**Supplemental Text**

**The impact of inter- and intra-species spore density on germination of the food spoilage fungus *Aspergillus niger***

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**Supplemental Text 1: Selection of spore densities of *A. niger***

Conidia from 7-day-old *A. niger* cultures were monitored for swelling and germ tube formation at different densities using oCelloScope imaging and data modeling. The maximum number of spores that had swollen (Pmax swelling) or formed germ tubes (Pmax germ tube formation) was estimated based on the asymmetrical model (Dantigny et al., 2011). Also, the time (τ) to reach 0.5 Pmax swelling and 0.5 Pmax germ tube formation was estimated as was the degree of heterogeneity in the germination response (d). Conidia were introduced into wells of 96-well plates containing medium supplemented with 10 mM of alanine, proline, or arginine, along with 25 mM Na-phosphate buffer and 2 mM MgSO4. Introducing > 40.000 spores in these wells hampered the assignment of individual spores as objects, while the germlings rapidly overgrew each other making it impossible to further track the individual germinating spores. On the other hand, introducing < 5000 spores resulted in a too low spore number for a robust germination analysis. Therefore, we used densities of 5,000, 10,000, 20,000, and 40,000 spores in each well.

The d value was not affected by spore density. Spore density also had no effect on Pmax ofswelling in arginine and in proline but did have an effect in alanine (Supplemental Table 1). In the latter case, Pmax of swelling was lower in the case of 40,000 spores (71.01%) compared to the other densities (84.14-95.98%). Spore density did not affect τ swelling in the case of arginine but did impact τ in the case of proline and alanine. For instance, a higher τ was observed in proline at 5000 spores (4.55 h) when compared to the higher densities (3.59–4.15 h).

Spore density did not impact the d value of germ tube formation for any of the tested amino acids. The Pmax of germ tube formation in arginine was only different between 5,000 spores (16.46%) and 10,000 spores (4.00%). In the case of alanine, Pmax was markedly higher at the lowest density (91.20 % at 5,000 spores) and decreased progressively to 49.57% at the highest density of 40,000 spores. In the case of proline, the 40,000 spores showed a lower Pmax of germ tube formation (47.32%) when compared to the other spore densities (75.50-108.03%). τ of germ tube formation was not affected by density in the case of proline and arginine. In contrast, it progressively increased from 11.37 to 15.00 h when density increased from 5,000 to 40,000 spores in the case of alanine.

Together, these data show that 5,000 and 40,000 spores are representative for use for quantitative analysis of swelling and germ tube formation at low and high densities.

**Supplemental Table 1.** Parameter estimates of the asymmetrical model describing swelling and germ tube formation of *Aspergillus niger* when adding 5000, 10000, 20000, or 40000 spores in wells supplemented with 10 mM of alanine, proline, or arginine, 25 mM NaPO4 buffer pH 6.0, and 2 mM MgSO4. Confidence intervals are indicated between brackets, N represents the number of objects at t = 1 h, while M represents the number of objects that could no longer be monitored between 2 and 16 h. RMSE represents the root mean square error of the modeled data.

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| **­­­** | **AA** | **Density** | **Pmax (%)** | **τ (h)** | **d (-)** | **RMSE** | **N** | **M** |
| Swelling | Ala | 5000 | 95.98[92.44;99.51] a | 6.93[6.59;7.26] a | 3.83[3.16;4.50] a | 0.82 | 373 | 10 |
| Swelling | Ala | 10000 | 90.81[86.43;95.18] a | 5.28[4.84;5.72] b | 3.42[2.49;4.35] a | 1.17 | 979 | 37 |
| Swelling | Ala | 20000 | 84.14[78.58;89.69] a | 5.77[5.17;6.37] b | 3.36[2.24;4.48] a | 1.35 | 1112 | 66 |
| Swelling | Ala | 40000 | 71.91[61.67;82.14] b | 6.31[5.06;7.57] b | 2.55[1.31;3.79] a | 1.58 | 1301 | 89 |
| Swelling | Arg | 5000 | 25.06[-0.83;50.96] a | 15.00[1.47;28.53] a | 2.66[-0.16;5.48] a | 0.78 | 626 | 94 |
| Swelling | Arg | 10000 | 16.61[5.63;27.60] a | 15.00[7.02;22.98] a | 2.94[0.80;5.08] a | 0.37 | 1026 | 19 |
| Swelling | Arg | 20000 | 13.69[4.32;23.05] a | 15.00[-0.44;30.44] a | 1.41[0.67;2.15] a | 0.16 | 1372 | 14 |
| Swelling | Arg | 40000 | 11.25[-1.93;24.43] a | 15.00[-12.95;42.95] a | 1.32[0.16;2.48] a | 0.21 | 1408 | 105 |
| Swelling | Pro | 5000 | 94.95[91.22;98.69] a | 4.55[4.19;4.91] a | 3.70[2.69;4.71] a | 1.18 | 526 | 7 |
| Swelling | Pro | 10000 | 95.82[91.38;100.25] a | 3.59[3.18;4.00] b | 2.88[1.99;3.77] a | 1.32 | 857 | 17 |
| Swelling | Pro | 20000 | 90.33[87.04;93.63] a | 3.68[3.35;4.01] b | 3.34[2.42;4.26] a | 1.09 | 1142 | 30 |
| Swelling | Pro | 40000 | 82.59[77.95;87.24] a | 4.15[3.64;4.66] b | 3.34[2.06;4.62] a | 1.44 | 1306 | 38 |
| Germ tube formation | Ala | 5000 | 91.20[88.12;94.28] a | 11.37[11.11;11.63] a | 8.94[7.30;10.58] a | 0.77 | 373 | 10 |
| Germ tube formation | Ala | 10000 | 79.89[75.76;84.02] b | 12.48[12.10;12.87] b | 8.75[6.66;10.84] a | 0.88 | 979 | 37 |
| Germ tube formation | Ala | 20000 | 67.95[64.63;71.27] c | 13.72[13.36;14.08] c | 6.90[5.87;7.93] a | 0.45 | 1112 | 66 |
| Germ tube formation | Ala | 40000 | 49.57[45.72;53.41] d | 15.00[14.39;15.61] d | 5.54[4.71;6.37] a | 0.29 | 1301 | 89 |
| Germ tube formation | Arg | 5000 | 16.46[7.61;25.30] a | 15.00[10.12;19.88] a | 4.35[0.79;7.91] a | 0.48 | 626 | 94 |
| Germ tube formation | Arg | 10000 | 4.00[2.20;5.81] b | 15.00[11.82;18.18] a | 6.83[-0.64;14.3] a | 0.18 | 1026 | 19 |
| Germ tube formation | Arg | 20000 | 3.79[-1.35;8.94]b,a | 15.00[-11.23;41.23] a | 1.67[-0.16;3.50] a | 0.10 | 1372 | 14 |
| Germ tube formation | Arg | 40000 | 4.64[1.17;8.10]b,a | 15.00[3.17;26.83] a | 2.11[0.69;3.53] a | 0.08 | 1408 | 105 |
| Germ tube formation | Pro | 5000 | 108.03[93.02;123.04] a | 13.61[12.14;15.09] a | 3.27[2.61;3.93] a | 0.77 | 526 | 7 |
| Germ tube formation | Pro | 10000 | 97.39[83.92;110.87] a | 15.00[13.77;16.23] a | 4.51[3.52;5.50] a | 0.77 | 857 | 17 |
| Germ tube formation | Pro | 20000 | 75.50[65.95;85.05] a | 15.00[13.93;16.07] a | 4.82[3.79;5.85] a | 0.60 | 1142 | 30 |
| Germ tube formation | Pro | 40000 | 47.32[42.93;51.71] b | 15.00[14.31;15.69] a | 6.13[4.90;7.36] a | 0.38 | 1306 | 38 |

**Supplemental Text 2: Impact of density on heterogeneity in the germination response of *A. niger***

*Supplemental Text 2A*

The d value of swelling and germ tube formation of *A. niger* conidia was not affected by spore density in the case of arginine and alanine (Table 1). This was also the case for the swelling response in proline, but d was affected by density during germ tube formation in the presence of this amino acid. In the latter case, d values were 5.45 and 8.19 for low and high spore density, respectively. Together, the impact of density on heterogeneity in the germination response is low, if present at all.

*Supplemental Text 2B*

The d value of swelling of *A. niger* spores in the presence of either arginine, alanine or proline was affected after applying the selection criteria to distinguish the *A. niger* spores from those of *A. oryzae* (Table 2). This was also the case for proline for the *A. niger* subset to distinguish these spores from those of *A. clavatus*. The d of germ tube formation was only affected after applying the selection criteria to distinguish the *A. niger* spores from those of *A. oryzae* in the case of proline. In all other cases, the selection criteria did not impact d of swelling and germ tube formation. Together, the selected subpopulations are representative of the whole population of *A. niger* spores except for the subpopulation selected for co-culturing with *A. oryzae* or, to a lesser extent, *A. clavatus.*

*Supplemental Text 2C*

The d value of swelling of *A. niger* spores was not affected by the presence of other aspergilli in arginine, while it was affected in all cases in the presence of proline. Also, *A. niger* co-cultured with *A. clavatus* showed a difference in heterogeneity when compared to the mono-culture (4.46 versus 3.28) in the presence of alanine. In the case of germ tube formation, d was not affected by the presence of other aspergilli in arginine, while it was affected in all cases in proline and alanine. In fact, d was reduced in these inducing amino acids in the presence of another *Aspergillus*, implying germ tube formation of *A. niger* becomes more heterogeneous when exposed to other aspergilli. This also applies to swelling of the *A. niger* spores in proline.