Growth kinetics of *Agaricus bisporus* mycelium on solid substrate (mushroom compost)

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Growth of *Agaricus bisporus* was studied on sterile compost and on sterile compost pre-grown with the thermophilic fungus *Scytalidium thermophilum*. Early growth was characterized by depressed, slowly growing hyphae. The mycelial radius extended exponentially in this stage. Mycelium on sterile compost switched to a fluffy growth type of radially orientated hyphae that extended at a linear rate, K_r , of 5 mm d⁻¹. On compost pre-grown with *S. thermophilum*, early growth continued up to 30 mm, then extension became linear at $K_r = 7.2$ mm d⁻¹. If cultures were well ventilated, K_r was higher. A new growth function was derived to combine both growth phases. The width of the peripheral growth zone, w , of *A. bisporus* mycelium was 3 mm. The exponential specific growth rate, μ , was calculated from $K_r/w = 2.4$ d⁻¹, which is much higher than a previous estimate of 0.19 d⁻¹. In addition to growth-promoting effects, *S. thermophilum* had inhibitory effects; the period of early growth was reduced if *S. thermophilum* was inactivated in pre-grown compost. Under commercial conditions, maximum growth rates are not exploited. The introduction of selected strains of thermophilic fungi in compost and the development of a new kind of spawn inoculum of *A. bisporus* might lead to improvements in commercial practice.

Introduction

In commercial mushroom cultivation two phases of mycelial growth of *Agaricus bisporus* may be recognized. The first phase is started by inoculating compost with cereal grains colonized with mushroom mycelium, i.e. spawn. This spawn is seeded randomly at 0.5% (v/w) in compost. After 12 d incubation, the compost is colonized with mushroom mycelium. The second phase is started with the supplementation of a 5 cm thick casing layer of peat and calcium carbonate on top of the colonized compost. This casing layer is invaded vertically by mushroom mycelium in about 10 d. Then the development of fruit bodies is stimulated by lowering the CO₂ concentration and temperature of the air in the growing room.

A better understanding of growth might reveal suboptimal culture procedures in commercial practice. Duration of mycelial growth is not only of direct economic importance, but also non-fully colonized compost and casing soil are sensitive to fungal and bacterial infections, usually resulting in reduced yield. Moreover, a precise knowledge of different phases of growth is necessary to analyse growth anomalies like

‘stroma’ in mycelial cultures that are presumably linked with degeneration of *A. bisporus* strains (Fritsche, 1966, 1970; Elliott, 1985).

Olivier & Guillaumes (1979) reported low growth rates of about 3 mm d⁻¹, although few details were given, while Straatsma *et al.* (1989) found much higher rates of about 8 mm d⁻¹. However, fast growth was preceded by a long period of accelerating growth until the mycelium was 30 mm away from the site of inoculation. The long accelerating phase of growth could mean that cells 30 mm behind the front contribute to the expansion of the front. This would be peculiar because in other fungi the widths of the peripheral growth zone responsible for maximum growth are much lower, i.e. about 2 mm (Trinci, 1971). Experimentally determined values for the extension rate, K_r , and the width of the peripheral growth zone, w , can be used to calculate the specific growth rate, μ ($\mu = K_r/w$; Trinci, 1971). Chanter & Thornley (1978) estimated the doubling time as 3.73 d, i.e. $\mu = \ln 2/3.73 = 0.19$ d⁻¹, during growth and crop development.

In the present study of the growth of *A. bisporus*, mainly sterilized compost fully colonized by the thermophilic fungus *Scytalidium thermophilum* was used as a

model substrate. The stimulatory effect of *S. thermophilum* on mycelial growth of *A. bisporus* (Olivier & Guillaumes, 1979; Straatsma *et al.*, 1989) was further analysed.

Methods

Strains. Commercial millet grain spawn of *A. bisporus* strain Horst® U1 (Somyel spawn company, Langeais, France) and *S. thermophilum* strain 15.8 (= CBS 671.88, ATCC 66938) were used.

Culture of *S. thermophilum* and preparation of model mushroom compost. Stocks and inoculum were grown on yeast/glucose agar at 45 °C in the dark. Model compost was prepared according to Straatsma *et al.* (1989) from 100 g rewetted dried and ground mushroom compost, and sterilized in small polycarbonate containers (150 × 105 × 78 mm, 1 × w × h; Twyford, Glastonbury, UK). If required, *S. thermophilum* was inoculated and cultured for 3 d. Inactivation of fully grown cultures was achieved by the addition of benomyl (10 mg active ingredient kg⁻¹) or by autoclaving cultures for 10 min at 100 °C (then *S. thermophilum* cannot be recovered from compost when tested on agar).

Culture of *A. bisporus* and growth recording. Sterile compost and sterile compost fully grown with *S. thermophilum* was dispensed in 9 and 14 cm Petri dishes at respectively 30 and 72 g per dish. Small dishes were inoculated centrally and big dishes centrally or at the edge. The lids of the Petri dishes were fixed with adhesive tape and marked with radii that crossed the inoculum: four or eight radii in the case of centrally placed inoculum and two in the case of inoculum at the edge. Dishes were routinely placed in plastic bags to prevent desiccation and were incubated at 24 °C in the dark. The position of the mycelial front was marked under a dissection microscope (12.5 ×) with a needle scratch on the lid every 1 or 2 d. When cultures were fully grown the marks were measured from the periphery of the inoculum with a ruler at 1 mm accuracy and growth parameters were calculated.

Growth was also studied in tubes according to Straatsma *et al.* (1989), and in small containers. The latter were inoculated in a vertical profile on the narrow side of the box. Growth was marked at 2–3 d intervals with a marker. In these cases early growth is difficult to measure and growth parameters of this period are indicative only.

The peripheral growth zone, *w*, was determined according to Trinci (1971).

Conditions resembling commercial cultivation. Untreated fresh mushroom compost and casing soil were used. Small containers and 1 litre glass beakers (9 cm inner diam.) were filled with 300 g compost randomly seeded with 1.1 g spawn (about 115 grains) and compacted to 700 ml. Cultures were loosely covered and incubated at 24 °C in the dark. In small containers the presence of *A. bisporus* on washed compost particles was determined (Straatsma *et al.*, 1989) at 2 d intervals. Casing soil (225 g) was placed on top of the compost in beakers after 0, 4, 8, 12 and 15 d. Casing soil invasion was marked every 2 d with a marker.

Growth parameters and calculus. The mycelial radius extended nearly exponentially in the early stage of growth and linearly 30 mm behind the point of inoculation. Growth rates are expressed as α (d⁻¹) and K_r (mm d⁻¹) and were calculated by fitting growth functions $r = r(t=0) \cdot e^{\alpha \cdot t}$ or $r = r(t=0) + K_r \cdot t$. The validity of the fitted exponential function was determined by comparing the extrapolated $r(r=30)$ with the actual value that was calculated by linear regression on data directly around $r=30$ mm. For linear functions, the actual values for $t(r=30)$ are given instead of intercepts $r(t=0)$. The

peripheral growth zone, *w*, (Trinci, 1971) is given in mm. The specific growth rate μ (d⁻¹) was calculated from K_r/w .

Colonization of compost under conditions resembling commercial cultivation occurs three-dimensionally (radial extension is one-dimensional). Its initial rate β (d⁻¹) was calculated by fitting a logistic function to data on mycelial presence:

$$p = p_{\max} / [1 + ((p_{\max}/p(t=0)) - 1) \cdot e^{-\beta \cdot t}]$$

where *p* is mycelial presence (%); $p_{\max} = 100\%$.

* Exponential and logistic functions were fitted by regression of linearized growth data (Causton, 1978).

Replication of results. Within experiments, each treatment had four to six replicates. Experiments were repeated at least once. Means of *n* experiments are given with standard deviations (SD).

Results

Growth on sterile compost

Early growth of *A. bisporus* on sterile compost in dishes was characterized by slowly- and flat-growing mycelium. Later, faster-growing sectors were formed, characterized by dense fluffy mycelium consisting of radially orientated hyphae. Ultimately, sectors appeared all over the colonies' circumference (Fig. 1). When a single spawn grain was used as an inoculum, the first sectors were formed 17 mm away from the inoculum, after 12 d incubation. Whole colonies showed fluffy growth 29 mm away from the inoculum, after 19 d. The extension rate of flat mycelium increased slowly to the constant rate of fluffy parts (Table 1). Fluffy sectors were formed earlier when 10 spawn grains were used as a single inoculum source; actual lag times, $t(r=30)$, were 3.4 ± 0.8 d shorter than the 18.2 ± 2.0 d of controls with one spawn grain ($n=2$). Sectors were formed progressively earlier in a substrate moisture range between 55 and 80% (v/w) at steps of 5% (data not shown; confirmation of results of Kligman, 1943).

When fluffy mycelium was subcultured, the fluffy growth characteristic was stable. However, reversion to slow and flat growth occurred when the amount of substrate subcultured was low, i.e. single compost particles. Reversion was also found if larger substrate lumps were stored at room temperature for 5 d prior to subculture.

In tubes the extension rate, K_r , was about 5 mm d⁻¹ but it was not truly constant. However, in small containers K_r was constant (4.7 ± 0.3 mm d⁻¹, $n=6$). The lag time lasted about 13 d, similar to that in dishes inoculated with 10 spawn grains.

Growth on compost pre-grown with *S. thermophilum*

The density of *A. bisporus* mycelium in dishes was very low initially when single spawn grains were used as

Fig. 1. Mycelium of *A. bisporus* on sterile compost. (a) Flat growth only, 11 d after inoculation; (b) sectors around almost the entire circumference 5 d later (separate cultures within the same experiment).

Table 1. Lag times and linear extension rates of *A. bisporus*

Conditions	Lag time, $t(r = 30 \text{ mm})$ (d) $\pm$ SD	Extension rate, K_r (mm d ⁻¹) $\pm$ SD	No. of expts
<i>Sterile compost</i>			
One grain in centre of small dish in bag	17.0 $\pm$ 1.3	ND	23
(Fluffy growth monitored in detail)	17.4 $\pm$ 1.5	3.6 $\pm$ 0.4	7 of 23)
<i>Compost pre-grown with S. thermophilum</i>			
One grain in centre of small and big dish in bag	13.0 $\pm$ 1.6	ND	25
Ten grains in centre of big dish in bag	11.1 $\pm$ 1.4	7.0 $\pm$ 0.5	6
Ten grains at edge of big dish in bag	12.8 $\pm$ 1.2	7.2 $\pm$ 0.3	7
Ten grains at edge of big ventilated dish	11.3 $\pm$ 0.5	7.9 $\pm$ 0.3	3
<i>S. thermophilum inactivation</i>			
Benomyl (one grain, small dish in bag)	11.9 $\pm$ 1.6	6.5 $\pm$ 0.2	3
10 min 100 °C (one grain, small dish in bag)	11.1 $\pm$ 0.6	5.8 $\pm$ 0.9	5

ND, Not determined.

inoculum. The colony was only visible under a dissection microscope. After a few days, growth in parts of the colony [in $31 \pm 19\%$ of monitored radii ($n = 25$)] was interrupted. Extending parts showed an increase in mycelial density and bulged into the empty space left by non-growing parts. As a result colonies were not circular.

Exponential functions fitted to the period of accelerating growth (up to 30 mm beyond the point of inoculation) were characterized by $r(t = 0) = 1.2 \pm 0.3 \text{ mm}$ and rate $\alpha = 0.27 \pm 0.05 \text{ d}^{-1}$. The extrapolated lag times $t(r = 30)$ were $1.0 \pm 0.8 \text{ d}$ ($n = 25$) lower than the actual ones (Table 1), indicating that extension was not truly exponential.

If compost was pre-grown with *S. thermophilum* strain 15.1 (from our own collection), or with CBS 184.64 (which has the synonymous name *Humicola grisea* var. *thermoidea*), *A. bisporus* grew more regularly than when the standard strain 15.8 was used. Values for $t(r = 30)$ were 3.1 ± 1.4 and $3.2 \pm 2.6 \text{ d}$ shorter, respectively, than controls with strain 15.8 of 13.7 ± 1.4 and $13.7 \pm 2.3 \text{ d}$ (both $n = 3$).

When 10 spawn grains were used as a single inoculum source, initial growth was regular; $r(t = 0)$ and α were $1.1 \pm 0.5 \text{ mm}$ and $0.31 \pm 0.08 \text{ d}^{-1}$, respectively. After this early growth phase, mycelium extended linearly (Fig. 2, Table 1). Occasionally growth was also monitored at the bottom of dishes. After about 10 d, mycelium at the bottom and at the surface reached equally far. When dishes were almost fully grown, the mycelium at the bottom was up to 9 mm closer to the edge of the dish than surface mycelium. Dishes placed directly in a humidified incubator ('ventilated incubation', Table 1) showed higher growth rates than dishes in plastic bags. When gas exchange was severely reduced by sealing the Petri dishes with Parafilm, growth of *A. bisporus* was completely prevented. CO_2 concentrations were at ambient levels in ventilated dishes, about 4% in dishes in

Fig. 2. Growth data of *A. bisporus* mycelium in 14 cm Petri dishes containing compost pre-grown with *S. thermophilum*, inoculated at the edge with 10 spawn grains (representative experiment). Actual lag time $t(r=30)=13.1$ d (linear interpolation between data around $r=30$ mm). The dashed line represents the linear function fitted to growth data above 30 mm: $r=-60+6.8 \cdot t$. The drawn curve fits to the functions in the Appendix: initially $r=1.15 \cdot e^{0.27 \cdot t}$ until $t=9.2$ d and $r=13.6$ mm ($=w$), then a second function with additional parameters $C=0.54$ and $b=5.54$ (interpolated $t(r=30)=12.6$ d).

bags, and above 15% in sealed dishes (0.3 ml samples analysed by gas chromatography).

In contrast to growth of single spawn grains in dishes, initial growth in tubes and small containers was regular; $t(r=30)$ values were just over 9 d. The ultimate extension rates, K_r , were 7.3 ± 0.7 ($n=21$) and 7.9 ± 0.6 mm d⁻¹ ($n=10$), respectively. The CO₂ level in small containers was below 1%. Growth in loosely capped glass cylinders (22 mm internal diam.) with a ventilation channel (11 mm diam.) along the substrate was characterized by a lag phase of more than 11 d and a high extension rate, K_r , of 8.6 ± 0.5 mm d⁻¹ ($n=5$).

In tubes, compost pre-grown with all 41 different strains of *S. thermophilum* from our collection and from the collections of CBS, CMI and ATCC caused regular and fast growth of *A. bisporus*. No differences in effects between strains were found. Only tests in dishes will help to select strains with optimal growth-promoting characteristics for composting studies.

Subculture in tubes of *A. bisporus*-colonized compost, whether originally sterile or pre-grown with *S. thermophilum*, resulted in the absence of a lag phase. Mycelium extended almost instantaneously at its maximum rate. A normal lag period was found when the compost inoculum had been stored at room temperature for 5 d or more. In dishes, subcultured compost resulted in a moderately long lag period. When single, freshly colonized compost particles were tested in dishes, some outgrowth was seen, but growth stopped within a few days.

The width of the peripheral growth zone, w , was determined in dishes by cutting peripheral mycelium.

Cutting resulted in disturbance of the particulate substrate that carried the mycelium. Therefore, our measurement of 3 mm for w is an overestimate. According to Trinci (1971), the specific growth rate, μ , of *A. bisporus* was calculated from the data found for the linear extension rate K_r and w . $\mu = K_r/w = 7.3/3 = 2.4$ d⁻¹, the doubling time $t_d = \ln 2/2.4 = 0.3$ d.

Effects of inactivation of *S. thermophilum*

The observations described above indicated inhibitory effect(s) of *S. thermophilum* on *A. bisporus* (interrupted growth, inhibited outgrowth of small compost particles carrying *A. bisporus* mycelium, and inhibited outgrowth in dishes lacking ventilation), in addition to its growth-promoting effect. Therefore the effect of inactivation of *S. thermophilum* in pre-grown compost was studied in dishes. When the fungicide benomyl was used as selective inactivator, single spawn grains of *A. bisporus* showed regular outgrowth and values for $t(r=30)$ were low (Table 1). K_r values were also low but were higher than for sterile compost. When *S. thermophilum* was completely inactivated by treatment at 100 °C for 10 min, outgrowth was even denser but the growth parameters were very similar to those for the benomyl treatment (Table 1). Similar growth parameters were found if compost pre-grown with *S. thermophilum* was autoclaved for 1 h. However, autoclaving for 2 h on two successive days (standard procedure to sterilize compost), resulted in the same growth rates as on sterile compost. Apparently growth promotion is caused, at least partly, by a non-volatile, rather heat-insensitive factor.

Alternative mathematical model

After the formation of a mycelial front in dishes, growth is two-dimensional, whereas it is one-dimensional in tubes and in small containers (following our methods). The extension rates in dishes were rather low, which might be due to the geometry of the growing colonies. A growth model was developed in which the colonized area, rather than the radius, increased at a constant rate (see Appendix). The model predicts an early period of exponential radial extension, until a peripheral zone, w , has developed. However, the calculated values of w of about 14 mm (see Appendix) were much higher than the experimentally determined value of 3 mm. The concept also predicts an increasing extension rate when the area to be colonized is narrowing. This condition occurs (for parts of the colonies' periphery only) when dishes are inoculated at the edge. However, an increased growth

Fig. 3. Presence of *A. bisporus* mycelium during growth of spawn grains dispersed in compost as in commercial cultivation. Presence is expressed as the percentage of compost particles showing recovery of mycelium on agar plates. Fitted logistic function: $p = 100 / (1 + 10.4 \cdot 10^3 \cdot e^{-0.98t})$, where p is presence (representative experiment, run simultaneously with the experiment of Fig. 4).

rate at the edge was not noticed. Colonies remained essentially circular.

Conditions resembling commercial cultivation

Under commercial cultivation conditions, spawn grains are dispersed in mushroom compost at a concentration of 5 litres per tonne (weight of non-pasteurized substrate), which corresponds to one millet grain per 6 cm³ of ready-to-spawn substrate. This volume might be considered as a sphere of 11 mm radius. According to the growth functions mentioned above, mycelia in compost will touch after 8.4 d.

Samples were taken at intervals from randomly inoculated fresh compost in small containers and the presence of *A. bisporus* mycelium was counted on small washed particles plated on agar. Logistic functions were calculated for these data (Fig. 3). The initial colonization rate, β , of *A. bisporus* was $0.79 \pm 0.12 \text{ d}^{-1}$ ($n = 3$). In parallel experiments the colonization of a casing layer on top of inoculated fresh compost in 1 litre glass beakers was measured. When the casing layer was applied immediately after inoculation or within a period of 8 d, no invasion could be seen until $9.8 \pm 1.9 \text{ d}$ ($n = 4$) after inoculation. Then, the casing soil was invaded with dense mycelium with a linear growth rate, K_r , of $6.9 \pm 0.5 \text{ mm d}^{-1}$ ($n = 4$). Casing soil applied after 8 d was colonized immediately (Fig. 4). Fusion in the compost of small mycelia, developing from single spawn grains into one large mycelium, may have occurred. This could coincide with a shift from preliminary colonization to extensive substrate exploitation.

When mycelium in compost was ready to colonize the casing layer, *A. bisporus* was present on $61 \pm 20\%$ of the compost particles ($n = 3$).

Fig. 4. Invasion of *A. bisporus* mycelium into casing soil placed on top of inoculated compost at days 0, 4, 8, 12 and 15. Growth rates were 7.0, 6.6, 6.3, 6.0 and 6.0 mm d⁻¹, and lag periods were 10.2, 9.7, 9.8, 12.5 and 15.7 d, respectively (representative experiment, run simultaneously with the experiment of Fig. 3).

Discussion

Sterile compost

Fluffy sectors (Fig. 1) always developed. Sectoring was stimulated by increasing the amount of inoculum or the moisture content of the substrate. These observations fully agree with those of Kligman (1943). Stability and reversion after subculture of sectors could be checked. Sectoring seems to be a normal developmental process in *A. bisporus* mycelium, in which no change in the genome or degeneration is involved. Degeneration of mushroom strains (Elliott, 1985) and 'stroma' formation in the casing soil have been linked with the presence of fluffy mycelium in grain spawn. However, when spawn originated from fluffy subcultures, similar cropping results as with normal spawn were found by Kligman (1943). This was largely confirmed by Fritsche (1966, 1970); degeneration was exceptional. Sigel & Sinden (1953) postulated a 'true' form of sectoring linked with strain degeneration.

Apparently *A. bisporus* shows a mode switch during mycelium growth, as observed in other fungi (Rayner & Coates, 1987; Coggins *et al.*, 1980; Sharland *et al.*, 1986). Switches in the extension rate in other fungi were found by Plomley (1959), Trinci (1974) and Butler (1984). Further research on mode switching in *A. bisporus* might help to develop methods to prevent 'stroma' formation in casing soil.

Compost pre-grown with *Scytalidium thermophilum*

Growth of single spawn grains in dishes on compost pre-grown with *S. thermophilum* (Fig. 2) was a good model for

growth in commercial practice. Extension slowly increased during 13 d and then became linear at the maximum rate of almost 8 mm d⁻¹ (Table 1). Since mushroom growers use an incubation time of 12 d, they cannot exploit the maximum extension rate. The specific growth rate, μ , was calculated to be 2.4 d⁻¹ (a doubling time of 0.3 d). Whole mycelia exploit this rate only at the periphery.

Subcultured lumps of compost carrying young *A. bisporus* mycelium caused instantaneous fast growth in tubes. Probably the lumps contained an intact peripheral growth zone that continued to extend at the maximum rate. Instantaneous fast growth depended on the size and age of the subcultured lump rather than on a *S. thermophilum* treatment of the original culture. This indicates that only a certain type of *A. bisporus* mycelium, i.e. the fluffy type, is receptive for growth stimulation caused by *S. thermophilum*. The time necessary for switching from flat to fluffy growing mycelium, together with the development of a peripheral growth zone, explains the long lag period that occurs when spawn grains are used as inoculum. It is a challenge to develop new spawns that give rise to instantaneous fast-growing mycelia.

Ventilation of dishes and tubes caused higher extension rates than observed in non-ventilated controls. Apparently volatiles produced by either *S. thermophilum* or *A. bisporus* inhibit growth of the latter. Complete inhibition coincided with high CO₂ concentrations of 15% (sealed dishes), slight inhibition with 4% (dishes in bags), while optimal growth coincided with CO₂ concentrations below 1% (small containers and ventilated dishes and tubes). Tschierpe (1959) found growth inhibition of *A. bisporus* on sterile substrate by CO₂ concentrations of 2% and higher.

The lower extension rates observed in dishes, even if ventilated, than in tubes and containers remain unexplained. This was also found for other fungi by Dickson (1935) and Trinci (1973).

Alternative mathematical model

The model based on a continuous area increase (see Appendix) predicts exponential growth for a peripheral zone, w , which agrees with the model of Trinci (1971). Calculated lag times $t(r=30)$ fitted closely to actual values and curves were well fitting (Fig. 2). However, early growth was not really exponential and the experimentally determined value for w was much lower than the value calculated in the model. Apparently hyphal branching, required for colonization of an expanding area in dishes, does not occur at the expense of the extension rate. Branching of fungi typically occurs beyond newly formed septa, possibly as a response of

inhibited apical transport of hyphal tip 'softeners' (Prosser & Trinci, 1979).

Conditions resembling commercial cultivation

After 9.8 d incubation, compost was colonized to such an extent that it was ready for subsequent colonization of a casing layer (Fig. 4). This period is only 1.4 d longer than the calculated time required for colonies to touch. Colonization of compost occurred at an initial specific rate, β , of 0.79 d⁻¹ (Fig. 3), which might be expected from the exponential extension rate, α , of 0.27 d⁻¹ (Fig. 2) during early growth (α to be multiplied by 3 for volume increase of a sphere).

The rate of compost dry matter loss and CO₂ production during casing soil invasion and cropping is initially 0.15 d⁻¹ [calculated from data of J. G. M. Amsing (1984 and personal communication)]. Chanter & Thornley (1978) estimated a very similar value for the specific growth rate, μ , of 0.19 d⁻¹. These rates are much lower than we found for the exponential colonization rate, β , (0.79 d⁻¹) or the calculated specific growth rate, μ , (2.4 d⁻¹).

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Appendix

A growth model was developed in which the colony's area, A , rather than its radius, r , increases at a rate, C , proportional to a peripheral growth zone with area P and constant width w (Pirt, 1966; Trinci, 1971). Thus $dA/dt = C \cdot P$.

One-dimensional growth in tubes with width F :

if $r < w$ then $F \cdot dr = C \cdot F \cdot r \cdot dt$, $dr/dt = C \cdot r$,
 $r = r(t=0) \cdot e^{C \cdot t}$

if $r > w$ then $b \cdot dr = C \cdot b \cdot w \cdot dt$, $dr/dt = C \cdot w$; this function is identical to $K_r = \mu \cdot w$ (Trinci, 1971), $dr/dt = K_r$, $= 7.3$ mm d⁻¹ (in text).

Two-dimensional growth in dishes:

if $r < w$ then $2\pi r \cdot dr = C \cdot \pi r^2 \cdot dt$, $dr/dt = (1/2) \cdot C \cdot r$, or
 $r = r(t=0) \cdot e^{(1/2) \cdot C \cdot t}$ (Righelato, 1975) (eq. 1)

if $r > w$ then $2\pi r \cdot dr = C(\pi r^2 - \pi(r-w)^2) dt$, or
 $dr/dt = C \cdot (2rw - w^2)/2r$, or

$dt = 1/C(r/(rw - (1/2) \cdot w^2)) \cdot dr$, and after integration:
 $t = 1/C(r/w + (1/2) \cdot \ln(r - (1/2) \cdot w)) + b$ (eq. 2)

(this function has no analytical inversed form).

[If r is big, i.e. limit $w/r \rightarrow 0$, $dr/dt = C \cdot w$ (one-dimensional growth).]

Early growth data of experiments in dishes are used to fit eq. 1. Then C is known and w can be calculated from $C \cdot w = 7.3 \text{ mm d}^{-1}$ (iteratively only growth data not exceeding w were used). In addition $t(r = w)$ is extrapolated and used in eq. 2. Then b in eq. 2 can be calculated by substituting values for $t(r = w)$, C , and w , and $r = w$.

Combined eq. 1 and 2 resulted in smooth curves. Combinations of exponential and linear functions, as described in the text, always resulted in curves with discrete gaps. Smooth curves were also given by Hsu *et al.* (1989), but were based on a different model that required a numerical solution. Fitted values of w were $10.0 \pm 1.6 \text{ mm}$ and $10.0 \pm 1.2 \text{ mm}$ in the case of one and ten spawn grains as inoculum ($n = 25$ and $n = 7$ respectively). Interpolated values of $t(r = 30)$ were respectively 2.10 ± 1.34 and $1.70 \pm 0.47 \text{ d}$ lower than the actual values in Table 1. If growth data up to $r = 30 \text{ mm}$ were used to fit eq. 1 (ignoring exponential extension only up to $r = w$), good-fitting curves were obtained (Fig. 2). Interpolated values of $t(r = 30)$ are only 0.5 ± 0.8 and $0.3 \pm 0.2 \text{ d}$ lower than the actual values. However, values for w were even 3.7 ± 2.1 and $3.7 \pm 1.5 \text{ mm}$ higher than above.

Linear functions fit well to data from dishes above $r = 30 \text{ mm}$ (Fig. 2). Interestingly, a linear function is predicted when no peripheral growth zone is considered and growth depends on the periphery only; then

$$dA/dt = C' \cdot 2\pi r, \quad 2\pi r \cdot dr = C' \cdot 2\pi r \cdot dt, \quad \text{and} \quad dr/dt = C'$$

This might be valid if mycelial branching, necessary for filling space in two-dimensional growth, occurs behind the peripheral growth zone.

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