British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/fbr

Review

Phenotypic heterogeneity in fungi: Importance and methodology

*Sarah K. HEWITT, David S. FOSTER, Paul S. DYER, Simon V. AVERY**

School of Life Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

ARTICLE INFO

Article history:
Received 5 August 2016
Received in revised form
15 September 2016
Accepted 15 September 2016

Keywords:
Aspergillus spp.
Cell individuality
Cell–cell variation
Hyphal heterogeneity
Non-genotypic heterogeneity
Fungal biodiversity
Yeast

ABSTRACT

Phenotypic heterogeneity describes the variation that exists between individual cells, spores or other biological entities within genetically-uniform populations of fungi or other organisms. Studies over the last 10–15 years have successfully used laboratory- and modelling-based approaches to demonstrate the prevalence of phenotypic heterogeneity and characterise the molecular bases of the phenomenon (primarily centred around heterogeneous gene expression). In contrast to progress in these areas, the relevance of phenotypic heterogeneity for the competitive success of organisms in different natural scenarios, although widely speculated upon, has only recently begun to be investigated. This review addresses this latter question as tackled in recent studies with yeasts and filamentous fungi. We concentrate on the relevance to fungal activities such as survival against environmental stressors, pathogenesis, and spoilage. We also discuss methodologies for interrogating phenotypic heterogeneity in fungi. The emerging prevalence and apparent importance of fungal phenotypic heterogeneity provides a timely reminder that certain, potentially core aspects of fungal biology still remain widely under-explored.

1. Introduction

In the context of microbiology, 'phenotypic heterogeneity' refers to the phenomenon whereby individual cells within clonally-derived populations, that have a uniform genetic background, can nevertheless display differences in phenotype (i.e., heterogeneity). This phenomenon (also termed 'non-genotypic heterogeneity') is likely to be observable in almost any phenotype. One classic example is evident when clonally-derived microbial cells are exposed to harmful stressors (Sumner *et al.*, 2003; Levy *et al.*, 2012; Holland *et al.*, 2014; Guyot *et al.*, 2015). In this situation it is frequently observed that not all of the cells of a population will lose viability simultaneously. Instead, a minority of cells often survive at levels of exposure that kill most of the sibling cells, despite all of the cells being genetically uniform. In the case of fungi, phenotypic heterogeneity may encompass variation seen between genetically-uniform populations of single cells, such as those formed by ascomycete and basidiomycete yeasts, between genetically identical mitospores, and between genetically uniform hyphal compartments of filamentous fungi. Several mechanisms underlying heterogeneity have been described, which are largely manifested via differential gene expression (gene expression noise). That is, if one or

* *Corresponding author.*
E-mail address: Simon.Avery@nottingham.ac.uk (S. V. Avery).
http://dx.doi.org/10.1016/j.fbr.2016.09.002

more genes influencing a particular phenotype are expressed at a different level in different cells or spores, heterogeneity in the phenotype is likely to result. Therefore, heterogeneous phenotypes are usually traceable to heterogeneity in levels of particular transcripts or in their (post-)translational regulation. The molecular bases for heterogeneity have been reviewed in depth elsewhere (Avery, 2006; Munsky *et al.*, 2012; Coulon *et al.*, 2013; Symmons and Raj, 2016).

Phenotypic heterogeneity is potentially of great significance in fungal biology and ecology. For example, it may result in the survival of a subset of cells or hyphae which may then go on to mount adaptive responses and allow re-establishment of populations, critical for the survival of species (Avery, 2006). Furthermore, given that the degree of phenotypic heterogeneity can vary between different strains of the same species, it can provide an extra, often not appreciated, level of variation conferring an adaptive advantage upon which natural selection can act (Blake *et al.*, 2006; Yvert *et al.*, 2013; Holland *et al.*, 2014).

Phenotypic heterogeneity is also potentially of great applied significance. In addition to survival in response to environmental stress, the phenomenon might also be important in terms of virulence whereby certain cells within a population might display enhanced pathogenicity and/or resistance to antifungal drugs (LaFleur *et al.*, 2006; Halliwell *et al.*, 2012; Pierce and Kumamoto, 2012; Bezerra *et al.*, 2013). Similarly, with respect to food spoilage certain cells or spores might be able to survive treatment by preservatives that otherwise kill all other members of the population (Steels *et al.*, 2000; Stratford *et al.*, 2013). Finally, there might also be biotechnological applications if certain cells within populations produce higher levels of desirable metabolites or proteins (Papagianni, 2004; Krijgsheld *et al.*, 2013; Xiao *et al.*, 2016).

2. Methods for determining phenotypic heterogeneity in fungi

The ability to examine single cells microscopically and to culture them discretely as single colonies has been available for nearly as long as the field of microbiology has existed. It is only with the emergence of phenotypic heterogeneity as a major field of study over the last 10–15 years that a range of new methods have been developed to examine phenotypic variability between single yeast and other fungal cells. Phenotypic variability may be present in a population as part of a normal distribution, skewed distribution or biphasic distribution, reflective of the underlying mechanism or evolutionary strategy. Therefore, examining the shape as well as the extent of the phenotype's distribution can help resolve the source and role of phenotypic heterogeneity in a population. Colony forming ability provides a classic binary assessment of single cell viability (Table 1). A quantitative measure of heterogeneity in response to a drug or stressor can be achieved by comparing the ability of single cells to form colonies over a range of doses (Fig. 1). The gradient of the dose–response curve depicts the extent of population stress-response heterogeneity, revealing

Table 1 – Methods for quantifying phenotypic heterogeneity in fungal populations.

Approach	Method	References
Dose-response analysis	% Colony forming units (CFUs) in microtiter wells	Steels *et al.* (2000), Stratford *et al.* (2013), De Brucker *et al.* (2016)
	% CFUs on agar dishes	Sumner *et al.* (2003), Stratford *et al.* (2013), Holland *et al.* (2014), De Brucker *et al.* (2016)
Flow cytometry	Gene expression reporters	Blake *et al.* (2006), de Bekker *et al.* (2011b), Levy *et al.* (2012), Liu *et al.* (2015)
	Metabolic staining	Kell *et al.* (1991), Lloyd *et al.* (1996), Davey *et al.* (2004), Noda (2008), Guyot *et al.* (2015)
	Live/dead staining	Wenisch *et al.* (1997), Attfield *et al.* (2001), Guyot *et al.* (2015)
	Intracellular cytoplasmic-pH measurement	Weigert *et al.* (2009), Stratford *et al.* (2014)
	Single cell X-ray	Crawford *et al.* (2016)
Single cell, hyphal and colony imaging	High throughput single cell microscopy	Levy *et al.* (2012), Bauer *et al.* (2015)
	Microfluidics	Fehrmann *et al.* (2013), Nobs and Maerkl (2014), Hansen *et al.* (2015), Zhu *et al.* (2015), Lee *et al.* (2016)
	Macrocolony size/growth rate variation	Stratford *et al.* (2014)
	Microcolony size/growth rate size variation	Levy *et al.* (2012), Ziv *et al.* (2013)
	Filamentous fungal macrocolony imaging	Bleichrodt *et al.* (2012), Vinck *et al.*, (2005, 2011)
	Membrane-fluidity probing	Guyot *et al.* (2015)
	Fluorescence markers of single-cell growth	Di Talia *et al.* (2007), Carlquist *et al.* (2012)
	BONCAT-FISH[a]	Hatzenpichler *et al.* (2014)
	Nonlinear spectral microscopy (NLSM)	Knaus *et al.* (2013)
Mass spectrometry	Single cell ICP-MS	Groombridge *et al.* (2013), Wang *et al.* (2015)
	Microarrays for mass spectrometry (MAMS) platform	Ibanez *et al.* (2013)
	NanoSIMS[a]	Zimmermann *et al.* (2015)
Other	Single-cell RNA-seq[a]	Tang *et al.* (2010), Fan *et al.* (2015)
	Single-hypha transcriptomics	De Bekker *et al.* (2011a)
	Zonal secretomics in fungal mycelium	Krijgsheld *et al.* (2013)

a Method developed in other cell systems, but with potential for application in fungi.