Micro-colony heterogeneity in liquid cultures

Brand Reuter, Wieke Teertstra, Han Wosten

Utrecht University, Faculty of Science, Department of Biology, Molecular Microbiology, Padualaan 8, 3584 Ch Utrecht, The Netherlands

Introduction

Aspergillus niger is an important cell factory for enzymes and organic acids. The fungus is grown in large scale bioreactors in industry. Fungal morphology can range from dispersed mycelium to mm-scale micro-colonies and is a key contributor for productivity. Here we studied the morphology of A. niger mycelium in liquid shaken cultures using a COPAS-plus device, equipped with a 1 mm nozzle.

Heterogeneity of micro-colonies is influenced by spore age and inoculum density

COPAS-analysis showed that micro-colonies are not uniformly distributed when conidia that were harvested 1 day after their formation were used for inoculation (Figure 1). Statistical analysis showed a population of large and small micro-colonies. In contrast, inoculation with spores that had been harvested 7 days after their formation resulted in a normal size distribution within the culture (Table 1A).

Initial spore concentration also impacted heterogeneity of the mycelium in a liquid shaken culture. When inoculated with a 7-fold higher spore concentration, smaller micro-colonies with a normal size distribution were formed (Table 1B).

Secondary aggregation is less frequent when compared to conidial aggregation

Aggregation may explain heterogeneity in liquid cultures. This was tested by mixing GFP and dTomato labelled spores. If both spore types were mixed at the moment of inoculation or 2 h after inoculation, all micro-colonies contained both spore types (Figure 2A). However, when these strains were mixed 8 h after inoculation, only 15% of the micro-colonies showed both types of fluorescence (Figure 2C).

References