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Modelling colony population growth in the filamentous fungus *Aspergillus nidulans*

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HIGHLIGHTS

- Mechanistic models for population growth are missing for filamentous fungi.
- We present such a mechanistic model for the species *Aspergillus nidulans*.
- The model is based on physiological parameters that influence colony growth.
- The model predicts the number of individual nuclei present in a colony through time.
- Fungal population size is most dependent on changes in mycelial growth rate.

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ABSTRACT

Filamentous fungi are ubiquitous in nature and have high societal significance, being both major (food-borne) pathogens and important industrial organisms in the production of antibiotics and enzymes. In addition, fungi are important model organisms for fundamental research, such as studies in genetics and evolutionary biology. However, mechanistic models for population growth that would help understand fungal biology and fundamental processes are almost entirely missing. Here we present such a mechanistic model for the species *Aspergillus nidulans* as an exemplar of models for other filamentous fungi. The model is based on physiological parameters that influence colony growth, namely mycelial growth rate and sporulation rate, to predict the number of individual nuclei present in a colony through time. Using population size data for colonies of differing ages, we find that our mechanistic model accurately predicts the number of nuclei for two growth environments, and show that fungal population size is most dependent on changes in mycelial growth rate.

1. Introduction

Filamentous fungi (moulds) are of considerable socioeconomic importance, being both vital as industrial tools of enzyme and antibiotic production (Santos and Linardi, 2004; Ward et al., 2004) and harmful as (food-borne) pathogens (Goto et al., 1996; Roze et al., 2007) and as pests of crops (Bosmans, 2009). In addition, fungi are used as model organisms in fundamental research, such as on the effect of sex and recombination (Bruggeman et al., 2003; Leslie and Klein, 1996) and the dynamics of adaptation (Schoustra et al., 2009). Despite their significance, we know little about the population biology of individual fungal colonies. Chiefly important for this understanding is the development of population growth models for filamentous fungi (Nielsen, 1992). Current models on fungal growth focus on directly describing biomass or product formation involved in particular industrial processes without regard to the underlying population growth driving the system (Mitchell et al., 2004). Although product formation models are useful for some industrially relevant processes, these models generally are inflexible to changes in growth physiology – e.g. how the number of individual nuclei or the density of hyphal tubes changes over time – and cannot be broadly applied, particularly to processes that directly depend on population size. Population growth models allow for 'predictive microbiology' and their utility is exemplified by various growth models for unicellular organisms such as bacteria and yeast in liquid media (Buchanan et al., 1997; McKellar, 1997; Zwietering et al., 1990) and on solid media, which is thought to more closely mimic the growth of microbes on food products (reviewed in Pipe and Grimson, 2008). For unicellular organisms, these models have been used in both applied and fundamental contexts, for example in the field of food microbiology to predict the number of bacteria

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over time in food products (Ferrer et al., 2009; McMeekin et al., 2008; Zwietering et al., 1990) and in evolutionary studies, for example by modelling the spread of faster-growing mutants arising within bacterial populations (Fitzsimmons et al., 2010).

The growth process of filamentous fungi differs significantly from the relatively simple process of binary fission employed by most unicellular organisms (Buchanan et al., 1997). Unlike unicellular populations, colonies of filamentous fungi are made of interconnected, differentiated structures with different functions. The complexity of fungal growth, particularly on solid surfaces, creates two challenges for developing population growth models. The first challenge is that the inherent interconnected structure of mycelium within fungal colonies blurs the division between 'individual' and 'population'. Product formation models have avoided this distinction by focusing instead on metrics of growth other than population size, such as biomass (Matcham et al., 1985), metabolite production (Edelstein and Segel, 1983) and resource uptake (Hamidi-Esfahani et al., 2007). To construct population-based models, biologically-relevant definitions for 'individual' and 'population size' are critical. In the context of population biology, the individual is typically defined as the smallest unit on which selection for evolutionary change can take place (Elena and Lenski, 2003), which usually is the smallest unit capable of reproducing (either sexually or asexually) (Schoustra et al., 2005). A fungal colony consists of nuclei in mycelium and spores, both of which are 'individuals' capable of forming a new colony, or 'colony forming units' (CFU). Population size is therefore defined as the total number of nuclei, with each nucleus as an individual member of the population. These definitions are congruent to those for unicellular organisms, where each cell is considered an individual (Zwietering et al., 1990). The second challenge for the development of population growth models is distilling the biology of colony growth into a parsimonious, but biologically-relevant, mathematical model. Colony growth is affected by a complex interaction between the environmental conditions (e.g. temperature, pH, salinity, etc.), the rates of nutrient uptake and conversion into biomass, and the biophysics of colony growth (Lew, 2011), however, modelling these effects directly would result in an overly complex model. Unicellular growth models combine environmental factors into several measurable parameters (e.g. lag time, rate of cell division, carrying capacity, etc.). The same can be done for filamentous fungi, keeping in mind that these parameters may have different values for each of the differentiated structures.

Taking inspiration from previously developed growth models, we developed a mechanistic population growth model for the total number of nuclei in a colony over time for the filamentous fungus *Aspergillus nidulans* growing on a solid surface. The model is based on the growth physiology of the asexual cycle (Adams et al., 1998; Mims et al., 1988; Timberlake, 1991), and is comprised of three growth phases differing in the rate of asexual spore production. We tested the fit of the model against CFU data for laboratory-grown colonies of various ages on two different growth media and find that our model provides an accurate representation of population size through time. Further, we investigate the parameter space of the model and discuss the effects of changes in physiology on growth.

2. Mechanistic model of population growth

2.1. Growth physiology and model parameters

Nuclei in asexual *A. nidulans* colonies are present in both the mycelium and in spores (Fig. 1A). Consisting of a branched network of hyphae, mycelium forms the basis of the colony and is in direct contact with the solid growth medium. Mycelia can arise from a single nucleus from either a conidiospore or fragment of another mycelium (here, we assume that spores and mycelial nuclei have the same capacity to form colonies and thus contribute equally to CFU). Mycelia expand radially by the formation of hyphae, with the apical tips of hyphae growing away from the origin of the colony at a constant mycelial growth rate (MGR, m; Table 1 lists all parameters used in this study). Apical cells contain approximately 40 mitotically active nuclei. Non-expanding, subapical, sections of hypha are subdivided by septa into cells, each containing around four metabolically active nuclei that are arrested in interphase and do not divide mitotically (Fig. 1B) (Wolkow et al., 1996; Momany and Taylor, 2000). We define the density of nuclei in mycelium, n, as uniformly distributed across area, because individual mycelia are narrow relative to the size of the entire colony (Pipe and Grimson, 2008).

Once the hyphae have matured (typically after 24 h), some subapical cells develop new sites of polarity to produce vertical multinucleate hyphae (conidiophores), each with a swollen "spore head" that supports an array of uninucleate cells that undergo serial mitoses to bud off chains of uninucleate asexual spores (conidia). Spore heads are distributed throughout the colony with uniform density p. After a brief period of development, termed 'competency time' (c), specialized cells on the conidiophore begin producing spores by repeated mitoses at a constant rate s, with each spore containing a single nucleus. Conidiophores will continue producing spores until they become saturated (where S_{sat} is the number of spores on a saturated conidiophore) at which time spore production ceases.

2.2. Mechanistic mathematical model.

The lag between growth of mycelium and conidiophore competency, and the saturation of conidiophores, makes it necessary to break the asexual growth cycle into three stages: the production of mycelium only, the production of spores and mycelium at a constant rate and the saturation of conidiophores. We model fungal colony population size on solid medium (N) as a three-part function of time, t, in hours. Before conidiophores have become competent ($t < c$), the only contribution to population size comes from mycelial nuclei (Fig. 2A). At this stage, the number of mycelial nuclei, N, is proportional to colony area and increases at a rate $dN/dt = 2\pi t m^2 n$. Here, we disregard the extension of mycelium into the growth substrate, which Trinci (1969) demonstrated occurs for *A. nidulans*. However, nuclei in these hyphae are trapped, with little prospect for dispersal in either natural or laboratory settings, and are thus unlikely to contribute extensively to population size. After conidiophores become competent, they begin producing spores at a constant rate. At the start of colony development, the density of spores is highest in the centre of the colony and declines toward the growing front. The delay in the acquisition of competency means that unlike mycelial nuclei, the density of spores is highest in the centre of the colony and declines toward the edges, with a band at the colony edge of around 0.4 cm where spores are absent (Adams et al., 1998). Spore density can be modelled as a cone centred at origin of the colony, where its height $sp(t-c)$ is the density of spores produced at t by the oldest conidiophores and its radius $m(t-c)$ corresponds to the radius of the spore-producing portion of the colony (Fig. 2B). Since mycelial nuclei also continue to be produced, at this stage the rate of population size increase is $dN/dt = 2\pi m^2 nt + \pi sp m^2 (t-c)^2$. Finally, conidiophores stop producing spores once they have become saturated with spores. As this will occur first at the centre of the colony, spore density can now be modelled as a conical frustum (a cone with the tip removed, Fig. 2C). The removed tip is also a cone, with radius proportional