Micro-colony heterogeneity in liquid cultures

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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[(1)]. Here we studied the morphology of *A. niger* mycelium in liquid shaken cultures using a COPAS-plus device, equipped with a 1 mm nozzle[(2)].

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 2AB). However, when these strains were mixed 8 h after inoculation, only 15% of the micro-colonies showed both types of fluorescence (Figure 2C).

Figure 1A

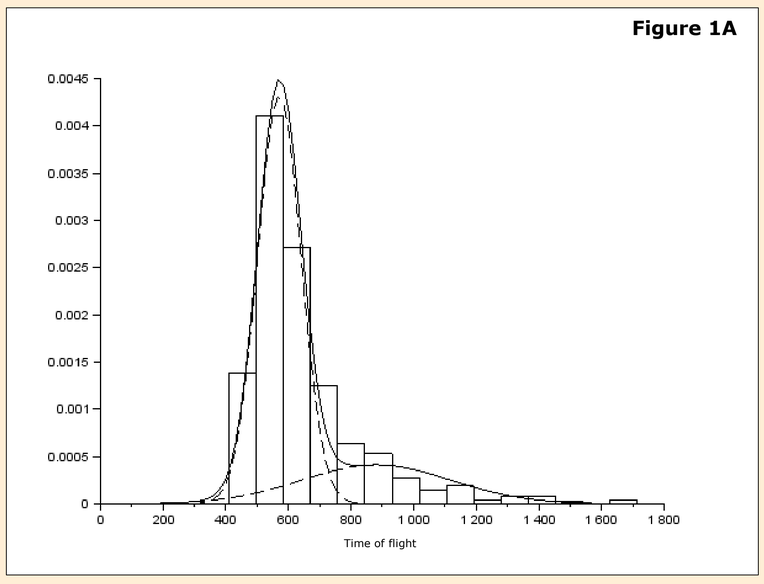


Figure 1B

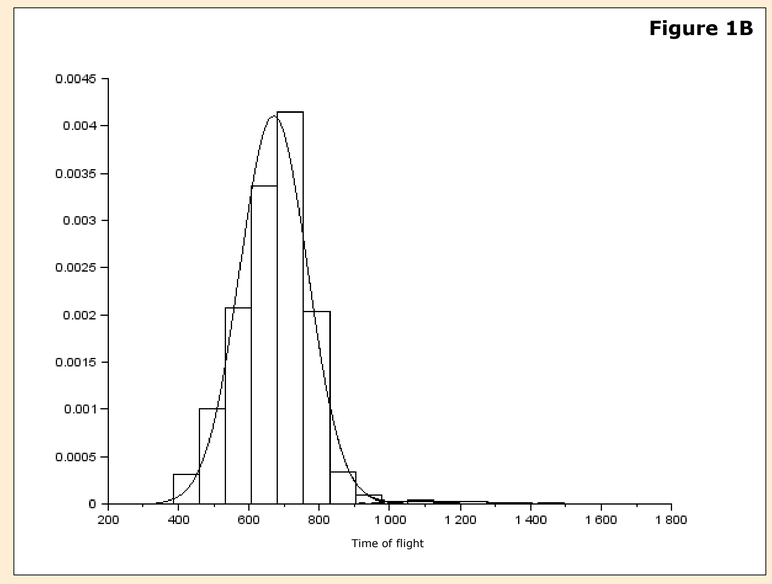


Figure 1A: Size distribution of micro-colonies from 1 day old spores

A normal distribution could not be fitted to Time of Flight data from micro-colonies from a shaken culture inoculated with 1 day old spores. Two populations were observed.

Figure 1B: Size distribution of micro-colonies from 7 day old spores

A normal distribution could be fitted to Time of Flight data from micro-colonies from a shaken culture inoculated with 7 day old spores. The second population contributed only to 3% of the total events.

Spore Age	Mean 1 (µm)	Mean 2 (µm)	Participation fraction
1-day	646 ± 74	813 ± 112	0,76 ± 0,17
4-days	631 ± 16	776 ± 109	0,77 ± 0,25
7-days	612 ± 56	817 ± 139	0,97 ± 0,01

Spore concentration (spores/ml)	Mean 1 (µm)	Mean 2 (µm)	Participation fraction
$0{,}1*10^7$	1098 ± 263	1166 ± 96	0,46 ± 0,06
$0{,}4*10^7$	646 ± 74	813 ± 112	0,76 ± 0,17
$2{,}8*10^7$	592± 34	747 ± 138	0,96 ± 0,01

Table 1A:

Size distribution of micro-colonies from spores of different age.

Table 1B:

Size distribution of micro-colonies resulting from different inoculation density

Conclusion

Maturation of spores and inoculum density are involved in heterogeneity of micro-colonies. When 7 day old spores were used, no heterogeneity could be discovered, while 1 day old spores did show heterogeneity in liquid shaken cultures. Reducing heterogeneity could improve control of productivity of the culture. When mixing strains with green or red fluorescence we determined that aggregation occurs between t=0 and t=6, afterwards only secondary aggregation is seen, this contributes to around 15% of micro-colonies

Figure 2ABC: Red and Green fluorescence of unmixed micro-colonies (Red and Green), and colonies mixed after 0, 2, and 8 hours after inoculation (Blue).

Spores containing cytosolic dTomato, or GFP, were grown for 0, 2 and 8 hours, before mixing in a 1:1 ratio. Cultures were grown for 24 h in total before COPAS analysis. When mixing at t=0, no overlap can be seen between the combined culture, and the red and green control, giving rise to completely mixed micro-colonies. When spores were mixed 8h after inoculation, when germination has already started, only 15% of micro-colonies show no overlap, indicating that a relatively small amount of micro-colonies undergo secondary aggregation.

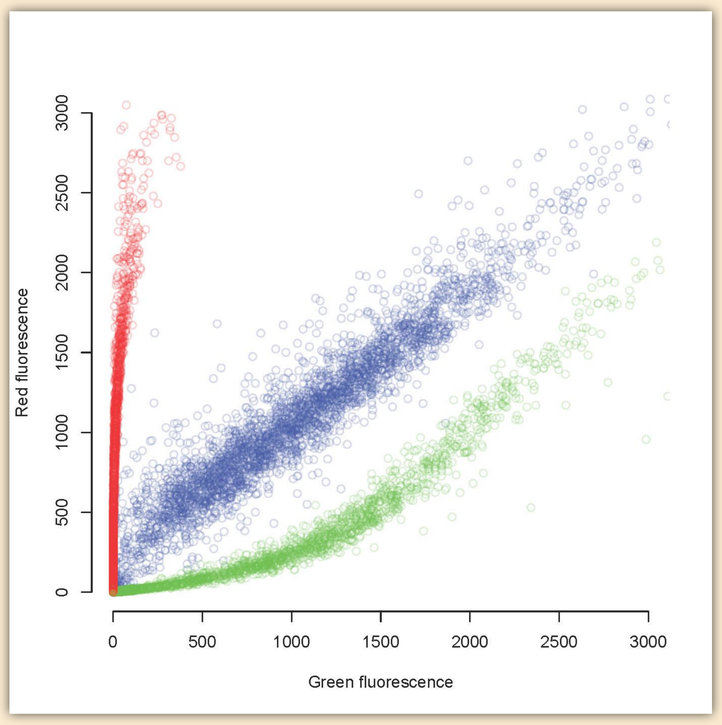


Figure 2B

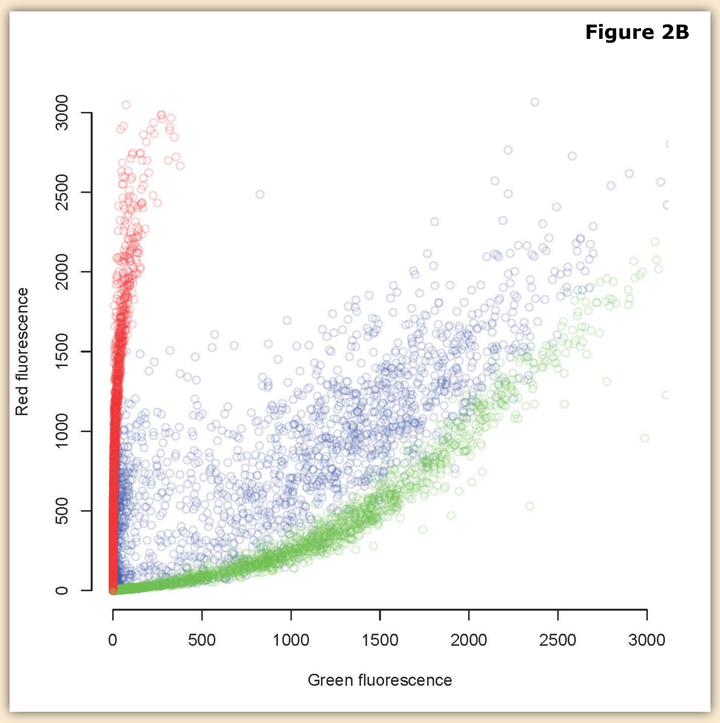
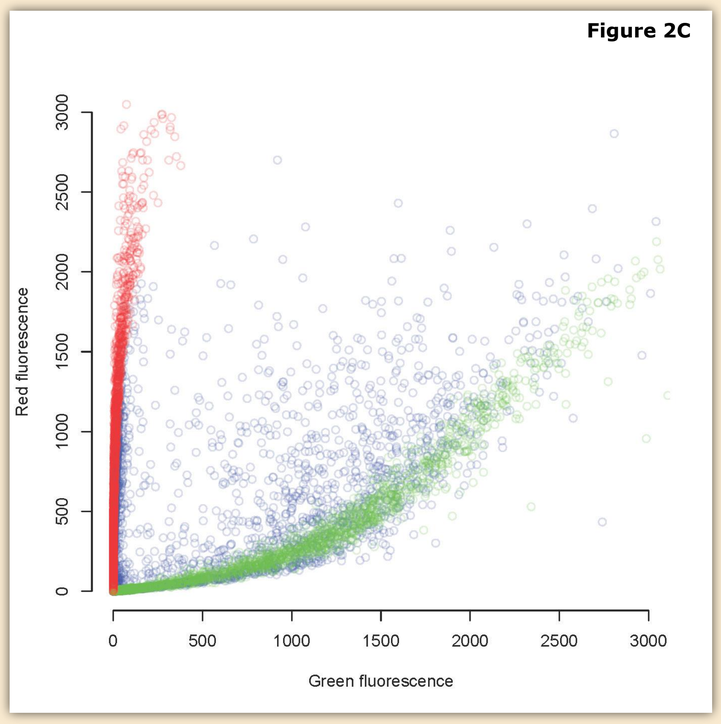


Figure 2C



References

1. Papagianni M. Fungal morphology and metabolite production in submerged mycelial processes. Biotechnol Adv. 2004 Jan;22(3):189-259. Review. PubMed PMID: 14665401.
2. van Veluw GJ, Teertstra WR, de Bekker C, Vinck A, van Beek N, Muller WH, Arentshorst M, van der Mei HC, Ram AF, Dijksterhuis J, Wösten HA. Heterogeneity in liquid shaken cultures of Aspergillus niger inoculated with melanised conidia or conidia of pigmentation mutants. Stud Mycol. 2013 Mar 15;74(1):47-57. doi: 10.3114/sim0008. Epub 2012 Sep 14. PubMed PMID: 23449476; PubMed Central PMCID: PMC3563290.