Basidiospore Germination in the Wood-destroying Fungus *Lenzites saepiaria*

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SUMMARY

Basidiospores of *Lenzites saepiaria* are not dormant and resemble vegetative mycelium in physiological properties and in the way they initiate outgrowth on a suitable substrate. Moistened spores respire significantly and autolyse in the absence of growth substrates; glucose, acetate or succinate are oxidized without lag. Optimal germinants, such as malt extract, allow outgrowth of 95 % of the spores in 4 to 5 hr.

Studies with [^{14}C]leucine, [^3H]uridine, [^3H]thymidine, [^{59}Fe]FeCl $_3$ and [^{32}P]NaH $_2$ PO $_4$ suggest that RNA and protein are synthesized at low rates which increase immediately after addition of a medium supporting germination; synthesis of DNA and Fe-porphyrins commences after approximately 2.5 hr. During germination and outgrowth, glucose is used primarily for synthesis of cellular material and little is converted to CO $_2$. Glucose is initially utilized by the hexose monophosphate shunt and the Embden–Meyerhof pathway becomes active as outgrowth progresses. Exogenous organic acids are utilized primarily as sources of energy or membrane synthesis; CO $_2$ evolution is high and incorporation of these substrates into cellular components is low. Much of the incorporation may involve the fixation of CO $_2$ produced from substrate oxidation.

INTRODUCTION

The prevalence of spore-forming organisms in nature attests to the survival advantage these structures confer. However, the roles that spores play in the life cycle of organisms may vary. The wood-rotting Basidiomycetes produce an astronomical number of spores. The great number of spores produced compared to the number that reach an environment in which they can grow would suggest that spores as dormant or resistant cells need not play a significant role in the life cycle of these organisms. If such is the case, it is reasonable to expect that 'spore germination' in these organisms is not a unique, irreversible event that transforms a metabolically inactive cell, but a process similar to the resumption of growth by vegetative hyphae. This report is concerned with spore germination in basidiospores of *Lenzites saepiaria* and the intracellular events involved in the transformation of a basidiospore into a growing hypha.

METHODS

Materials. All substrates were obtained commercially and used without further purification. Unless indicated otherwise, all compounds tested were dissolved in water and sterilized by autoclaving after adjusting pH to 5.4 to 5.5 with HCl or

KOH. The DuPont untreated cellulose film was soaked (12 hr) before use in 0.12 N-HCl and washed with distilled water. [^{32}P]sodium phosphate (monobasic), [5- ^3H]uridine, [methyl- ^3H]thymidine and [^{59}Fe]ferric chloride were obtained from New England Nuclear Corp., Boston, Mass. All other radioactive materials were obtained from Nuclear Chicago Corp., Des Plaines, Ill.

Spores of *Lenzites saepiaria* were collected from naturally produced fruiting bodies on rotting Southern Yellow Pine (*Pinus* sp.) and from laboratory-grown fruiting structures produced by a stock culture (FR-I). The spores were cast into a stream of dry air and caught on filter paper. Collected spores were stored over activated silica gel or CaSO_4 at 4°.

Mycelium of *Lenzites saepiaria* was grown by inoculating aseptically collected spores (10^6 to 10^8 /l. medium) into 2 l. of sterile 1 % malt extract. Aeration and agitation were provided by bubbling sterile filtered air (1 l./min.) through the medium. Mycelial pellets were harvested at 36 to 72 hr by filtration on a fritted glass filter, washed with 250 ml. of cold sterile distilled water, remoistened to allow complete swelling of the mass and stored at 4°. Mycelial pellets were starved to deplete endogenous reserves by shaking in sterile distilled water for 24 hr. All experiments were conducted at 30° in the dark except short-term respirometry or radio-respirometry experiments.

Germination experiments. Germination in water suspension was studied as described previously (Walkinshaw & Scheld, 1965) except that aliquots were withdrawn and plated on water agar to eliminate counting difficulties from movement of liquid films. Germination was also studied on 0.3 % purified agar (Difco) containing various substrate combinations. Agar was melted by steaming 30 min. and substrates were added after steaming. The viability of spores in all experiments was ascertained by plating samples on 1.5 % purified agar and incubating 5 hr; during this time, non-viable spores lysed. Dry weights are corrected for the percentage of non-viable spores present.

Respiration measurement. Respirometry procedures were those of Umbreit, Burris & Stauffer (1964); detailed conditions are given in the text. The continuous radio-respirometry procedures developed by Wang *et al.* (1958) were used to measure the evolution of [^{14}C]carbon dioxide from [^{14}C]labelled substrates. The column contained 10 ml. of 2+1 absolute ethanol+monoethanolamine and 200 μl . samples were removed at intervals for determination of [^{14}C]carbon dioxide evolved. Corrections were applied for decrease in column volume resulting from evaporation and sampling.

Substrate incorporation. Spores were germinated with labelled substrates according to conditions given in the text. Periodically samples were removed and washed on membrane filters (B-6 Schleicher and Schuell Co., Keene, N.H.) with cold 5 % trichloroacetic acid (TCA). The filters were placed in vials and dried before addition of scintillation fluid.

Fractionation. Radioactive mycelium or spores (about 0.1 mg.) on a 25 mm. millipore filter (Solvinert, pore size 0.45 μ) in a millipore microfiltration apparatus heated by passing hot water or steam through a jacket on the filter funnel was washed successively with 10 ml. cold water followed by: (1) four 3 ml. volumes of cold 5 % TCA; (2) a 3 ml. rinse with 95 % ethanol followed by four 3 ml. volumes of Bloors solution (ether+ethanol 1+3, v/v), the filter heated to 55° for 3 min. during each wash; (3) three washes with 3 ml. of 5 % TCA held at 90° for 15 min. and a final 3 ml.

rinse without heating; (4) four 3 ml. washes with concentrated formic acid at 90° for 3 min. during each wash; (5) four 1 min. washes with 4 % KOH at 100°. These procedures separate selectively small molecules, lipid, nucleic acids (Schneider, 1945), protein and amorphous wall fractions. Washings from each treatment were combined and 100 μ l. samples were counted for radioactivity. Wall material remaining on the filter after the hot KOH treatment was triturated overnight at 4° with 0.2 ml. 26N- H_2SO_4 , diluted to 1 ml. with water and 100 μ l. taken for radioactivity determination.

Radioactivity measurement. 12 ml. scintillation fluid [4 g. 2,5-diphenyloxazole and 0.1 g. 1,4-bis-2-(5-phenyloxazolyl) benzene/l.] was added to samples dehydrated with 2 ml. absolute ethanol, if necessary, and radioactivity assayed in a Mark I analyser (Nuclear Chicago Corp.).

Fig. 1. *Lenzites saepiarum* basidiospores at different stages of swelling and outgrowth. (1) Dry spores at 0 hr; (2) spores after wetting at 0 hr; (3) to (6) spores 1, 2, 3, 4 hr after addition of germinant.

RESULTS

Properties of basidiospores. Damp *Lenzites saepiarum* spores or dry spores exposed to moist atmosphere lost viability within a few hours. Spores in free water lost viability in 1 to 3 days at 30° or after several weeks at 4°. Thirty-six hr mycelium could be incubated at 30° in water for over a month without complete loss of viability.

Visible germination. Germination of the basidiospore appeared as a uniform swelling of the bean-shaped spore with subsequent elongation of one or both ends into a hypha (Fig. 1). At the optimal temperature (30 to 35°) and with malt extract as substrate, 80 to 90 % of the spores exhibited outgrowth after 4 hr; under less optimal conditions, equivalent growth took several days.

Respiratory activity. Powder dry spores had low respiratory activity and instantaneously began to respire rapidly (Fig. 2a) in water. Mycelium outgrowing in malt extract (Fig. 2b) had a respiration rate similar to spores (Fig. 3d) but a significantly

lower oxygen uptake. The Respiratory Quotient (R.Q.) calculated for spores respiring endogenously and germinating in malt extract suggested (Table 1) that the respiration substrates were lipid and carbohydrate respectively. Respiration was sensitive to cell concentration, particularly at a higher temperature (Table 2). Under the conditions the germination rate in a respirometer vessel lagged behind the rate achieved in more dilute suspension.

Fig. 2. Respiration by *Lenzites saeiparia* basidiospores before and after moistening (a) and by mycelium (b). For (a) desiccated spores were placed in the main chamber of a respirometer vessel and after 15 min. equilibration at 35° respiration was measured for 1 hr. Water was introduced through the vent plug, and after 15 min. equilibration, tipped into the main chamber. (b) Mycelium was grown from spores in 1% malt extract, harvested and resuspended to the original volume in water. After starvation for 24 hr the mycelium was filtered, placed in respirometer vessels at 3 to 4 mg. dry wt./vessel and incubated at 30°. ●, Endogenous; ○, malt extract.

Table 1. *Respiration of Lenzites saeiparia* spores on selected substrates

One to two mg. dry wt spores were suspended in 5 mM potassium phosphate buffer, pH 5.4, and equilibrated 15 min. before adding substrate to final vol. 2.4 ml. and measuring respiration at 30° for 60 min. Values represent averages of two to three trials of two replicate determinations. Oxygen uptake in µl./mg. dry wt./hr.

Substrate	Concentration mg./flask	Oxygen uptake	R.Q.
Endogenous	—	45	0.62
Glucose	36	61.5	0.75
Succinate (Na)	3.2	66	0.81
Acetate (Na)	1.6	82.5	0.87
Pyruvate (Na)	2.2	72	1.12
Malt Extract	80	81	0.96

Synthetic activity. The synthesis of protein ($[^{14}\text{C}]$ leucine), ribonucleic acid (RNA) ($[^3\text{H}]$ uridine) and phosphate-containing compounds ($[^{32}\text{P}]\text{NaH}_2\text{PO}_4$) was initiated immediately on wetting spores while synthesis of deoxyribonucleic acid (DNA) ($[^3\text{H}]$ thymidine) and respiratory pigments ($[^{59}\text{Fe}]\text{FeCl}_3$) started only after hyphal elongation was evident (Fig. 3). Synthesis by 36 hr mycelium under comparable conditions was similar.

Contribution of exogenous substrates to germination. Carbohydrates in water solution generally supported germination while amino acids and organic acids did not (Tables 3 and 4). Intermediate and short-chain fatty acids and some sugars, including cellobiose, inhibited germination in the presence of maltose.

Fig. 3. Studies on the metabolic events occurring during the germination and initial out-growth of *Lenzites saepiaria* basidiospores. For all experiments with labelled substrates, 0.7 mg. spores were shaken in 250 ml. flasks containing 70 ml. 1 % malt extract and labelled substrate. At intervals 5 ml. samples were removed and washed with water and then cold 5 % TCA on membrane filters. Labelled compounds were: (a) 0.05 μC . $^{59}\text{Fe}[\text{FeCl}_3]$; (b) 5 μC . $^{32}\text{P}[\text{NaH}_2\text{PO}_4]$; (c) 0.05 μC . $[\text{U-}^{14}\text{C}]\text{leucine}$; (e) 10 μC . $^3\text{H}[\text{uridine}]$; (f) 10 μC . $^3\text{H}[\text{thymidine}]$. In (c) mycelium was grown for 36 hr from spores in 1 % malt extract, harvested, starved by shaking for 24 hr in water and added at 3 mg. dry wt/vessel. For the respirometer experiments in (d), 2 mg. spores were suspended in water in a respirometer vessel. After 15 min., 1.2 ml. 8 % malt extract was added from the side arm. Per cent germination was measured in flasks removed at the indicated times.

Purified agar at 1.5 % contained adequate nutrients to support germination. Agar at 0.3 %, melted by a brief steaming, did not support germination; in this, tricarboxylic acid cycle intermediates had a marked effect on the germination rates in contrast to their inactivity in water (Fig. 4). Maltose or glucose added together with stimulatory compounds accelerated germination and subsequent outgrowth further. High levels of CO₂ replaced organic acids when maltose was present.

Table 2. *Respiration of glucose by Lenzites saepiarum spores at various cell densities*

Spores were suspended in 2 ml. 5 mM potassium phosphate buffer, pH 5.4. 200 μ moles glucose added from the side arm after 15 min. equilibration. Oxygen uptake as μ l. O₂/mg. dry wt/hr. Values are means of triplicate determinations.

Spores (mg./vessel)	Oxygen uptake	
	At 30°	At 35°
0.85	37	48
1.70	44	59
3.40	47	52
6.80	44	27

Table 3. *Germination of Lenzites saepiarum basidiospores with various carbon sources*

Spores from wild or laboratory-grown fruiting bodies were cast directly on to 1 mm. films of distilled water in glass Petri dishes and incubated at 30°. Carbohydrates at 0.1 M, amino acids (adjusted to pH 5.4 with KOH) at 0.01 M. Mean % germination recorded after 18 hr for four to six separate trials.

Carbon source	% germination
Maltose	95
Fructose	70
Glucose	65
Mannose	60
Cellulose (Cellophane)	60
Starch (soluble)	95
Xylose	55
Sucrose	55
Ribose	45
Galactose	45
Gluconic acid	35
Lactose	25
Glutamic acid	90
Aspartic acid	75

Utilization of uniformly labelled carbon compounds. Seventy-five to 80 % of the [U-¹⁴C]glucose metabolized by spores germinated in malt extract was incorporated (Fig. 5). Spores incubated with glucose in buffer (20 μ moles glucose/mg. spores) behaved similarly. Outgrowth experiments employing continuous collection procedures indicated a similar incorporation pattern by spores which had outgrown for 6 hr; there was a reduced ratio in 36 hr mycelium (Fig. 6). Spores germinated in uniformly labelled succinate or acetate incorporated 50 % or more of the labelled substrate metabolized during the first 60 min. of germination. After 60 min. incorporation declined abruptly and the spores began to evolve a major portion of the labelled substrate as CO₂ (Fig. 7). An appreciable lag in CO₂ evolution during the first 60 min. of growth of starved mycelia (Fig. 6) suggested that the same change in ratio of uptake of labelled substrate to CO₂ evolution occurred.

Table 4. *Tests of various compounds for synergy with maltose in the germination of Lenzites saepiarum spores*

The compounds were tested under the conditions given in Table 3 in maltose in water and in maltose in 0.3 % purified agar. Germination was arbitrarily considered significant if 20 % or more were germinated at 18 hr.

Stimulatory compounds	Inhibitory compounds	Compounds having no effect
Lactate	Cellobiose	Mannitol
Pyruvate	Sorbose	Sorbitol
Acetate	Rhamnose	Arabinose
Citrate	Trehalose	Glucosamine HCl
α -Oxo-glutarate	Caprate	<i>N</i> -Acetyl- α -D-glucosamine
Succinate	Caprylate	Turanose
Malate	Caproate	Myristate*
Oxalacetate	Valerate	Laurate*
Oleate*	Butyrate	
Stearate*	Propionate	
Palmitate*	Cysteine	
Leucine		
Lysine		
Alanine		
Glutamine		
Phenylalanine		
Tryptophan		
Methionine		
Arginine		

* Tested at 0.001 M.

Table 5. *Fates of [UL-¹⁴C]glucose and [UL-¹⁴C]acetate added to proliferating mycelium and germinating spores of Lenzites saepiarum*

Mycelium from a spore inoculum was harvested from malt extract culture after 36 hr, washed and incubated in 5 mM potassium phosphate buffer, pH 5.5, with labelled substrate and extracted after 4 hr. Spores germinated in malt extract plus the indicated substrates were harvested and extracted after 6 hr. Recorded as % total activity incorporated in mycelium or germinated spores.

Fraction	Mycelium		Spores	
	[UL- ¹⁴ C]glucose	[UL- ¹⁴ C]acetate	[UL- ¹⁴ C]glucose	[UL- ¹⁴ C]acetate
Cold TCA	3.9	25.2	8.1	34.6
Ethanol-ether	6.0	21.7	18.7	34.5
Hot TCA	33.0	10.7	23.1	9.8
Hot formic	27.0	26.9	21.6	11.3
Hot KOH	10.6	7.3	12.0	2.4
Residue (cell wall)	19.5	8.2	16.4	7.3

In both spores and 36 hr mycelium, a high proportion of the metabolized glucose was incorporated into nucleic acid and wall fractions. A high proportion of the incorporated acetate was found in lipid and protein fractions (Table 5). Some label from glucose was incorporated into the KOH-insoluble wall fraction in less than 1 hr after initiation of germination. During the first hr of incubation, most of the acetate incorporated was in the lipid fraction (Fig. 7). Succinate and propionate were incorporated in the same manner as acetate.

Fig. 4

Fig. 5

Fig. 4. Germination of *Lenzites saepiarum* basidiospores with various carbon sources. Agar (0.3 %) was melted by steaming for 30 min. and cooled to 55° before addition of substrates. Carbohydrates were added directly to 1 % (w/v); amino acids and organic acids were added as 0.1 M solutions to 0.01 M. Spores were cast directly on to the agar surface. Test no. 3 was made in a desiccator from which 25 % of the air was withdrawn and replaced with CO₂. Incubation at 30°. Counts were made of 100 spores/plate in 6 replicates. (1) 1 % malt extract; (2) maltose + acetate; (3) maltose + 25 % CO₂; (4) glucose + acetate; (5) glutamic acid; (6) Na acetate; (7) Na α -oxo-glutarate; (8) maltose; (9) Na succinate; (10) glucose; (11) glycine.

Fig. 5. Utilization of [¹⁴C]glucose by *Lenzites saepiarum* basidiospores germinating in malt extract. Spores germinated at 30° in 4 % malt extract in respirometer vessels with 1 μ C. (1 μ C/ μ mole) [¹⁴C]glucose. [¹⁴C]carbon dioxide was trapped in 20 % KOH on filter papers in the centre well. At intervals, germination in selected flasks was stopped by adding 0.2 ml. 2N-H₂SO₄ from the side arm. Papers were removed, damp-dried, soaked in 2 ml. of absolute ethanol and counted. Organisms were washed with cold water (2 $\times$ 5 ml.) followed by cold 5 % TCA (2 $\times$ 5 ml.) on membrane filters. Filters were dried and counted.

Table 6. Evolution of [¹⁴C]carbon dioxide by basidiospores of *Lenzites saepiarum* incubated with specifically labelled glucose at different stages of outgrowth

Spores germinated in dilute suspension in a large volume of malt extract broth. Duplicate samples containing about 2 mg. spores were withdrawn at intervals, centrifuged, washed twice and resuspended in 10 ml. of 5 mM phosphate buffer, pH 5.4. After 10 min., 1 μ mole specifically labelled glucose (0.1 μ C./ μ mole) was added and the CO₂ evolved was collected continuously.

Hours after initiation of germination	c.p.m. evolved as [¹⁴ C]carbon dioxide after 2 hr exposure to labelled substrate at 30°	
	[1- ¹⁴ C]glucose	[3,4- ¹⁴ C]glucose
0	10,774	817
1	12,000	1,237
3	18,897	2,746
8	11,872	35,567

Utilization of specifically labelled carbon compounds. In ungerminated spores and during the early stages of outgrowth most of the CO₂ evolved from glucose originated from the C-1 position indicating that the pentose phosphate shunt was the predominant route of glucose metabolism. As outgrowth progressed, there was an increasing proportion that originated from the C-3 and C-4 positions suggesting that glycolytic activity became dominant (Table 6).

Fig. 6. Utilization of [¹⁴C]glucose and succinate by *Lenzites saepiaria* mycelium outgrowing in malt extract. Mycelium was grown from spores in 1 % malt extract, harvested at 36 hr, starved by incubation for 24 hr in water and 4 mg. added to 25 ml. medium in 125 ml. Erlenmeyer flasks fitted with side arms and an inlet tube for scrubbing gas. The medium was 1 % malt extract with 1 μ C. [U-¹⁴C]glucose or 0.75 μ C. [U-¹⁴C]succinate. After 10 min. equilibration the mycelium was added from the side arm. [¹⁴C]CO₂ evolution was measured continuously (see Methods). Incorporation of label was measured at the end of the experiment by filtering a sample and washing with cold 5 % TCA on a membrane filter and counting. CO₂ evolution as % total carbon utilized.

Table 7. Evolution of [¹⁴C]carbon dioxide from specifically labelled propionate by spores of *Lenzites saepiaria* germinating in malt extract

After 10 min. equilibration, spores (about 2 mg.) were dumped from the side arm of a 125 ml. flask into the main compartment containing 25 ml. of 1 % malt extract and 0.5 μ moles Na propionate. The flask was fitted with an inlet tube for scrubbing gas which flowed into an absorption column. The absorption column contained initially 10 ml. of 2:1 ethanol-ethanolamine and samples for CO₂ estimation were withdrawn hourly. Incorporation was measured at the end by withdrawing a sample of spores, filtering onto a membrane filter, washing with cold 5 % TCA and counting. CO₂ evolution as % total carbon taken up (CO₂ evolved + propionate incorporated) in 4 hr.

Incubation (hr)	[¹⁴ C]carbon dioxide derived from position		
	C-1	C-2	C-3
1	2.6	0.1	2.1
2	23.7	6.9	12.6
3	48.4	21.1	24.5
4	82.2	41.0	35.5

The high R.Q. values suggested that CO_2 originated from simple decarboxylation of organic acids. Experiments with several specifically labelled organic acids, including propionic acid (normally considered an inhibitor of fungal growth), indicated that utilization was via normal pathways (Table 7).

Fig. 7

Fig. 8

Fig. 7. Utilization of $[^{14}\text{C}]$ succinate and acetate by *Lenzites saepiaria* basidiospores germinating in malt extract. Spores germinated at 30° in 4% malt extract in respirometer vessels with $0.75 \mu\text{C}$. ($1 \mu\text{C}/\mu\text{mole}$) $[U-^{14}\text{C}]$ succinate or $[U-^{14}\text{C}]$ acetate. $[^{14}\text{C}]$ Carbon dioxide was trapped in 20% KOH on filter papers in the centre wells. At intervals, germination in selected flasks was stopped by adding 0.2 ml. 2N- H_2SO_4 from the side arm. Centre well papers were damp-dried, soaked with 2 ml. absolute ethanol and counted. Parallel samples were filtered on Teflon membrane filters and washed with cold water ($2 \times 5 \text{ ml.}$) followed by cold 5% TCA ($2 \times 5 \text{ ml.}$) or with cold water and TCA followed by hot (50°) ethanol-ether, 2:1 ($3 \times 3 \text{ ml.}$). Filters were oven dried then counted.

Fig. 8. Incorporation of $[^{14}\text{C}]$ glucose by germinating spores and outgrowing mycelium of *Lenzites saepiaria*. Approximately 0.7 mg. spores or 3.0 mg. of 36 hr mycelium were added to 250 ml. flasks containing 70 ml. of 1% malt extract and $1 \mu\text{C}$. $[U-^{14}\text{C}]$ glucose and shaken at 30° in a water bath. At intervals 5 ml. samples were washed with cold water and cold 5% TCA on membrane filters. Filters were oven dried and counted.

Fixation of CO₂ in germination. In all experiments involving CO₂ collection a lag in early CO₂ evolution was observed which implied fixation of the metabolic CO₂. Added [¹⁴C]carbon dioxide was fixed in germinating spores; incubation under 25 % CO₂ decreased incorporation of [¹⁴C]acetate by 30 % during the first hr of germination.

Utilization of reserves during germination. Lipids were apparently present in basidiospores but no significant amount of carbohydrate reserve. The absence of carbohydrate reserves was reflected in the abrupt glucose incorporation curves exhibited by both spores and mycelium (Fig. 8).

DISCUSSION

The term germination used throughout this report is probably inappropriate when applied to the process whereby *Lenzites saepiaria* basidiospores initiate outgrowth. Germination usually implies a unique and irreversible event in a specialized cell. The basidiospores of this organism appeared relatively unspecialized in spite of their origin and distinct morphology. They exhibited no remarkable degree of resistance to a harsh environment, excepting desiccation, and in all other respects were less able to survive than vegetative hyphal cells.

The physical appearance of germinating basidiospores also suggested an absence of specialization. There was no clearly defined transition point between spore swelling and hyphal elongation, and the original spore cell quickly became indistinguishable from the elongating hypha. Ultrastructurally basidiospores and mycelium are very similar (Hyde & Walkinshaw, 1966). Basidiospores contain a full complement of cell organelles and do not synthesize additional ones until outgrowth occurs. Further, no striking changes in cellular or cell wall organization occurred upon outgrowth.

Exposure of basidiospores to germination (outgrowth) conditions did not result in respiration or synthesis patterns expected of cells which must undergo metamorphosis in order to grow. Respiratory rates were high in ungerminated spores and increased only as the amount of cell material increased. There was no apparent need for synthesis of protein before outgrowth began. Incorporation curves which served as indices of RNA and protein synthesis (Fig. 3) suggest exponential growth curves having their origins at zero time. Moreover, the abrupt glucose incorporation curve suggested that enzyme systems required for the utilization of this substrate were present and fully active.

Qualitatively there was little difference in the patterns of respiration, synthesis of cellular components, or utilization of substrates by outgrowing basidiospores and vegetative mycelium. The few qualitative differences and the generally lower activity of mycelium probably reflected the presence of a substantial number of older cells with a decreased metabolic rate in the mycelium.

Most general studies of spore germination include data on respiration. The impression gained from the present studies was that respiration as measured was useful only as a general indication of relative activity. Any experiment with *Lenzites saepiaria* spores under the crowded conditions of a respirometer vessel was subject to error and should be interpreted with caution. The oxygen uptake for *L. saepiaria* spores was higher than those reported for other fungal spores (Barash, Conway & Howard, 1967; Niederpruem, 1964; Gottlieb & Caltrider, 1963; Tokoro & Yanagita, 1966; Walkinshaw, 1968). It is possible that spores of other fungi respire at a lower rate, but spore respiration has usually been measured in much heavier suspensions than

were found optimal for this organism. Outgrowth in a respirometer vessel lagged behind that in dilute suspension even though the highest oxygen uptake was observed in the denser suspensions (Table 2). Also, there was no difference in rate of outgrowth at 30° and 35° although a significant difference in oxygen uptake was observed. Respiration of various substrates and the rate of outgrowth may not be directly related (Walkinshaw, 1968).

A shift in the pathways of glucose utilization in outgrowing spores has been noted for other fungi (Newburgh & Cheldelin, 1958). While this change may represent a transformation from spore metabolism to mycelial cell metabolism, an alternative explanation is that the pentose phosphate activity remains associated with the hyphal tip, and that glycolytic activity develops in cells or parts of the cell behind the differentiating apex. In support of this, the pentose phosphate activity (as measured by the preponderance of CO₂ originating from the C-1 of glucose) during the first 8 hr remained essentially constant as did the volume of functional hyphal tip. Glycolytic activity increased steadily, paralleling the increase in cell volume. It is reasonable to expect localization of activity at or near the growing tip since this is where the requirement for pentose for nucleotides, as well as reducing power for membrane synthesis, would be greatest. Yanagita & Kogane (1963) in the *Aspergilli*, demonstrated that there is no forward transport of [³²P]-labelled compounds; further, there are large pools of nucleotides in the apex (Nishi, Yanagita & Maruyama, 1968). A similar situation might be expected in *Lenzites saepiararia*.

During the first hour of incubation in a germination medium, most of the label from exogenously added [¹⁴C]organic acids was extracted from spores in the lipid fraction. This suggested that lipid synthesis was one of the early events in basidiospores that began outgrowth. Membrane synthesis or changes in configuration would be expected as a preliminary to wall synthesis. The stimulatory effect of organic acids could thus be explained, although the high endogenous respiration and apparently adequate lipid reserve was inconsistent with a requirement for exogenous materials. CO₂ alone could stimulate germination in the same manner as organic acids and an increased CO₂ tension (Fig. 4) displaced the incorporation of [¹⁴C]acetate into cells. Obviously metabolic CO₂ was rapidly fixed. Since membrane synthesis occurred concomitantly with the initiation of outgrowth, it was likely that the CO₂ was required for lipid and membrane synthesis as suggested by Choe & Bertani (1968). One of the main roles of exogenous organic acids could be the generation of CO₂.

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