

Influence of the Width of the Peripheral Growth Zone on the Radial Growth Rate of Fungal Colonies on Solid Media

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SUMMARY

Growth of colonies of *Rhizopus stolonifer*, *Mucor racemosus*, *Actinomucor repens*, *Absidia glauca*, *Geotrichum lactis*, *Penicillium chrysogenum*, *Aspergillus wentii*, *A. niger* and two strains of *A. nidulans* were studied. The radial growth rate (K_r) of these colonies was found to be a function of the length (w) of the leading hyphae spanning the colony's peripheral growth zone and the specific growth rate (α) of these hyphae. Thus

$$K_r = \alpha w.$$

The peripheral growth zone is the region in which hyphae are able to contribute protoplasm to the apical extension of the colony's leading hyphae. The width of the peripheral growth zone remained constant with time but varied from 423 μm . for *G. lactis* to 8660 μm . for *R. stolonifer*. The specific growth rate of the hyphae in the colony's peripheral growth zone appeared to be identical to the organism's maximum specific growth rate in submerged culture.

The width of the peripheral growth zone was not influenced by temperature or by adding an inhibitor to the medium but did vary with glucose concentration. An observed difference between the radial growth rates of the two strains of *Aspergillus nidulans*, which had almost identical specific growth rates in submerged culture, was found to be correlated with a difference between the widths of their respective peripheral growth zones. Although there was a significant difference in the length of the apical cells and hyphal compartments of the leading hyphae of these strains, the growth zone of the hyphae of each strain contained 16 septa; it is suggested that the strains differ in the frequency of septum formation but not in the rate at which the septa become plugged.

INTRODUCTION

The radius of fungal colonies (Trinci, 1969) and the length of unbranched hyphae (Trinci & Banbury, 1967) increase linearly with time (i.e. they grow at a constant rate, K_r). Thus

$$R_1 = R_0 + K_r (t_1 - t_0), \quad (1)$$

where R_1 = colony radius or hyphal length at time t_1 ; R_0 = colony radius or hyphal length at t_0 , and K_r = the growth rate constant. However, filamentous fungi grow exponentially in submerged shake-flask culture (Zalokar, 1959*a*; Trinci, 1969) as long as all nutrients are present in excess and growth inhibitors are not accumulated. Thus

$$\frac{dM}{dt} = \alpha M, \quad (2)$$

where M = organism mass/unit volume, t = time, and α is a constant known as the specific growth rate. Integration of (2) gives

$$\ln M_1 = \ln M_0 + \alpha (t_1 - t_0), \quad (3)$$

where \ln = natural logarithm, M_0 = mould dry weight at time t_0 , and M_1 = mould dry weight at time t_1 . Thus through hyphal branching the observed linear increase in colony radius (or length of unbranched hypha) is compatible with the exponential nature of the organism's growth in submerged culture. Specific growth rate is a direct measure of the exponential rate of mould growth and is related to the organism's doubling time (T_d) by the expression,

$$T_d = \frac{\ln 2}{\alpha}. \quad (4)$$

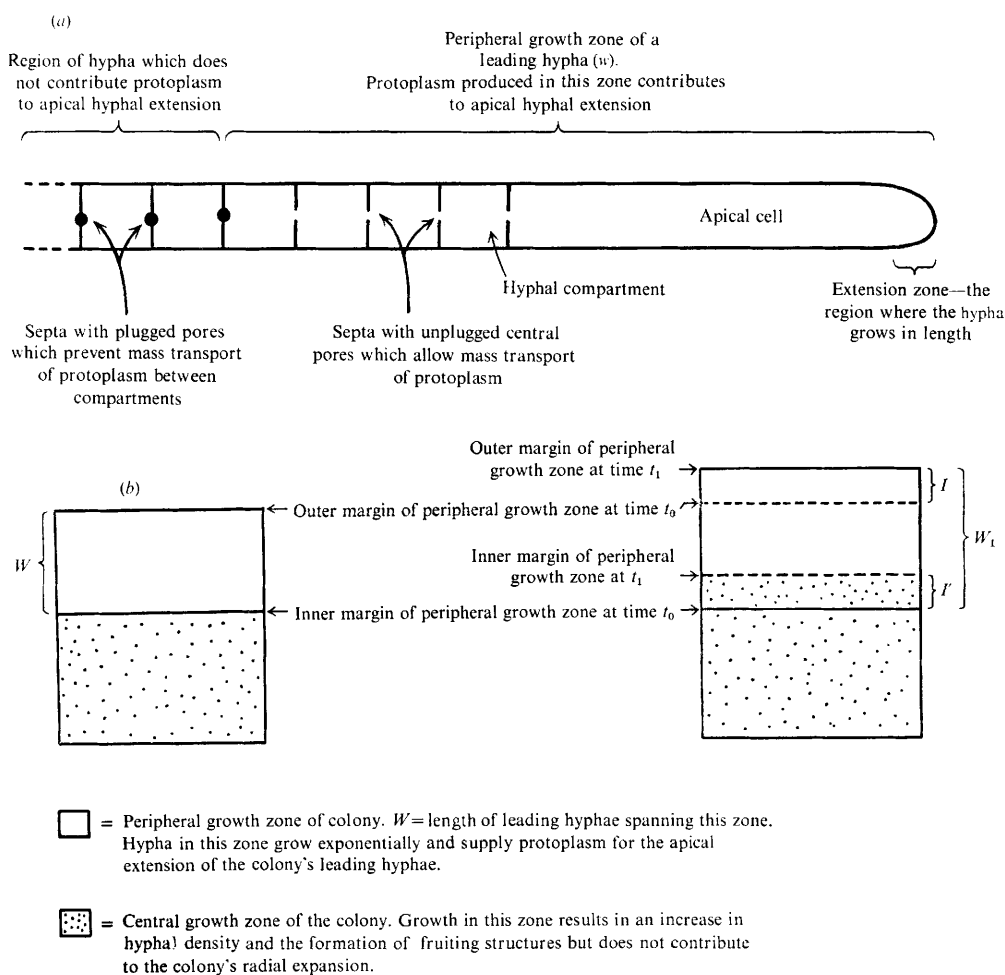


Fig. 1. (a) Diagrammatic representation of the extension and peripheral growth zones of a septate hypha. (b) Diagrammatic representation of growth at the margin of a colony during a given time interval ($t_1 - t_0$). W = experimentally determined width of colony's peripheral growth zone; W_t = distance from the inner margin of peripheral growth zone at t_0 to its outer margin at t_1 . Because $I = I'$ the width of the peripheral growth zone remains constant.

Fungal hyphae increase in length only at their apices (Fig. 1*a*). The apical extension zone of *Aspergillus nidulans* hyphae is at most only about 35 μm . long (Trinci, 1970) but at 37° this organism has a colony radial growth rate of 297 μm ./h. and a doubling time in submerged culture of about 2 h. (Trinci, 1969). Thus although growth in length occurs in only the extension zone it is clear that protoplasm in a much longer portion of the hypha must increase in volume in order to supply the advancing tip. Zalokar (1959*b*) calculated that the terminal 12 mm. portion of a *Neurospora crassa* hypha was involved in synthesizing the protoplasm necessary to maintain the observed hyphal extension rate. Thus the rate of extension of a hypha (or of a colony, since its radial extension is governed by the growth of its leading hyphae) is probably a function of the length of the terminal portion of the hypha contributing protoplasm (including wall precursors) to apical extension and the rate of duplication of protoplasm within this region (Fig. 1*a*). Since the radius of a colony (or length of unbranched hypha) increases at a linear rate, it is possible that the width of the peripheral growth zone does not vary significantly with time (Fig. 1*b*); as an increment of hyphal growth is added at the outer margin of the peripheral growth zone an equivalent amount is removed from its inner margin (i.e. it ceases to contribute protoplasm towards the apical extension of leading hyphae). The length of a colony's peripheral growth zone (Fig. 1*b*) may be determined experimentally (Ryan, Beadle & Tatum, 1943; Clutterbuck & Roper, 1966; Lhoas, 1968) by severing hyphae at different distances from their apices and determining the shortest hyphal length consistent with an unaltered extension rate. The maximum rate of duplication of protoplasm within the peripheral growth zone could well be the same as the organism's specific growth rate in submerged culture. If so, then it should be possible to obtain an estimate of the radial growth rate of a colony by calculation from experimental determinations of the width of its peripheral growth zone and the organism's specific growth rate; this estimate will be called the theoretical radial growth rate (K_{rt}) of the colony.

Pirt (1966) suggested that mycelial pellets would increase linearly in radius according to the expression

$$\frac{dr}{dt} = \alpha w, \quad (5)$$

where r = the radius of the pellet, w = width of the pellet's peripheral growth zone after the pellet had exceeded a certain diameter, and α = the organism's specific growth rate. On integration this becomes

$$r_1 = \alpha w (t_1 - t_0) + r_0, \quad (6)$$

where r_0 = radius of the pellet at time t_0 and r_1 = radius of the pellet at time t_1 . Thus the constant K_r in equation (1) = αw .

In the present study the theoretical radial growth rate (K_{rt}) of a colony in μm ./h. was calculated from the expression

$$K_{rt} = \alpha w, \quad (7)$$

where w = the experimentally determined width of the colony's peripheral growth zone in μm . and α = the mould's maximum specific growth rate in submerged shake-flask culture in h^{-1} .

The validity of equation (7) may be tested by comparing the observed (K_r) and theoretical (K_{rt}) radial growth rates of fungal colonies.

METHODS

Organisms. The organisms used were *Penicillium chrysogenum* WIS 54-1255; *Geotrichum lactis* QEC, F1; *Rhizopus stolonifer* (+) QEC, Z5; *Mucor racemosus* QEC, Z10; *Absidia glauca* (+) QEC, Z8; *Aspergillus niger* QEC, A1; *Aspergillus wentii*, QEC, Z9; *Aspergillus nidulans* BWB 224, y, ve; *Aspergillus nidulans* BWB 480, y, mo C96, ve. Abbreviations: QEC = from Queen Elizabeth College collection; BWB = from Dr B. W. Bainbridge.

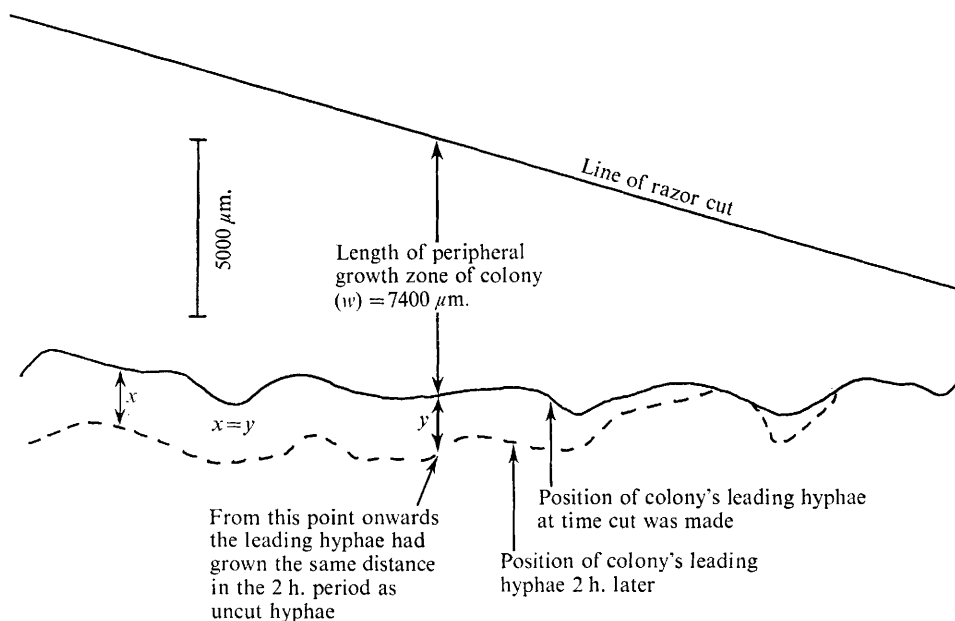


Fig. 2. Effect on the growth of leading hyphae of a *Rhizopus stolonifer* colony of cutting them at varying distances from their apices. Tracings made from a colony magnified by the Shadowmaster.

Media. The two *Aspergillus nidulans* strains were grown on DAN medium (Trinci, 1969). All other organisms were grown on DM medium (g./l.): D-glucose, 10; KH_2PO_4 , 3.4; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 8.9; $(\text{NH}_4)_2\text{SO}_4$, 6; EDTA, 0.6; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25; CaCl_2 , 0.05; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.02; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.005; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; Na_2SO_4 , 0.5; $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.005. Vitamins (mg./l.): *m*-inositol, 1.25; thiamine, 0.25; riboflavin, 0.25; calcium pantothenate, 0.25; *p*-aminobenzoic acid, 0.125; pyridoxin, 0.25; D-biotin, 0.0125.

The ammonium sulphate was added to the phosphates which were prepared at four times the final concentration and adjusted to pH 6.8. Glucose, EDTA-chelated trace elements and the vitamin solution were prepared separately at five, 20 and 80 times their final concentrations respectively.

The vitamin and cycloheximide solutions were sterilized by membrane filtration and the other solutions by autoclaving at 121° for 15 min. Solid medium was prepared by adding 15 g. of agar to 1 l. of medium.

Determination of colony radial growth rate on agar medium and specific growth rate in submerged culture

Spores for inocula were harvested with 0.1 % (v/v) Tween 80 from cultures grown at 25°, washed and then suspended in sterile distilled water or 0.1 % Tween 80.

Plate cultures containing 20 ml. of agar medium were inoculated at three points by means of a repette, a special pipette (Pirt, 1967), and the radial growth rates of the colonies were determined (Trinci, 1969).

Submerged shake-flask cultures were prepared as described previously (Trinci, 1969) and specific growth rates were calculated from the early part of the exponential phase of growth, usually before an organism concentration of 1 to 2 mg. dry wt./ml. of medium had been exceeded. Unless stated otherwise, all cultures were grown at 25°.

Determination of the width of the peripheral zone involved in the radial extension of colonies

Plate cultures were inoculated across their diameter and when the colonies were in the linear phase of growth they were cut diagonally with a sterile razor blade as shown in Fig. 2; care was taken that the razor blade did not reach the bottom of the medium in the plate in

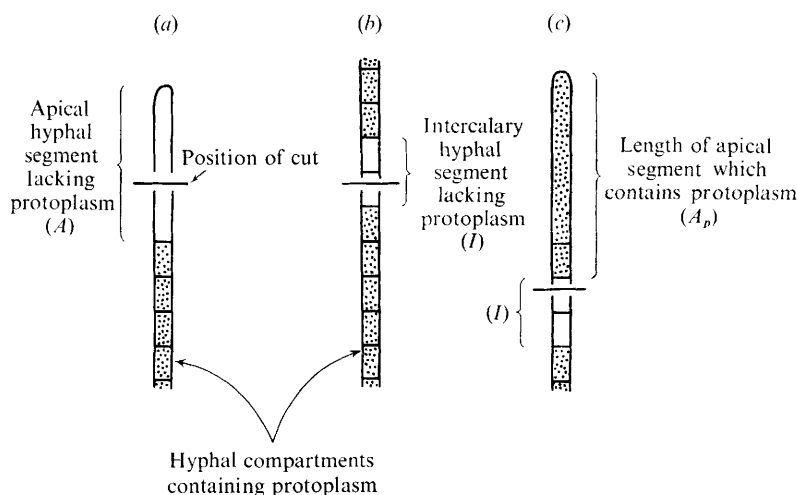


Fig. 3. Diagrammatic representation of the morphological effects of cutting septate hyphae at varying distances from their apices. The shaded areas represent protoplasm.

order to prevent the ensuing separation of the cut halves. The plate was then inverted on the stage of a microscope, and the severed colony was photographed at 15 min. intervals with a 35 mm. time-lapse camera. The shortest length of severed hypha which had an unaltered growth rate was determined as shown in Fig. 2. The distance 'w' represents the maximum width of the peripheral growth zone involved in radial extension of the colony. The peripheral growth zone of *Rhizopus stolonifer* was determined by severing colonies and tracing their circumference with a Shadowmaster (Pirt, 1967) before and after a 2 h. incubation period (Fig. 2).

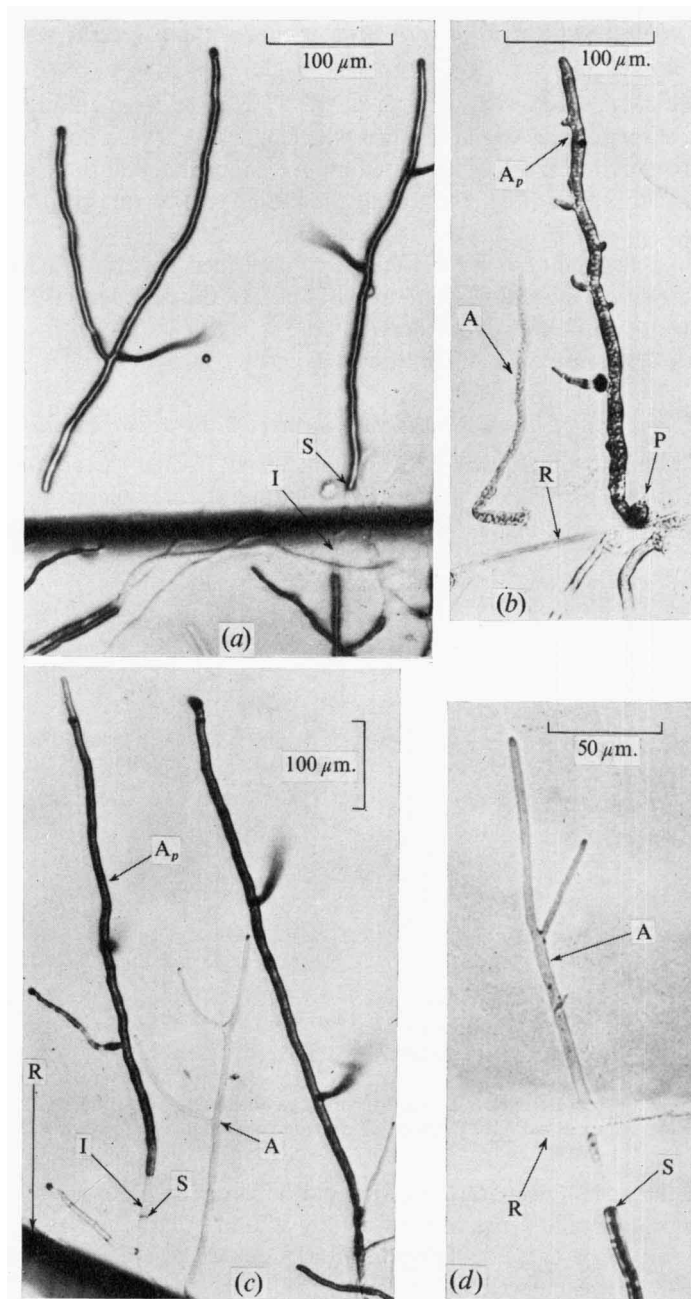


Fig. 4 to 6. *Abbreviations used:* A, apical segment lacking protoplasm; Ac, apical cell; Ap, apical hyphal segment containing protoplasm; I, intercalary hyphal segment lacking protoplasm; P, protoplasm; R, position of razor cut; S, septum.

Fig. 4. (a) Severed hyphae of *Aspergillus niger*. (b) Severed hyphae of *Absidia glauca*. (c) Severed hyphae of *A. wentii*. (d) Severed hyphae of *Geotrichum lactis*.

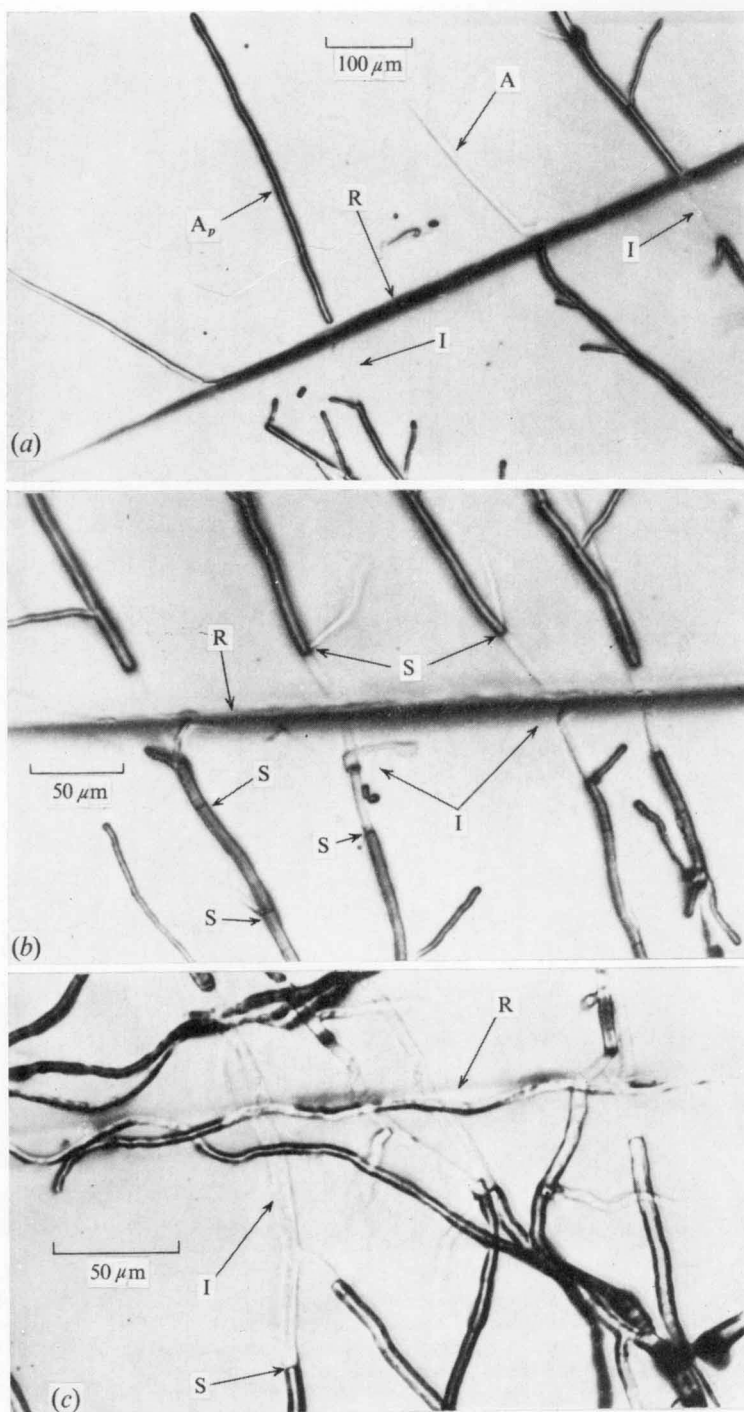


Fig. 5. (a) Severed hyphae of *Geotrichum lactis*. (b) Severed hyphae of *G. lactis*. (c) Severed hyphae of *Aspergillus niger*.

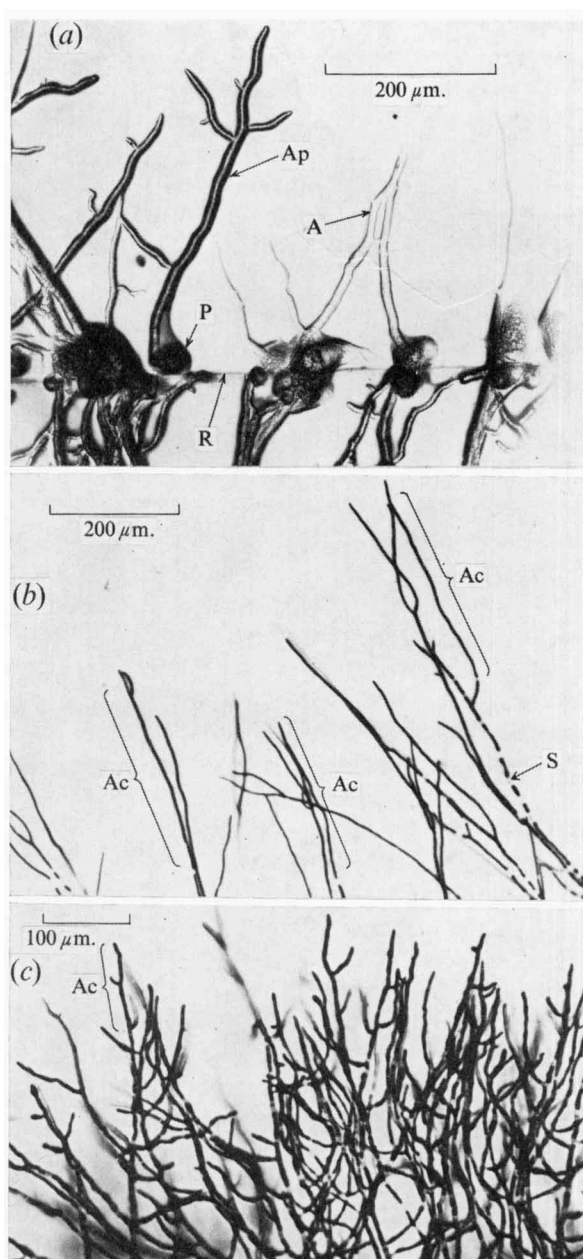


Fig. 6. (a) Severed hyphae of *Rhizopus stolonifer*. (b) Surface hyphae at the periphery of a colony of *Aspergillus nidulans* BWB 224 grown on media containing 0.2 g./l. of glucose. Stained with trypan blue. (c) Surface hyphae at the periphery of a colony of *A. nidulans* BWB 480 grown on media containing 0.2 g./l. of glucose. Stained with trypan blue.

Morphological effect of severing hyphae with a razor blade

Plate cultures of *Rhizopus stolonifer*, *Absidia glauca* and *Actinomucor repens* were grown on medium containing 10 g./l. of glucose, and other organisms on media containing 0.2 g./l. of glucose; the morphological effects of severing septate hyphae were more clearly observable in sparse colonies. Hyphae at the colony's margin were cut with a razor blade and photographed. The following measurements were made on the prints: (1) The length of apical hyphal segment which lacked protoplasm (Fig. 3a; 4c, d). (2) The length of intercalary hyphal segment which lacked protoplasm (Fig. 3b; 4a; 5a-c). (3) The length of the shortest apical segment which contained protoplasm (Fig. 3c; 4a-c; 5a).

Determination of the lengths of the apical cell and hyphal compartments of septate species

Colonies were stained with trypan blue in lactophenol (Righelato, Trinci, Pirt & Peat, 1968), photographed (Fig. 6b, c) and the lengths of the apical cell and hyphal compartments measured (Fig. 1a).

Determination of hyphal density

Variation in hyphal density with distance from the periphery of *Rhizopus stolonifer* colonies was determined by staining them with trypan blue in lactophenol and photographing the surface hyphae at the colony's margin. Hyphal densities were determined by measuring the total length of surface hyphae in successive $100 \times 1000 \mu\text{m}$. rectangles drawn at $500 \mu\text{m}$. intervals from the colony's periphery. Hyphal densities are expressed as total hyphal length (in μm .) per mm^2 of surface area.

Hyphal growth into the medium was determined as described by Trinci (1969); the colonies were grown on plates containing 45 ml. of medium. Student's 't' test was used to assess the significance of the results. Moroney's text (1956) was the source of statistical methods.

RESULTS

Determination of the organism's specific growth rate in submerged culture and the width of the colony's peripheral growth zone

The specific growth rate of each mould in submerged culture was determined (Table 1). *Geotrichum lactis* and *Rhizopus stolonifer* grew filamentously in submerged culture whilst *Mucor racemosus* formed a mixture of filamentous hyphae and small pellets. Other organisms only formed small pellets in submerged culture. All organisms grew exponentially during at least the early stages of batch culture before an organism concentration of about 1 to 2 mg./ml. of medium had been exceeded. However, there was often a decline from the maximum specific growth rate as the maximum yield was approached (Fig. 7).

The mean width of the peripheral growth zone of the colonies of each mould was also determined (Table 1). The width of this zone varied from $423 \mu\text{m}$. for *Geotrichum lactis* to $8660 \mu\text{m}$. for *Rhizopus stolonifer*; this represents a 20-fold range for the organisms studied. Lhoas (1968) has previously reported that *Aspergillus niger* hyphae have a peripheral growth zone of $1200 \mu\text{m}$., a figure similar to that found in the present study. Colonies of non-septate fungi had significantly wider peripheral growth zones than those of septate fungi (Table 1).

Table 1. *Width of the peripheral growth zones of colonies (w), specific growth rates (α) in submerged shake-flask culture, observed (K_r) and theoretical (K_{rt}) colony radial growth rates at 25° on DM medium*

The theoretical radial growth rates were calculated from equation (7).

Organism	Growth in submerged culture*		Mean width of peripheral growth zone of colonies (w, $\mu\text{m.}$)†	Mean observed radial growth rate of colonies (K_r , $\mu\text{m./h.}$)‡	Theoretical radial growth rate of colonies (K_{rt} , $\mu\text{m./h.}$)	$\frac{K_r}{K_{rt}} \times 100$
	Mean specific growth rate (α , h.^{-1})	Mean doubling time (T_d , h.)				
<i>Rhizopus stolonifer</i> (+)	0.135 (± 0.026)§	5.2 (± 0.68)	8,660 (± 946)	970 (± 76)	1,169	83
<i>Mucor racemosus</i>	0.102 (± 0.032)	7.2 (± 1.6)	3,406 (± 635)	433 (± 16)	347	125
<i>Actinomucor repens</i>	0.181 (± 0.032)	4.0 (± 0.71)	2,479 (± 532)	486 (± 18)	449	108
<i>Absidia glauca</i> (+)	0.124 (± 0.026)	5.8 (± 0.86)	2,487 (± 567)	379 (± 14)	308	123
<i>Geotrichum lactis</i>	0.353 (± 0.017)	2.0 (± 0.09)	423 (± 129)	200 (± 8)	149	134
<i>Aspergillus wentii</i>	0.147 (± 0.017)	4.8 (± 0.65)	1,342 (± 270)	157 (± 6)	197	80
<i>A. niger</i>	0.119 (± 0.018)	5.9 (± 0.71)	1,137 (± 313)	133 (± 4)	135	99
<i>Penicillium chrysogenum</i>	0.164 (± 0.019)	4.3 (± 0.46)	496 (± 84)	76 (± 3)	81	94
<i>Neurospora crassa</i>	0.353	2.0	10,000¶	4,400¶	3,530	125

* α , Each rate is the mean of three separate experiments.

† w, Each peripheral growth zone is the mean of five to eight separate experiments.

‡ K_r , Each radial growth rate is the mean of four to nine colonies.

§ Standard deviation.

|| From Zalokar (1959a) (at 30°).

¶ From Ryan, Beadle & Tatum, 1943 (at 30°).

Correlation coefficient (r) K_r/K_{rt} (excluding *N. crassa*) = +0.98 ($P < 0.001$). Thus a highly significant correlation exists between K_r and K_{rt} .

Table 2. *Lengths of the apical cell (length from hyphal tip to first observable septum) and hyphal compartments of surface hyphae at the periphery of fungal colonies*

Organism	Strain no.	Temperature (°)	Glucose concn. (g./l.)	Apical cell (μm.)	First hyphal compartment (μm.)	Second hyphal compartment (μm.)	Third hyphal compartment (μm.)	Fourth hyphal compartment (μm.)	Mean of all hyphal compartments (μm.)	No. of septa		Colony growth
										In peripheral growth zone (w) of leading hyphae	Formed/h.	
<i>Geotrichum lactis</i>	F 1	25	10	290 (± 99)*	81 (± 7)	76 (± 5)	75 (± 4)	— †	77	2	2·6	
<i>Aspergillus niger</i>	A 1	25	10	426 (± 181)	65 (± 22)	59 (± 30)	75 (± 20)	—	66	11	2·0	
<i>A. wentii</i>	A 9	25	10	449 (± 99)	66 (± 22)	60 (± 30)	60 (± 20)	—	62	14	2·5	
<i>Penicillium chrysogenum</i>	WIS-54	25	0·2	136 (± 16)	29 (± 11)	28 (± 11)	32 (± 8)	28 (± 5)	29	13	2·6	
	1255											
<i>A. nidulans</i>	BWB 224	25	10	299 (± 66)‡	52 (± 19)‡	69 (± 19)	(43 ± 10)	53 (± 14)	54	6	2·7	
<i>A. nidulans</i>	BWB 224	30	10	300 (± 64)‡	55 (± 22)‡	61 (± 14)	—	—	58	7	3·7	
<i>A. nidulans</i>	BWB 224	37	10	327 (± 54)‡	48 (± 13)‡	51 (± 17)	46 (± 7)	—	48	7	5·6	
<i>A. nidulans</i>	BWB 224	37	0·2	270 (± 38)§	43 (± 18)	46 (± 25)	—	—	45	16	6·7	
<i>A. nidulans</i>	BWB 480	37	0·2	81 (± 31)§	25 (± 9)	26 (± 8)	23 (± 7)	24 (± 8)	25	16	8·4	

* Standard deviation.

† Not determined.

‡ $P > 0·5$. Therefore there is no significant difference between either the length of the apical cells or the length of the first hyphal compartments.

§ $P < 0·001$. Therefore there is a significant difference between the lengths of these two apical cells.

Length of the apical cell and hyphal compartments of septate species

The lengths of the apical cell (length from hyphal tip to first observable septum) and hyphal compartments (Fig. 1*a*) of the surface leading hyphae were determined (Table 2). It was calculated that hyphae which spanned the colony's peripheral growth zone would possess 2 to 16 septa, depending upon the species (Table 2).

Assessment of the damage caused by severing hyphae with a razor blade

Since determination of the width of a colony's peripheral growth zone involved severing hyphae with a razor blade, it was clearly important to assess the extent of the damage caused by cutting.

Table 3. *Assessment of damage caused by severing hyphae at varying distances from their apices*

The organisms were grown at 25° on media containing 0.2 g./l. of glucose.

Organism	<i>I</i> *	<i>H</i> †	<i>A</i> ‡	<i>A/I</i>	<i>A_p</i> §
<i>Geotrichum lactis</i>	77 (± 22)	1	275 (± 31)	3.7	409 (± 62)
<i>Aspergillus niger</i>	91 (± 31)	1 or 2	320 (± 59)	3.5	323 (± 37)
<i>A. wentii</i>	98 (± 16)	1 or 2	426 (± 125)	4.3	384 (± 60)
<i>A. nidulans</i> BWB 224	131 (± 49)	2 or 3	412 (± 90)	3.1	333 (± 15)

* Mean length of the intercalary zone of a hypha which lacked protoplasm after it had been cut (*I*, $\mu\text{m.}$).

† Estimated no. of hyphal compartments in the intercalary zone lacking protoplasm. Calculated from Table 2 and length of the *I* zone (1st col.).

‡ Mean length of the apical segment which lacked protoplasm after hyphae had been cut apically (*A*, $\mu\text{m.}$).

§ Minimum length of intact apical segments of hyphae which contained protoplasm after the hyphae had been cut just behind their apices (*A_p*, $\mu\text{m.}$).

|| Standard deviation.

The damage (as indicated by loss of protoplasm) caused by severing hyphae varied with distance from the apex; apical regions were more subject to damage than distal hyphal regions. This difference is indicated (Table 3) by the ratio, length of apical hyphal segment devoid of protoplasm after cutting:length of intercalary hyphal segment devoid of protoplasm after cutting (*A/I*).

When septate hyphae were cut apically the mean length of the region which lost its protoplasm (Figs. 3*a*; 4*c*, *d*) was almost identical with the length of its apical cell (Tables 2, 3). Thus when the apical cell of a hypha was severed, the protoplasm from the whole cell was exuded; usually, however, there was no significant loss of protoplasm from the hyphal compartment immediately behind the apical cell (Fig. 4*d*). This suggests that the central pore in the septum delimiting the apical cell from the adjacent hyphal compartment was either plugged prior to the treatment or became plugged immediately after the cut was made. When a hypha was cut behind the apical cell (Fig. 3*c*; 4*a*, *c*) the apical cell usually remained intact (Table 3). Severing hyphae behind the apical cell resulted in loss of protoplasm from one to three hyphal compartments in the region of the cut (Table 3).

It was more difficult to assess the damage caused to non-septate hyphae when they were cut. When a *Rhizopus stolonifer* hypha was severed about 320 $\mu\text{m.}$ from its apex, the whole apical segment became devoid of protoplasm. However, hyphae of *Absidia glauca* and *R. stolonifer* still contained protoplasm after they had been cut 330 and 420 $\mu\text{m.}$ from their apices respectively (Fig. 6*a*). The protoplasm which was retained by the apical portion of the cut hyphae often developed an increased degree of vacuolation. When non-septate

hyphae were cut some distance behind their apices, the protoplasm which was initially exuded apparently acted as a plug (Fig. 4b) preventing further loss. It has been shown that the ends of *Phycomyces blakesleeanus* sporangiophores which have been plucked from the mycelium are 'plugged' in a similar manner; such severed sporangiophores continue to elongate for many hours when their cut ends are placed in water (Bergman *et al.* 1969).

Comparison between the observed radial growth rates (K_r) of colonies and their theoretical radial growth rates (K_{rt}) calculated from the width of their peripheral growth zones (w) and the organism's specific growth rates in submerged culture (α)

The theoretical radial growth rate (K_{rt}) of each colony was calculated from the width of its peripheral growth zone (w) and the organism's specific growth rate in submerged culture (α) using equation (7). The theoretical and observed (K_r) colony radial growth rates are compared in Table 1. The correlation coefficient (0.98) between these two sets of values was highly significant ($P < 0.001$). This result indicates that the hypothesis that the radial growth rate of a colony is a function of the width of its peripheral growth zone and the organism's specific growth rate in submerged culture is probably correct.

The theoretical and observed radial growth rate of *Neurospora crassa* colonies at 30° are also compared in Table 1. This organism has a specific growth rate in submerged shake-flask culture of 0.353 (Zalokar, 1959a) and its colonies have a peripheral growth zone of 10,000 μm . (Ryan *et al.* 1943). There was a close similarity between the observed (4400 $\mu\text{m}/\text{h}$.) and the theoretical (3530 $\mu\text{m}/\text{h}$.) colony radial growth rate of this organism.

The results obtained (Table 1) reinforce the view (Trinci, 1969) that colony radial growth rate is not a suitable parameter to use to assess the relative growth rates of different mould species, e.g. although colonies of *Rhizopus stolonifer* had a radial growth rate of nearly 1 mm./h., the organism's specific growth rate in submerged culture was less than half that of *Geotrichum lactis*, an organism whose colonies grew at a rate of only 200 $\mu\text{m}/\text{h}$. (Table 1).

Comparison between an organism's specific growth rate (α) in submerged culture and the theoretical specific growth rate (α_t) of hyphae in the colony's peripheral growth zone

If the radial growth rate (K_r) and the width of the colony's peripheral growth zone (w) are known equation (7) may be used to estimate the specific growth rate of the hyphae in the colony's peripheral growth zone. Thus

$$\alpha_t = K_r/w, \quad (8)$$

where α_t is the theoretical specific growth rate of hyphae in the colony's peripheral growth zone.

The correlation coefficient (0.98) between the mould's specific growth rate in submerged culture and the theoretical specific growth rate of hyphae in the colony's peripheral growth zone was highly significant (Table 4). Furthermore, the observed and calculated specific growth rates of *Neurospora crassa* hyphae were also similar (Table 4). Thus hyphae in the peripheral growth zone grow exponentially in length at a rate more or less identical to the mould's specific growth rate in submerged culture.

Influence of temperature and cycloheximide concentration on the width of the peripheral growth zone of colonies

It has been established that the radial growth rate of a colony is a function of the width of its peripheral growth zone and the specific growth rate of the hyphae within that zone. It thus follows that radial growth rate may be altered by varying either the width of the

peripheral growth zone or the specific growth rate of the peripheral hyphae. The fact that the radial growth rate of fungal colonies is directly related to the organism's specific growth rate in submerged culture when the latter is altered either by varying the temperature (Trinci, 1969) or by incorporating an inhibitor in the medium (Trinci & Gull, 1970) suggests that the width of the peripheral growth zone is not influenced by temperature or inhibitor concentration. These predictions were tested experimentally.

Table 4. Comparison between the organism's specific growth rate (α) in submerged culture and the theoretical growth rate (α_t) of hyphae in the colony's peripheral growth zone

Organism	Theoretical specific growth rate of the hyphae in the colony's peripheral growth zone (α_t , h ⁻¹)*	Observed specific growth rate of the mould in submerged culture (α , h ⁻¹)	$\frac{\alpha}{\alpha_t} \times 100$
<i>Rhizopus stolonifer</i> (+)	0.112	0.135	121
<i>Mucor racemosus</i>	0.127	0.102	80
<i>Actinomucor repens</i>	0.196	0.181	92
<i>Absidia glauca</i> (+)	0.152	0.124	82
<i>Geotrichum lactis</i>	0.426	0.353	83
<i>Aspergillus wentii</i>	0.117	0.147	126
<i>A. niger</i>	0.117	0.119	102
<i>Penicillium chrysogenum</i>	0.153	0.164	107
<i>Neurospora crassa</i>	0.440†	0.353‡	125

* Calculated from equation (8).

† From Zalokar (1959a).

‡ Calculated from Ryan, Beadle & Tatum (1943).

Correlation coefficient (r) α/α_t (excluding *N. crassa*) = +0.98 ($P < 0.001$). Thus a highly significant correlation exists between α and α_t .

The specific growth rate of *Geotrichum lactis* and the width of the peripheral growth zone of its colonies were determined over a range of cycloheximide concentrations (Table 5). There was no significant difference ($P > 0.7$ to > 0.9) in the width of the peripheral growth zone over the range of cycloheximide concentration tested and the theoretical colony radial growth rates (K_{rt}) did not differ significantly from the observed (K_r) colony radial growth rates (Table 5).

The width of the peripheral growth zone of *Aspergillus nidulans* BWB 224 colonies did not vary significantly ($P > 0.4$) with temperature (Table 6). Clutterbuck & Roper (1966) have previously reported that *A. nidulans* hyphae have a peripheral growth zone of 500 to 600 μm ., a figure similar to that observed in the present study. The apical cell and the first hyphal compartment of *A. nidulans* BWB 224 hyphae also did not vary significantly ($P > 0.5$) with temperature (Table 2).

Influence of glucose concentration and genetic changes on the width of the peripheral growth zone of colonies

It has previously been established that the specific growth rate of an organism in submerged culture is not directly related to the radial growth rate of its colonies when the latter is varied by changing the glucose concentration in the medium (Trinci, 1969); the initial maximum specific growth rate of an organism in submerged culture was identical at glucose concentrations from 1 to 5% (w/v). Similarly, an observed difference in the radial growth rate of

colonies of two strains of *Aspergillus nidulans* was not correlated with a corresponding difference in their specific growth rates in submerged culture (Bainbridge & Trinci, 1970). For these two conditions one would predict that the observed differences in colony radial growth rate resulted from a variation in the width of the colony's peripheral growth zone.

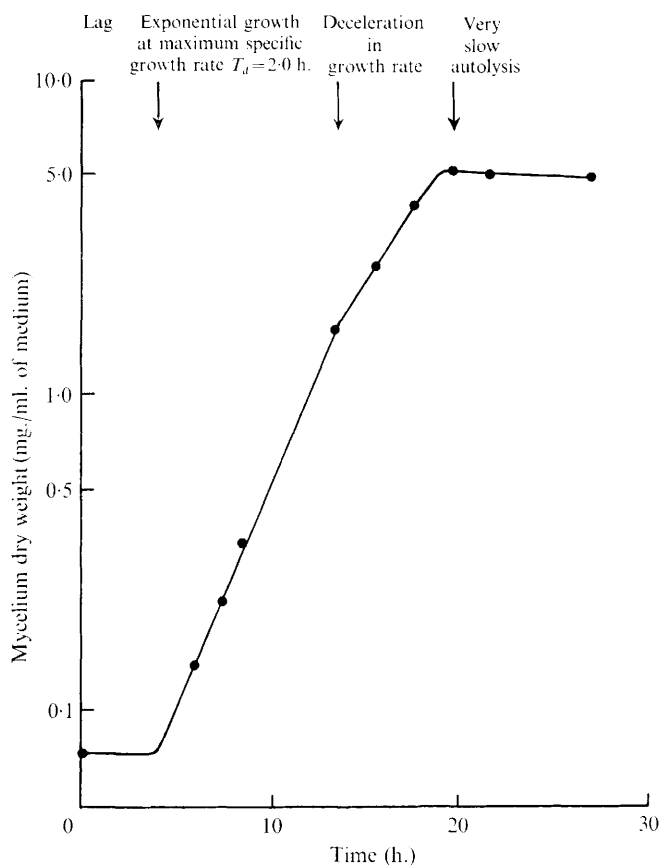


Fig. 7. Kinetics of a batch culture of *Geotrichum lactis* at 25°. The deceleration phase may be due to the supply of oxygen becoming rate limiting. Growth stops when the glucose in the medium is exhausted.

Table 5. Influence of cycloheximide concentration on the width of the peripheral growth zone of *Geotrichum lactis* colonies

Concn. of cycloheximide in the medium ($\mu\text{g./ml.}$)	Mean width of peripheral growth zone of colonies (w , $\mu\text{m.}$)	Growth in submerged shake-flask culture		Mean observed radial growth rate of colonies (K_r , $\mu\text{m./h.}$)	Theoretical radial growth rate of colonies (K_{rt} , $\mu\text{m./h.}$)	$\frac{K_r}{K_{rt}} \times 100$
		Specific growth rate (α , h.^{-1})	Doubling time (T_d , h.)			
0	423 (± 130)*†	0.353	2.0	196	149	132
100	417 (± 115)†	0.223	3.1	128	93	138
500	407 (± 106)†	0.204	3.4	79	83	95

* Standard deviation.

† $P > 0.7$. Therefore there is no significant difference between the width of the peripheral growth zones.

The width of the peripheral growth zone of *Aspergillus nidulans* BWB 224 colonies on media containing 0.2 g./l. of glucose was significantly ($P < 0.02$) wider (Table 6) than those of colonies grown at the same temperature on media containing 10 g./l. of glucose. The length of the apical cell of *A. nidulans* BWB 224 hyphae was also found to vary significantly with glucose concentration (Table 2).

Table 6. *Influence of temperature, glucose concentration and strain type upon the width of the peripheral growth zone of Aspergillus nidulans upon DAN medium*

Organism	Temperature (°)	Glucose conc. in medium (g./l.)	Mean width of colony's peripheral growth zone (w, $\mu\text{m.}$)	Specific growth rate in submerged culture (α , h.^{-1})	Observed radial growth rate of colonies (K_r , $\mu\text{m./h.}$)
<i>A. nidulans</i> 224	25	10	663 (± 115)*	0.148†	146†
<i>A. nidulans</i> 224	30	10	683 (± 91)*	0.215†	215†
<i>A. nidulans</i> 224	37	10	684 (± 95)*‡	0.360†	297†
<i>A. nidulans</i> 224	37	0.2	950 (± 143)§‡	0.289	303
<i>A. nidulans</i> 480	37	0.2	460 (± 62)§	0.257	209

* $P > 0.4$. Therefore there is no significant change in the width of the peripheral growth zone with temperature.

† From Trinci (1969).

‡ $P < 0.02$; therefore there is a significant difference in the width of the peripheral growth zone with glucose concentration.

§ $P < 0.001$; therefore there is a significant difference between the widths of the peripheral growth zones of strains 224 and 480.

|| From Bainbridge & Trinci (1970).

The mean width of the peripheral growth zone of *Aspergillus nidulans* BWB 224 colonies was significantly ($P < 0.001$) wider than that of colonies of strain *A. nidulans* BWB 480 (Table 6). The apical cell and hyphal compartments of *A. nidulans* BWB 480 were significantly shorter ($P < 0.001$) than those of *A. nidulans* BWB 224 (Table 2). Furthermore, *A. nidulans* 480 formed a mycelium which was much more densely branched than that of *A. nidulans* BWB 224 (Fig. 6*b, c*).

Thus the observed differences in colony radial growth rate caused by altering the glucose concentration in the medium or by genetic changes were largely due to variation in the width of the colony's peripheral growth zone and not to significant changes in the organism's specific growth rate.

Variation in hyphal density at the periphery of fungal colonies

Variation in the hyphal density with distance from the periphery of a *Rhizopus stolonifer* colony grown at 25° was determined (Fig. 8). The total length of surface hyphae per unit area increased exponentially in the peripheral fringe of the colonies.

With the exception of *Rhizopus stolonifer* the density of surface hyphae 500 $\mu\text{m.}$ from the circumference of colonies did not vary significantly between the organisms (Table 7).

Hyphal growth into the medium

Hyphae in a fungal colony not only grow over the surface of the substrate but also penetrate the medium (Trinci, 1969). Vertical sections of the agar medium were cut at the colony's periphery and the depth attained by the hyphae was determined (Table 7).

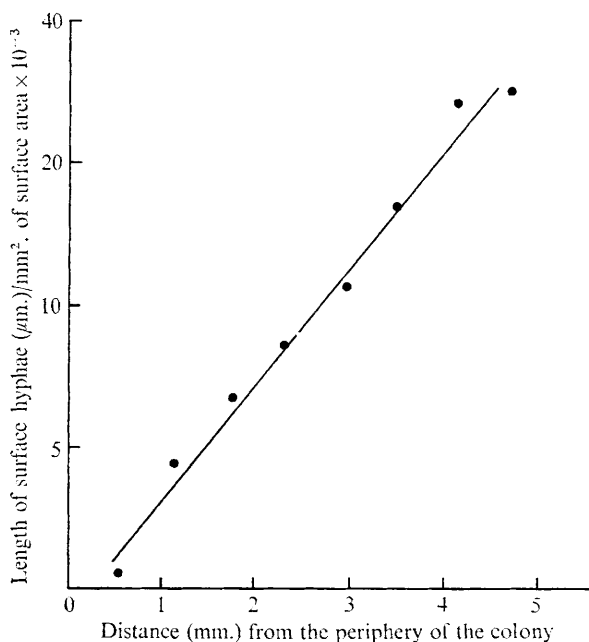


Fig. 8. Variation in the density of surface hyphae with distance from the periphery of a *Rhizopus stolonifer* colony grown at 25°. Mean of three separate determinations.

Table 7. Comparisons of (1) the density of the surface hyphae of colonies 500 $\mu\text{m.}$ from their circumference; (2) the depth attained by hyphae at the periphery of the colonies

Organism	Total hyphal length per mm. ² of surface area ($\mu\text{m.} \times 10^{-2}$)	Penetration into the medium attained by hyphae at the periphery of colonies ($\mu\text{m.}$)
<i>Rhizopus stolonifer</i> (+)	96 (± 26)*	6,156
<i>Mucor racemosus</i>	Too dense to count	7,695
<i>Actinomucor repens</i>	183 (± 83)	—†
<i>Absidia glauca</i> (+)	166 (± 44)	6,441
<i>Geotrichum lactis</i>	168 (± 29)	—
<i>Aspergillus wentii</i>	220 (± 43)	5,814
<i>A. niger</i>	210 (± 27)	—
<i>Pencillium chrysogenum</i>	Too dense to count	—

* Standard deviation. † Not determined.

DISCUSSION

The observed correlations between the theoretical and observed radial growth rates (Table 1) and the theoretical and observed specific growth rates (Table 4) support the hypothesis that the growth rate of a colony is a function of the width of its peripheral growth zone and the specific growth rate of the hyphae within that zone. The exponential nature of hyphal growth within the peripheral zone is further indicated by the exponential increase in hyphal density at the margin of *Rhizopus stolonifer* colonies (Fig. 8). A similar exponential increase in hyphal density has also been observed at the fringe of *Chaetomium* colonies (Plomley, 1959).

The linear growth in radius of a colony may be compared with the linear increase in the mass of organism in the overflow vessel of a steady-state continuous culture growing at the organism's maximum specific growth rate. Although the hyphae in a colony's peripheral growth zone are growing exponentially, the mass (or density) of hyphae in this region of the colony, like the mass of organism in a chemostat, remains constant because there is a continual balance between addition of hyphal mass at the outer margin of the peripheral growth zone and removal of hyphal mass at its inner margin (Fig. 1*b*). The fact that the peripheral hyphae of a colony are always growing into 'fresh medium' enables them to maintain their maximum specific growth rate. Exponential hyphal growth is probably maintained in only the outer margin of the colony. In the centre of the colony growth (if it occurs at all) is probably limited by nutrient concentration (including oxygen) or the accumulation of 'staling' products; the deceleration from the exponential rate of growth may even begin in the inner margin of the peripheral growth zone.

It was expected that the experimental method used to determine the width of the peripheral growth zone would overestimate its true length due to the damage caused to the hyphae in the region of the cut. This factor may have been compensated for by the method used to determine the length of the hyphae spanning the peripheral growth zone; the distance y (the width of the peripheral growth zone) in Fig. 2 is probably less than the actual length of the hyphae spanning this region as it is unlikely that these hyphae grow in an exact straight line.

The present model for colony growth will probably have to be modified in the light of subsequent results. In particular no account is taken of the role of branch formation in the peripheral growth zone. It is possible, although unlikely (Trinci, 1970), that branches grow independently of their supporting hyphae. The interchange of protoplasm between branches and main hyphae may, however, be of little significance when compared with overall production of protoplasm in the peripheral growth zone of leading hyphae.

The rate of growth of a fungus is most precisely defined in terms of its doubling time or specific growth rate in submerged culture. Although colony diameter and radial growth rate are frequently used to measure the rate of growth of fungi, few critical studies have been made to evaluate these growth parameters. It is possible to predict from the present results that for any given environmental variable, colony radial growth rate will be directly related to the organism's specific growth rate in submerged culture (and hence be a valid parameter of growth) only when the width of the peripheral growth zone is not influenced by the variable. Since the length of the peripheral growth zone did not vary with either temperature or the concentration of an inhibitor, colony radial growth rate may be used to determine the optimum temperature for growth of a mould or to study the effect of an inhibitor on growth. However, since the width of the peripheral growth zone did vary with nutrient concentration and between organisms (species or strains), colony radial growth rate should not be used to study the effect of nutrient concentration on growth or to compare the growth of different organisms. The common observation that colonies of non-septate fungi usually grow faster than those of septate fungi is explained by the present theory; colonies of non-septate fungi had wider peripheral growth zones than those of septate species, although they often had lower specific growth rates in submerged culture (Table 1).

Since mass transport of protoplasm can only occur through unplugged septa (Fig. 1*a*) the width of the peripheral growth zone of a septate hypha is regulated by the frequency and rate of occlusion of its septa. In some organisms (e.g. *Geotrichum lactis*) the septa clearly became occluded soon after their formation, but in others (e.g. *Aspergillus nidulans* and *Penicillium chrysogenum*) the number of septa present in the hyphal peripheral growth zone

suggests that the septa remain unplugged for some time after their formation (Tables 1, 2); it is assumed that all septa in the peripheral growth zone of a hypha are unplugged. In other organisms the septa may remain unplugged for even longer periods after their formation. The peripheral growth zone of a *Neurospora crassa* hypha is about 10,000 μm . (Ryan *et al.* 1943) and after the apical 150 μm . septa occur every 64 μm . (Zalokar, 1959*a*); the peripheral growth zone of this hypha possesses about 154 unoccluded septa. Thus the high growth rate of *N. crassa* hyphae is correlated with a long peripheral growth zone which contains numerous unplugged septa.

Variation in glucose concentration may influence the radial growth rate of *Aspergillus nidulans* colonies (Trinci, 1969) by influencing the rate at which septa become occluded and hence the width of the peripheral growth zone. Similarly, any mutation which influences either the rate of formation of septa or their occlusion will influence the length of its hypha's peripheral growth zone and hence its colony radial growth rate. The fact that the peripheral growth zone of hyphae of *A. nidulans* BWB 224 and *A. nidulans* BWB 480 both contain 16 septa (Table 2) and that the apical cell and hyphal compartments of the latter strain are considerably shorter than those of the former (Table 2) probably indicates that a mutation has influenced the frequency of septa formation in strain BWB 480 and not the rate at which the septa become plugged. Thus this study indicates that colony size or radial growth rate should not be used as a criterion to select mutants with an altered growth rate. The more branched habit of *A. nidulans* BWB 480 hyphae (Fig. 6*c*) as compared with those of *A. nidulans* BWB 224 (Fig. 6*b*) may be correlated with the reduced length of the peripheral growth zone of its hyphae. The increased hyphal density (Fig. 6*c*) which apparently results from the decrease in the length of the peripheral growth zone of *A. nidulans* 480 hyphae may be of considerable significance since the development of pseudotissues (e.g. sclerotia, rhizomorphs and fruit bodies) appears to be restricted to organisms with septate hyphae. There may be a correlation between the ability of a fungus to produce pseudotissues and the length of the peripheral growth zone of its hyphae.

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