-glucosidic linkages were active as were some monosaccharides including glucose, fructose, mannose, galactose and xylose. None of the sugar alcohols including mannitol and arabitol supported basidiospore germination, as evaluated by absorbancy, during this time period. The possibility that mannitol could be inhibitory to spore germination appeared ruled out by combination experiments with glucose, in which mannitol (7 mg/ml) was essentially without effect.

The failure of various sugar alcohols to either stimulate basidiospore respiration or sustain germination over the 30-hr incubation period employed in the present work is not in keeping with the occurrence of

certain enzymes of polyol metabolism in ungerminated spores of *S. commune* (NIEDERPRUEM, HAFIZ and HENRY, 1965). Consequently, a more critical evaluation of sugar alcohols and all other poor carbon sources was next performed in minimal medium employing $(NH_4)_2SO_4$ instead of

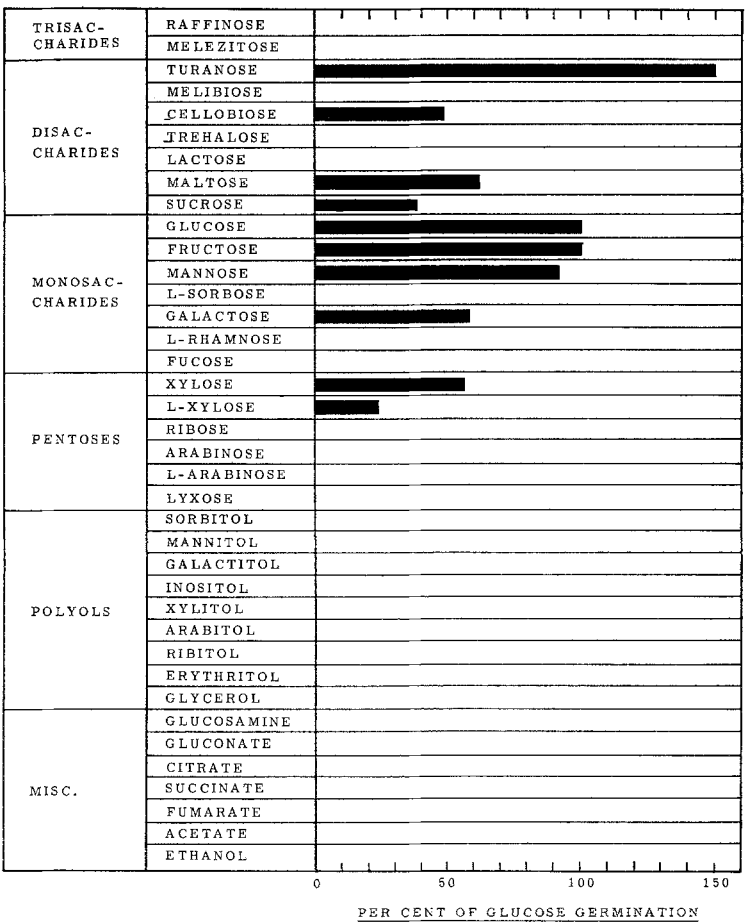

Fig. 4. Effect of carbon sources on basidiospore germination in *Schizophyllum commune*. Incubation period, 30 hr

L-asparagine as sole nitrogen source, and direct cell measurements of population changes were compared to absorbancy kinetics as well as to terminal dry weights obtained after conditions of prolonged incubation (i.e. 7 days). Attendant changes in cell length with representative carbon sources are compared with absorbancy measurements in Fig. 5. Early germination occurred with glucose while absorbancy changes prior to 30-hrs were negligible for sorbitol and only 10—25% of the cells showed signs of

microscopic elongation under the latter condition. Between 30 and 60 hrs of incubation, several individual sugar alcohols including sorbitol, mannitol, ribitol, xylitol, arabitol, erythritol and glycerol sustained absorbancy increases and basidiospore elongation. Similar results were obtained with mannitol-asparagine broth. Terminal dry weights relative to glucose were erythritol (100%), sorbitol, mannitol, ribitol and xylitol (60—75%) and

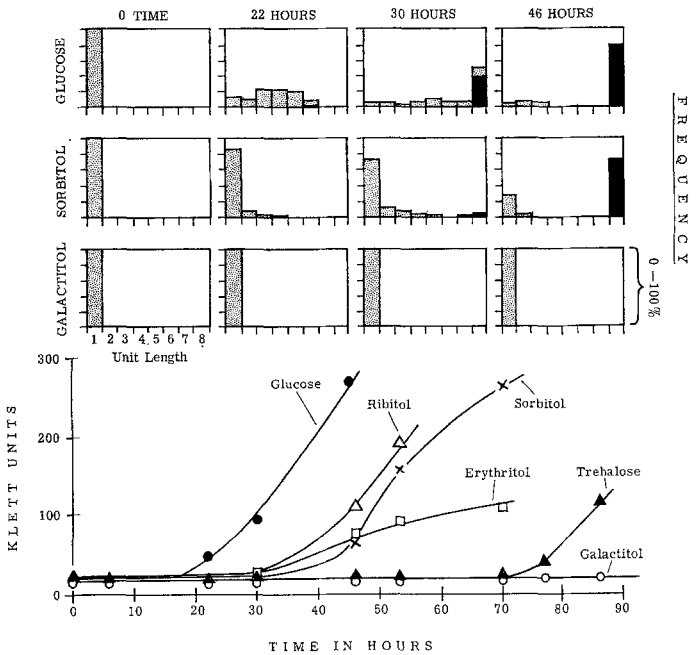

Fig. 5. Changes in size distribution and absorbancy of elongating basidiospores of *Schizophyllum commune*. Unit length 1 through 8 represents length of cells 5—10 μ, 11—20 μ, 21—30 μ, 31—40 μ, 41—60 μ, 61—80 μ, 81—100 μ and those greater than 100 μ, respectively. Black bars indicate percentage of cells having septations

D-arabitol and L-arabitol (30—50%). A second category of carbohydrates active between 30 hrs and 7 days of culture included lactose, sorbose, raffinose, melezitose, trehalose (see Fig. 5) ribose, melibiose, xylose and arabinose. A third group of carbon compounds was completely inactive after 7 days incubation and these included galactitol, inositol, succinate, acetate, gluconate, citrate, fumarate, rhamnose, fucose and inulin.

D. Effects of Nitrogen Sources

The role of various nitrogen sources was also studied individually for ability to sustain basidiospore germination with glucose as a primary carbohydrate source over a 30-hr incubation period. These results are summarized in Fig. 6. A close correlation existed between good nitrogen

sources reported for mycelium growth and fruiting in *S. commune* (NIEDERPRUEM, HOBBS, and HENRY, 1964), and those materials active in basidiospore germination. Good nitrogen substrates included complex organic nitrogen sources, ammonium salts, and certain amino acids of the L-isomeric configuration which could readily yield ammonia by conventional deamination reactions and these included asparagine, glutamine

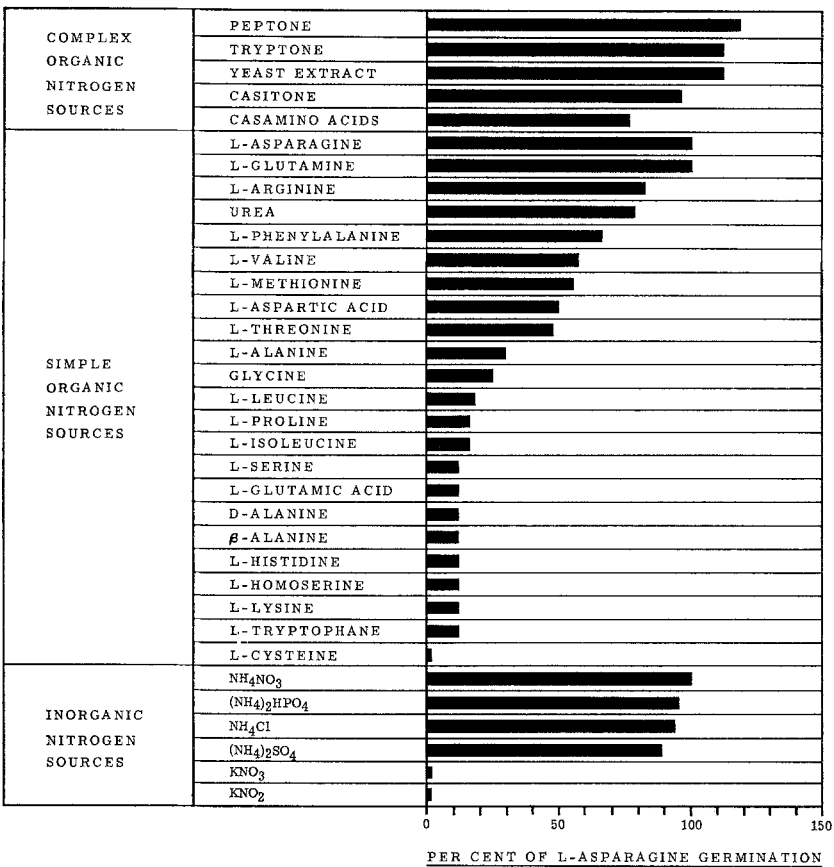

Fig. 6. Effect of nitrogen sources on basidiospore germination in *Schizophyllum commune*. Incubation period, 30 hr

and arginine. Urea also served as a good germination substrate when supplied in conjunction with glucose but failed to support germination in the absence of exogenous carbohydrate. Other intermediates of the urea cycle such as L-ornithine and DL-citrulline elicited 40 and 57% of the germination obtained with L-asparagine (not shown). Neither KNO_3 nor KNO_2 sustained germination and the latter substance was actually inhibitory to mycelium growth in *S. commune*.

Although no extensive, prolonged incubation-type experiments were performed with individual nitrogen sources, all poor nitrogen sources were also studied beyond 30-hrs and terminated at 50-hrs and from these studies it became apparent that L-leucine, L-isoleucine and L-serine were active over this extended time period while essentially all other poor nitrogen sources remained as categorized originally.

E. Effects of Inhibitors

Previous work has shown that the presence of alkali as a trapping agent inhibits basidiospore germination of *S. commune* on glucose-asparagine minimal agar medium; the additional finding that the amount of alkali required to arrest germination was related to the initial inoculum size provided some support for the idea that the fixation of metabolic carbon dioxide may be required for cell differentiation in this mushroom (HAFIZ and NIEDERPRUEM, 1963). In the present work, alkali (KOH, 2 N) included as a trapping agent also inhibited basidiospore germination in liquid medium. However, inhibition by alkali was clearly dependent upon the nature of the nitrogen source and the results of these experiments are summarized in Table 2. While inhibition of germination was seen with alkali in simple defined media employing $(NH_4)_2SO_4$, L-asparagine or L-glutamine as primary nitrogen sources, essentially no effect was observed with alkali in combination with complex organic nitrogen sources. This appears reasonable in view of the extensive biosynthesis that may be required for germination in glucose-$(NH_4)_2SO_4$ medium; the latter could lead to a serious drainage of carbon from the tricarboxylic acid cycle which ordinarily is balanced by carbon dioxide fixation in other systems. However, available evidence does not yet permit a final decision in this matter with respect to *S. commune*.

Table 2. *Effects of alkali as trapping agent on basidiospore germination in Schizophyllum commune**

Nitrogen Source	Inhibition (%)
$(NH_4)_2SO_4$	80
L-glutamine	60
L-asparagine	50
Peptone	10
Yeast Extract	0

* Cultures sealed and incubated with 4 ml of either H_2O or KOH (2 N) contained in center wells provided with fluted filter paper wicks for 30 hrs at 25° C. Glucose (15 mg/ml) constant in all cases.

The effects of various metabolic inhibitors on basidiospore germination in *S. commune* are summarized in Table 3. The inhibitory effects of the uncoupling agent 2,4-dinitrophenol as well as sodium azide stress the importance of energy-yielding reactions for germination while strong inhibition by the organic mercurial points to a requirement for essential sulfhydryl groups in differentiation. Inhibition by cycloheximide (actidione) and the amino acid analogues lends support to the idea that pro-

Table 3. *Effects of metabolic inhibitors on basidiospore germination in Schizophyllum commune on glucose-asparagine minimal medium*

Compound	Concentration	Inhibition (%)
Cycloheximide (Actidione)	10.0 μg/ml	93
	1.0 μg/ml	85
	0.1 μg/ml	36
L-ethionine	500 μg/ml	78
	100 μg/ml	62
	50 μg/ml	47
	10 μg/ml	28
p-fluoro-DL-phenylalamine	500 μg/ml	88
	100 μg/ml	85
	50 μg/ml	83
	25 μg/ml	76
	10 μg/ml	72
2-deoxy-D-glucose*	6.10×10^{-2} M	65
	3.05×10^{-2} M	58
	6.10×10^{-3} M	30
	3.05×10^{-3} M	12
Sodium azide	1×10^{-3} M	100
	1×10^{-4} M	100
Phenylmercuric acetate	1×10^{-4} M	100
	1×10^{-5} M	100
2,4-dinitrophenol	1×10^{-3} M	100
	1×10^{-4} M	0

* Tested in combination with D-glucose (6.10×10^{-2} M) held constant.

tein synthesis is a vital part of the germination process; the former substance also inhibits growth of vegetative mycelium of *S. commune* (PARAG, 1961). Complete reversal of germination inhibition by *p*-fluoro-DL-phenylalanine (100 μg/ml) was achieved with L-phenylalanine (500 μg/ml). Also noteworthy is the inhibitory action of 2-deoxy-D-glucose on basidiospore germination; whether this inhibition is related primarily to an effect on glucose uptake or rather to the subsequent metabolism of carbohydrate in *S. commune* remains to be established.

Discussion

Basidiospore germination in *S. commune* is described grossly as cell elongation, followed by nuclear division and cross-wall formation in the delimination of the vegetative hypha. The present work attempts to define the kinetics and nutritional requirements of the initial cell elongation phase of spore germination in this mushroom. Measurements of changes in absorbancy as well as dry weight revealed an apparent lag period of approximately 15—20 hrs, followed by a significant increase in the rate of both processes. Furthermore, continual microscopic observations of single basidiospores provided additional support for the

occurrence of an actual lag phase in individual basidiospore elongation also, although the time comprising the elongation lag phase was significantly shorter than the absorbancy lag period in germination. The heterogeneity of population dynamics revealed by measurements of elongation, could be a factor responsible for these differences. However, by all of these criteria, basidiospore elongation did not occur immediately upon contact with suitable substratum nor could the absorbancy lag period be eliminated by a variety of treatments, some of which are known to relieve self-inhibition of spore germination in other fungi. A possible clue to the physiological changes which occur during this early period of cell elongation in *S. commune* comes from the finding that a nicotinamide adenine dinucleotide phosphate (NADP)—coupled glutamate dehydrogenase increases 5—7 fold in specific activity during the initial 8—12 hrs in glucose—$(NH_4)_2SO_4$ minimal medium, as contrasted to only slight changes in either a nicotinamide adenine dinucleotide (NAD)—linked glutamate dehydrogenase or an NADP-dependent 6-phosphogluconate dehydrogenase in this mushroom (DENNEN and NIEDERPRUEM, 1965).

The evaluation of absorbancy changes offered a convenient procedure with which to determine the overall nutritional requirements of basidiospore germination in *S. commune* and these data may now be collated with carbohydrates shown to be active in the respiration of ungerminated basidiospores (NIEDERPRUEM, 1964). Substances stimulatory to spore respiration and germination included sucrose, maltose, glucose, fructose, mannose, galactose and xylose, therefore suggesting the presence of constitutive enzymes for the utilization of these particular carbon sources. On the other hand, the β-glucoside cellobiose did not enhance spore oxygen consumption yet supported germination to the extent of 45% of the glucose-medium within 20—30 hrs. The possibility that β-glucosidase activity is inducible in *S. commune* is supported further by the finding that a 20-fold increase in *p*-nitrophenyl-β-D-glycopyranosidase activity occurs in culture-filtrates of cellobiose-grown cells when compared to glucose-cultures of vegetative mycelium (WILSON and NIEDERPRUEM, 1966).

The acquisition of the ability to utilize exogenous sugar alcohols for cellular respiration during basidiospore germination on glucose-asparagine medium (NIEDERPRUEM, HAFIZ, and HENRY, 1965) and for spore germination after prolonged incubation on either mannitol-asparagine or mannitol-$(NH_4)_2SO_4$ media represent additional complexities. This situation differs from conventional induced enzyme systems, for certain enzymes of polyol oxidation already occur in ungerminated basidiospores of *S. commune*, and the cellular respiration of germlings of this mushroom is enhanced by specific sugar alcohols only after incubation in glucose-asparagine broth (NIEDERPRUEM, HAFIZ, and HENRY, 1965). Moreover,

growth of purely vegetative mycelium on mannitol medium does not lead to increased levels of mannitol dehydrogenase in *S. commune* (WILSON and NIEDERPRUEM, unpublished observations). Examination of the specificity of sugar alcohols stimulatory to germling respiration and spore germination shows that polyols (e.g. xylitol, mannitol and sorbitol) are active under both conditions while galactitol and inositol are completely inactive in both circumstances. Conceivably, permeability changes with respect to sugar alcohols may emerge under these conditions. Alternatively, multiple forms of enzymes required for polyol oxidation may be operative here or possibly ancillary enzymes of sugar alcohol utilization arise under these circumstances. Interestingly, mannitol supports mycelium growth but is inactive with spherules of the dimorphic fungus *Coccidioides immitis*, even after prolonged incubation (e.g. 96 hrs) while mannitol-1-PO_4 dehydrogenase activity was demonstrated in cell-free preparations of both forms (LONES and PEACOCK, 1964). In contrast, polyols including sorbitol, erythritol, arabitol and glycerol are also utilized after a considerable lag period for mycelium growth of *Pyrenochaeta terrestris* (WRIGHT and LETOURNEAU, 1965) while mannitol was detected in mycelium extracts after various nutritional conditions. Recent chromatographic studies of carbohydrates in *S. commune* show that mannitol and arabitol predominate in fruitbodies and basidiospores of this mushroom while reducing substances including glucose and fructose occur during basidiospore germination and mycelium growth (NIEDERPRUEM and HUNT, unpublished observations). The relation between intracellular carbohydrate pools and exogenous sugar alcohol utilization in *S. commune* and other fungi remains to be established.

Studies employing specific metabolic poisons show that basidiospore germination in *S. commune* involves energy-yielding reactions as well as protein synthesis. The definition of nitrogen sources required for germination stresses the importance of deamination reactions. Asparagine, glutamine and arginine are also good substrates for mycelium growth in several representative species of wood-rotting fungi (JENNISON, NEWCOMB, and HENDERSON, 1955). In contrast, a defined medium containing either proline or alanine as nitrogen sources supports spore germination in *Aspergillus niger* but fails to sustain mycelium growth of this fungus (YANAGITA, 1957).

A requirement for carbon dioxide in basidiospore germination of *S. commune* is implicated by the finding that alkali employed as a trapping agent strongly inhibits germination in a simple defined medium such as glucose-$(NH_4)_2SO_4$ while essentially no effect is observed with KOH in the case of complex organic nitrogen sources. Germination of conidiospores of *A. niger* is also affected by carbon dioxide (RIPPEL and BORTELS, 1927; VAKIL, RAGHAVENDRA RAO, and BHATTACHARYYA, 1961;

YANAGITA, 1957) and two different carbon dioxide-fixing enzymes have been described in *A. niger* (BLOOM and JOHNSON, 1962; WORONICK and JOHNSON, 1960).

Summary

The kinetics, nutritional requirements and inhibitor properties of basidiospore germination in the wood-rotting mushroom *Schizophyllum commune* were investigated. Measurements of changes in absorbancy and dry weight showed a lag period of approximately 15—20 hrs, followed by an abrupt increase in the rate of both processes. Individual basidiospore elongation also showed a lag phase and population changes were heterogenous in this regard.

Carbohydrates active for basidiospore germination were grouped into four categories. Those sugars active between 15 and 20 hrs included glycogen, turanose, cellobiose, maltose, sucrose, glucose, fructose, mannose, galactose and xylose. Several sugar alcohols were only active between 30 and 60 hrs incubation and these included mannitol, sorbitol, ribitol, xylitol, arabitol, erythritol and glycerol. A third category of carbohydrates active for germination required prolonged incubation between 30 hrs and 7 days and included lactose, sorbose, raffinose, melezitose, trehalose, ribose and melibiose. Compounds without activity after 7 days included galactitol, inositol, acetate, succinate, gluconate, citrate, fumarate, rhamnose, fucose and inulin.

Nitrogen sources active in basidiospore germination included complex organic nitrogenous substrates, asparagine, glutamine, arginine, urea and various ammonium salts.

Germination was inhibited by cycloheximide, L-ethionine, *p*-fluoro-DL-phenylalanine, sodium azide, 2,4-dinitrophenol, phenylmercuric acetate and 2-deoxy-D-glucose. Alkali as a trapping agent arrested germination in glucose-$(NH_4)_2SO_4$ medium but was without ill-effect in glucose peptone broth.

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