

# GROWTH OF FUNGAL BRANCHING SYSTEMS

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When we observe filamentous fungi with the naked eye, we most frequently see only evidence of reproductive structures, e.g. mushrooms or the pigmented spores of moulds on rotting food. These structures develop from a vegetative mycelium which consists of filamentous branching hyphae, visible only with the aid of a microscope. The kinetics of growth and branching patterns of vegetative mycelia were first studied in the 1920s and 1930s, for example by Smith (1924) and Buller (1931), who observed the colony of *Coprinus sterquilinus* (see back cover). More recently a number of research groups, particularly those of Trinci and Robinson, have greatly increased our understanding of the relationship between hyphal extension and branching, in both liquid culture and on solid media, and of the factors which lead to the remarkable organisation seen in Buller's colony of *Coprinus*. In addition to providing essential information on the kinetics of biomass production, of relevance to fungal physiologists and biotechnologists, this work is also fundamental to our understanding of the growth strategies of fungi in natural environments and of the differentiation processes which lead eventually to the formation of macroscopic structures such as mushrooms. References to much of the work discussed in this article may be found in reviews by Trinci (1979, 1984) and Prosser (1990).

Vegetative growth is most conveniently studied in the laboratory on Petri dishes containing nutrient medium solidified with agar. If such medium is inoculated centrally with a spore and incubated under condi-

tions suitable for germination and subsequent growth, a germ tube hypha is formed which extends across the agar. Growth of this, and all other hyphae growing on solid medium, occurs by incorporation of material at the hyphal tip in a region termed the extension zone (Fig. 1). The extension zone wall is considered to be relatively elastic, increasing in rigidity with distance from the tip, becoming completely rigid at the base of the extension zone beyond which hyphal diameter is constant. Wall precursors and enzymes involved in wall synthesis are produced in distal regions of the hypha and are transported in membrane bound vesicles to the tip, where their contents are released to form new wall material, while the membrane is incorporated into the membrane of the extension zone. Initially, extension of the germ tube occurs at an accelerating rate. This is partly due to mobilisation of spore reserves and also due to the synthesis of increasing amounts of material from an increasing length of germ tube hypha, i.e. growth is autocatalytic.

Although the tip exerts a high degree of polarity, a point is soon reached at which the hypha is of such a length that material synthesized in distal regions is not transported at a sufficiently fast rate to reach the extending tip. Subsequent hyphal extension is fuelled by a constant length of hypha ( $W$ ) termed the peripheral growth zone ( $PGZ$ ), which is the maximum length of hypha associated with tip growth. In aseptate hyphae,  $W$  is determined by the degree of tip polarity. In fungi which form complete septa, it is equivalent to the length of the apical compart-

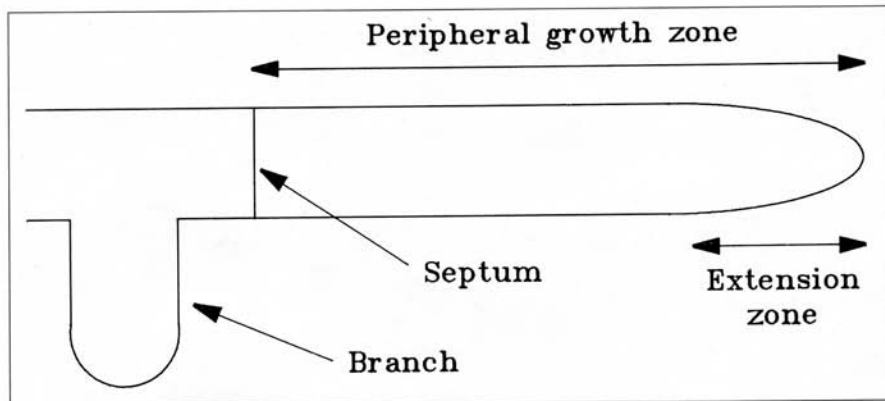


Fig. 1. The peripheral growth zone and extension zone of a branching hypha.

ment, i.e. the distance from the tip to the first septum (Fig. 1), which will provide a barrier to the tip. In fungi which form septa with central pores, which allow some passage of material,  $W$  is the distance from the tip to the first septum with a plugged pore. The length of the PGZ in part determines the rate of hyphal extension, but the extension zone must be capable of accommodating all the material supplied. Hyphal extension rate therefore also depends on the width and length of the extension zone. In *Neurospora crassa*, a fast growing fungus, extension rates reach  $3300 \mu\text{m h}^{-1}$ , with vesicles supplied by a PGZ  $6800 \mu\text{m}$  in length. Hyphal diameter is  $12.4 \mu\text{m}$ , and the length of the extension zone is  $29.2 \mu\text{m}$ .

Individual hyphae therefore extend, eventually, at a constant rate which may be maintained indefinitely if sufficient space is available, although in some species there is evidence that extension rate increases as hyphae 'mature' (see below). Hyphae behind the PGZ are still active and growing and the material being synthesized in these regions must be accommodated in some way. In addition, studies of the growth kinetics of unicellular yeasts and bacteria indicate that microbial biomass normally increases exponentially in the presence of ex-

cess nutrients. This will not result from a constant hyphal extension rate unless hyphal diameter increases, which is not normally the case. Both of these 'problems' are solved by the formation of branch hyphae, which in many ways is equivalent to cell division in unicellular organisms.

Branch formation is equivalent to the creation of a new tip, and therefore requires partial dissolution of existing cell wall material. In septate fungi this generally occurs behind and close to septa, probably as a result of accumulation of vesicles whose passage to the tip is blocked. Initially the parent hypha contributes to growth of the branch and extension occurs at an accelerating rate. Eventually a constant rate is reached which, in young mycelia, is equal to that of the parent hypha. As the germ tube extends, it subtends more branches, which in turn form branches, and this process leads to the development of a young mycelium.

In young mycelia, all hyphae have the same diameter. Total hyphal length is therefore equivalent to biomass and is found to increase exponentially. The total number of branches also increases exponentially, at the same rate, and both rates are equal to the specific growth rate of the organism in liquid medium of

equivalent composition. Exponential mycelial growth therefore results from exponential branch production. The similarity between the rates of increase of total mycelial length and branch number means that the ratio between these two properties becomes constant. This ratio is called the hyphal growth unit (HGU) and is the mean length, or more correctly volume (Robinson & Smith, 1979), of hyphae associated with each tip. In a sense this provides a mechanism for the timing of branch formation. When the actual ratio of length to branch number exceeds the HGU a branch must be formed somewhere in the colony to reduce the ratio.

The relationship between hyphal extension rate and branch formation illustrates an important advantage of the filamentous growth form. Unicellular organisms growing on solid media double in size and divide into two daughter cells, each of which grows in the manner of the parent, repeating the division process. Colonies are formed by cells pushing each other apart and piling on top of each other. Cells behave as individuals, there is no way of controlling or regulating biomass production and expansion of the colony ceases when the supply of nutrients by diffusion from surrounding media is insufficient to support further growth. Unicells therefore typically form small, discrete, self-limiting colonies. A mycelium, however, can regulate the distribution of biomass. This is most obviously seen in the supply of material from the PGZ to hyphal tips, which can explore new regions where nutrients may be absent or unavailable. This also provides an alternative to motility. In addition, a mycelium can regulate the distribution of newly synthesized biomass between hyphal extension and branch formation. Under poor nutritional conditions, branch formation is reduc-

ed and resources are concentrated in maintaining hyphal extension rate to maximise the spread of the mycelium, thereby increasing the chances of reaching fresh sources of nutrients. This decrease in branching results in an increase in HGU. Under more favourable nutritional conditions, branch production is increased, reducing HGU and providing a means of 'filling in' medium behind the outer exploratory leading hyphae, enabling full utilisation of nutrients. A reduction in nutrients within such a mycelium will reduce branch formation, thereby preventing complete exhaustion of nutrients and the self-limitation phenomenon typical of unicellular colonies. Consequently colonies of filamentous fungi can completely cover Petri dishes and can extend indefinitely given sufficient space. In some mutants, this control is lost and densely branched colonies are formed which become self-limiting. This phenomenon is exemplified by the *clock* mutants of *N. crassa* and the effect may be mimicked by growing the wild type strain on medium containing L-sorbose, which increases branch formation and gives rise to colonial morphology.

The relationship between extension rate, branching and specific growth rate ( $u$ ) can be quantified in the form of two equations. The first relates mean hyphal extension rate ( $E$ ) and the length of the hyphal growth unit ( $G$ ) in the equation:

$$E = uG.$$

The second relates the maximum extension rate ( $Kr$ ) and the length of the peripheral growth zone ( $W$ ):

$$Kr = uW.$$

A decrease in nutritional conditions is likely to reduce  $u$ , but will not affect  $E$  or  $Kr$  if  $G$  or  $W$ , respectively, can be increased i.e. if branching is suppressed and/or a greater length of hypha can supply the tip with new wall material.

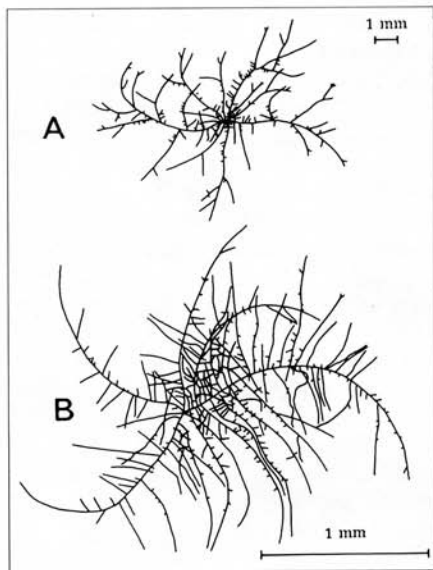


Fig. 2. Spiral growth in young colonies of *Neurospora crassa*. (A) 31 hours; (B) 36 hours after inoculation. (From Trinci et al. (1979). *Trans. Br. mycol. Soc.*, 73: 283-292).

These properties of mycelial organisms are of obvious value in natural environments, such as the soil, where available nutrients may exist in pockets or microenvironments, separated by barren areas. Mycelial growth provides a means of crossing such barren regions to reach new sources of nutrients, a strategy not available to unicells. The ability to regulate the degree of branching of vegetative hyphae may also be important in the early stages of formation of pseudotissues and differentiation structures, e.g. sclerotia, fruiting bodies, all of which develop from aggregates of mycelia.

The discussion so far has centred on the rates of hyphal extension, growth and branch formation. It is evident from the colony of *C. sterquilinus* illustrated on the cover that mycelia also exhibit a high degree of spatial organisation. The colony is circular, hyphae are oriented away from the centre, are well spaced and rarely

cross. Circular morphology becomes evident during early growth and is maintained during subsequent colony development when colonies become visible to the naked eye. Indeed, measurement of the rate of increase in colony radius, the colony radial growth rate, is frequently used to quantify the effects of particular environmental factors, inhibitors, etc., on fungal growth. Unfortunately, although this technique is very convenient, it only measures effects on extension rate, and does not take into account effects on branching and consequently on specific growth rate.

Hyphal avoidance reactions tend to occur when hyphae are brought within 10 - 30  $\mu\text{m}$  of each other. Although the mechanism for avoidance is not fully understood, it appears to be due to the existence of oxygen gradients around growing hyphae (Robinson 1973a,b). Any mechanism for the response of hyphal growth to chemical stimuli (chemotropism) must reside in the extension zone. This is the region in which new material is incorporated and is relatively elastic, while wall material behind the extension zone is rigid and unable to change direction. As two hyphae approach they will experience a reduction in oxygen concentration which will reduce extension rate. Respiratory activity within the extension zone on the side nearest to the approaching hypha will be reduced and vesicles and wall synthesizing activity will be concentrated at the opposite side. This will lead to increased extension and bulging of the wall in this region and extension towards the higher oxygen concentration, and away from the neighbouring hypha.

While avoidance reactions undoubtedly occur, hyphae in colonies are separated by distances greater than 10 - 30  $\mu\text{m}$  and computer simulations

of colony growth have shown that these reactions are not essential for formation of circular colonies. Hutchinson *et al.* (1980) recreated colonies of *Mucor hiemalis* on the basis of experimental measurements of three properties of fungal growth — hyphal extension rate, interbranch distance and branch angle. For each they determined the mean value and the statistical variation about the mean. They then reconstructed a colony from a small length of hypha, over a series of time intervals. At each interval they determined statistically the distance that each hypha would extend, whether and where a branch would be formed on each hypha and the angle that the branch hypha formed with the parent. Hyphal avoidance reactions were not incorporated but circular morphology was quickly achieved in simulated colonies. The formation of circular colonies can therefore be explained simply on the basis of statistical variation in the three properties which they investigated.

As a colony develops, conditions at the centre become less favourable for growth. Nutrients become scarce, end-products of metabolism accumulate, space becomes limited and the laws of growth and branching described above no longer apply. Exponential growth of the whole colony is not sustained, hyphal orientation mechanisms are less well defined and the colony begins to differentiate. Exponential growth does continue but it is restricted to a marginal ring, again termed the peripheral growth zone, equivalent to that of individual hyphae described above. Growth occurs behind the PGZ but does not contribute to colony radial expansion. Towards the colony centre, vegetative hyphae begin to autolyse, secondary metabolites, including antibiotics, are produced and reproductive structures are formed.

Vegetative mycelia in the PGZ of

such mature colonies are termed 'differentiated' and in some fungi their properties differ from those of 'undifferentiated' mycelia of young colonies. The situation is typified by *N. crassa*. Undifferentiated mycelia are characterized by hyphae of similar diameter, extension rate and extension zone lengths and branches are subtended at an angle of 90°. McLean & Prosser (1987) found that between 22h and 40h after spore germination several changes occurred. The first was a decrease in branch angle from 90° to 63°, with new branches oriented away from the colony centre. This was followed by development and establishment of a hierarchy in which leading hyphae, at the colony margin, subtended primary branches which had smaller extension zones, diameters and extension rates than their parents. Secondary branches subtended by these primary branches were themselves narrower and extended more slowly than their parents but all hyphae in the PGZ of differentiated mycelia were wider and grew faster than hyphae in undifferentiated mycelia of young colonies. These changes are believed to result from the effects of compounds produced at the colony centre, which then diffuse to the colony margin where they alter hyphal orientation and branch formation. In addition, there appears to be a continual developmental process in *N. crassa*, and in other fungi, leading to a gradual increase in hyphal diameter and extension rate, which may be due to improvements in transport mechanisms within hyphae.

Colonies of some fungi also display variations in morphology visible to the naked eye. Many species exhibit spiral growth, which involves coiling or bending of hyphae (Fig. 2) and results from rotation of the hyphal tip. This may be due to stretching or spiral structures in the wall of the extending zone under the influence of turgor

pressure. In the large aerial hyphae of sporangiophores of *Phycomyces blakesleeanus* rotation can be observed microscopically by placing starch grains on the hyphal tip, and the direction of rotation reverses during sporangiophore development. On solid medium, friction between the rotating tip and the medium causes the tip to roll over the surface, continually altering the direction of tip growth giving rise to a spiralling effect which can be seen macroscopically. Hyphae may spiral in either clockwise or anticlockwise directions, depending on the species (Madelin, Toomer & Ryan, 1978) and can be reduced by reducing the agar content of the medium, making it more 'slushy' and less resistant to frictional forces.

Rhythmic growth produces the characteristic alternate concentric bands of dense and sparsely branched mycelium. In *Podospora anserina* this is caused by increased branching of mycelia on the surface of the medium resulting in exhaustion of nutrients and self-limitation of colony expansion. Submerged hyphae branch less frequently and extend beneath the dense surface mycelium. They grow beyond the existing margin and then reach the surface of uncolonised medium, where they grow to produce sparsely and then densely branched mycelia and the process is repeated. Sectoring is also frequently observed in mature colonies. This results from genetic recombination events within the mycelium giving rise to regions of mycelium with altered extension rates, specific growth rates and branching properties. These differences can lead to growth of the mutant into the area which would normally be colonised by the parent, leading to bulging sectors, or alternatively overgrowth of the mutant by the wild-type may occur.

In summary, we can say that the

cellular mechanisms controlling hyphal growth and branching in young mycelia are well understood, although those controlling spatial distribution of branches and the direction of hyphal extension are less clear. This enables us to understand how fungi grow in natural environments and to optimise the quality and quantity of fungal biomass production in industrial processes. We know less of the more complex morphologies found in mature colonies, but all involve changes in branching patterns and hyphal extension rates. Similar processes occur during the initial events of many differentiation processes, and temporal and spatial control of branching are fundamental to the development of reproductive and other secondary structures.

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