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Genetic and environmental factors determining heterogeneity in preservation stress resistance of Aspergillus niger conidia
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CHAPTER 3

Interkingdom microbial variability in heat resistance

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Abstract

Microbial species are inherently variable, which is reflected in intraspecies genotypic and phenotypic differences. Strain-to-strain variation gives rise to variability in stress resistance and plays a crucial role in microbial ecology. Here, strain variability in heat resistance of asexual spores (conidia) of the fungal species *Aspergillus niger*, *Penicillium roqueforti* and *Paecilomyces variotii* was quantified and compared to variability found in the literature. After heat treatment, a 5.4- to 8.6-fold difference in inactivation rate was found between individual strains within each species, while the strain variability of the three fungal species was not statistically different. We hypothesised that the degree of intraspecies variability is uniform, not only within the fungal kingdom, but also between different microbial kingdoms. Comparison with three spore-forming bacteria and two non-spore-forming bacteria revealed that the variability of the different species was indeed in the same order of magnitude, which hints to a microbial signature of variation that exceeds kingdom boundaries.

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Introduction

Diversity of microbial species is key to adapt to environmental changes and to thrive in different niches. Intraspecies variability includes all variation within a species, including genotypic and phenotypic differences. Unravelling drivers for intraspecies variability has been a broadly studied subject the past decade including elucidating mechanistic differences between strains [1–3] and sources of phenotypic heterogeneity of genetic identical cell populations [4,5]. Strain diversity can have huge consequences on diagnostics, virulence and antimicrobial treatments in clinical microbiology, or on the efficacy of food preservation methods [6–10].

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As microbial species are inherently variable, strains of the same species may differ in their response to environmental stresses. Indeed, large differences in stress robustness have been reported in bacterial species [11]. This suggests that microbial stress robustness is a relevant trait to quantify strain variability. Recently, strain variability in heat resistance has been quantified for bacterial vegetative cells of the pathogen *Listeria monocytogenes* [12] and the food-borne organism *Lactiplantibacillus plantarum* [13] (previously known as *Lactobacillus plantarum* [14]) and for bacterial spores of the pathogen *Bacillus cereus* [11], and the food spoilers *Bacillus subtilis* [8] and *Geobacillus stearothermophilus* [15]. Notably, the quantified strain variability was high for the tested organisms and inactivation rates of the most heat sensitive and most heat resistance strains of the same species could differ a factor ten. This means that when a similar temperature/time regime is applied, the most heat resistant strain will be reduced with a factor 10, while the most heat sensitive strains will be reduced with a factor 10^{10}. This results in huge differences in heat treatment efficacies, depending on the heat stress robustness of the microbial contaminant [11].

Spores of bacteria and fungi are considered more stress resistant than vegetative cells [16]. The stress resistance of fungal spores varies strongly, ranging from spores that display stress resistance similar to that of vegetative cells to very high stress resistance that can be comparable to bacterial spores [16–18]. Filamentous Ascomyce-

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te fungi that belong to the order Eurotiales can produce asexual spores called conidia. Airborne conidia are resistant to various environmental stresses including ultra violet (UV) radiation, desiccation, cold and heat stress [16]. Conidia are an integral part of the fungal life cycle, and can be distributed in space by air, wind or other vectors and are abundantly present in the environment. For example, *Penicillium chrysogenum* conidia are so widespread that they are considered being cosmopolitan [19] and these airborne conidia are found in the air and soil in many different habitats [20]. Studies have shown that conidia can travel large distances. For instance, conidia of *Aspergillus sydowii* have been suggested to be transported over thousands of kilometres from the Sahara desert to the Caribbean reefs [21]. Being airborne and widely present, fungal airborne conidia are often related to food spoilage, leading to considerable losses of food and feed [22].

Recently, conidial heat resistance of various strains of the food spoilage fungus *Paecilomyces variotii* was reported to be highly variable. Decimal reduction times of thermal treatments at 60°C (D_{60}-values) ranged from 3.5 to 27.6 minutes when different strains were used of the same species [23]. This prompted us to study a larger selection of isolates and to quantify variability in conidial heat resistance among strains of *Pae. variotii* in detail. Furthermore, we also assessed strain variability in conidial heat resistance of the fermentative fungus *Penicillium roqueforti*, and for *Aspergillus niger*, which is commonly used as model fungus. Both species are also known as food spoilers. The quantified strain variabilities were compared between the fungal species, and also compared to strain variability in heat resistance of bacterial vegetative cells and spores, to assess strain variability across kingdom borders.

Results

Verification of strain identity

In total, 21 *A. niger*, 20 *P. roqueforti* and 20 *Pae. variotii* strains were selected (Table 3.S1) and their identity was verified by sequencing genetic marker genes according to the phylogenetic standards [24]. Based on the partial sequences of *caM*, all *A. niger*

strains grouped together with type strain *A. niger* NRRL 326, with *Aspergillus welwitschiae*, the most closely related species, being the sister clade (Fig. 3.1a). Similarly, the partial *benA* sequences of the *P. roqueforti* strains grouped with type strain CBS 221.30 and segregated from the closely related *Penicillium mediterraneum* (Fig. 3.1b). In agreement with previous studies [25,26], we found more intraspecies variation in the partial *benA* sequences of *Pae. variotii* compared to *A. niger* and *P. roqueforti*. However, all *Pae. variotii* strains clustered with type strain CBS 102.74, while *Paecilomyces brunneolus* was sister to this cluster (Fig. 3.1c).
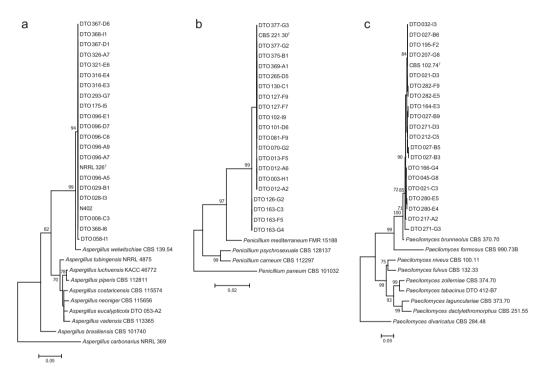
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Figure 3.1. Maximum likelihood trees for strain identification. (a) Phylogram based on partial *caM* sequence of studied *A. niger* strains, including type strain NRRL 326 and other closely related *Aspergillus* species with *Aspergillus carbonarius* used as outgroup. (b) Phylogram based on partial *benA* sequences of *P. roqueforti* strains, including type strain CBS 221.30 and other closely related *Penicillium* species with *Penicillium paneum* as outgroup. (c) Phylogram based on partial *benA* gene sequences of *Pae. variotii* strains, including type strain CBS 102.74 and other closely related *Paecilomyces* species with *Paecilomyces divaricatus* as outgroup.

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Quantification of heat resistance

Strains of *A. niger*, *P. roqueforti*, and *Pae. variotii* were heat-treated using biologically independent batches of conidia and technical duplicates. The differences between the technical replicates were rather small for all strains of the three species (Figure 3.2a, d, g). The differences between the biological replicates were clearly higher than those of the experimental duplicates (Figure 3.2b, e, h), but much higher differences were found between the individual strains per species (Figure 3.2c, f, i). Most inactivation kinetics, *i.e.* 237 out of 366, did not show a significant tailing or a shoulder curvature, and a linear model was used to calculate the *D*-value. For the other data sets the reparameterized Weibull model (Eq. 1) was used to calculate the average *D*-value (Table 3.S2).

The most heat-resistant *P. roqueforti* strain was DTO 013-F5, while the most heat-sensitive strain was DTO 130-C1 with D_{56}-values of 13.6 ± 3.0 and 1.6 ± 0.38 minutes, respectively. Similar to *P. roqueforti*, about an eight-fold difference was found between the most heat-resistant *Pae. variotii* strain DTO 195-F2 and the most heat-sensitive strain DTO 212-C5, with corresponding D_{60}-values of 26.6 ± 3.4 and 3.5 ± 0.30 minutes, respectively. This indicates that for this specific heat treatment, one out of ten cells will survive for the most resistant strain, while only one out of 10^8 cells will survive for the most sensitive strain. Three out of 21 *A. niger* strains, DTO 028-I3, DTO 029-B1 and DTO 058-I1, did not sporulate well after 7 days growth on MEA at 25°C. A better sporulation was achieved when cultivating at 30°C instead of 25°C, and therefore this temperature was used to culture conidia. Interestingly, these three strains belonged to the most heat sensitive strains, with D_{54}-values of 12.6 ± 1.7, 3.7 ± 0.60 and 9.9 ± 2.2 minutes, respectively. Impeded sporulation can be a sign of degeneration of a strain [27]. Indeed, these three strains were deposited more than six decades ago into the CBS culture collection and it cannot be excluded that the strains degenerated over the years or arrived in a degenerated state when deposited. Because growing cultures at higher temperatures can significantly enhance heat resistance in the case of *Aspergillus fumigatus* [28] and *P. roqueforti* conidia [29], it was decided to exclude DTO 028-I3, DTO 029-B1 and DTO 058-I1 for further analysis. This made DTO 367-D1 the most sensitive

and DTO 326-A2 the most resistant *A. niger* strain with D_{54}-values of 9.4 ± 0.85 and 50.4 ± 11.9 minutes, respectively.
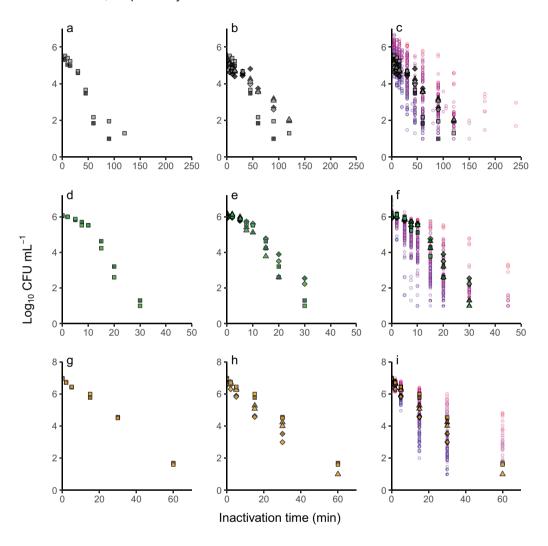

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Figure 3.2. Variability in thermal inactivation of species. Thermal inactivation was performed at 54°C for *A. niger* (a-c), 56°C for *P. roqueforti* (d-f) and 60°C for *Pae. variotii* (g-i). Experimental variability (a, d, g), biological variability (b, e, h) and strain variability (c, f, i) is depicted by the $\log_{10}$ CFU mL^{-1} data of each experiment. The strains *A. niger* DTO 316-E3 (black; a-c), *P. roqueforti* DTO 163-C3 (green; d-f) and *Pae. variotii* DTO 166-G4 (orange; g-i) are highlighted, showing two experimental replicates (dark fill, light fill) of three biological replicates (□, ◇ and Δ). All other strains (○; c, f, i) are coloured using a gradient from blue (heat-sensitive) to red (heat-resistant) based on the mean *D*-values presented in Table 3.S2.

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Quantification of variability

The experimental, biological and strain variability of the three species was quantitative-ly expressed in $\sqrt{MSE}$ (*i.e.* the standard deviation, σ) of the $\log_{10}$ *D*-values, which is a measure of variability (Fig. 3.3). Indeed, as observed in Fig. 3.2, experimental variability was the lowest variability factor with σ$_e$ values of 0.045, 0.044 and 0.033 for *A. niger*, *P. roqueforti* and *Pae. variotii*, respectively. Biological variability values were larger with a σ$_b$ of 0.092, 0.096 and 0.084 for *A. niger*, *P. roqueforti* and *Pae. variotii*, respectively. Strain variability was clearly higher, with σ$_s$ of 0.197, 0.179 and 0.230 for the three fungi, respectively. Interestingly, the 95% confidence intervals of the three species were over-lapping for each of the variability factors. This indicates that there are no differences in the magnitude of the variability between the species. However, the variability factors were clearly different, with strain variability being higher than biological variability, and both being higher than experimental variability.
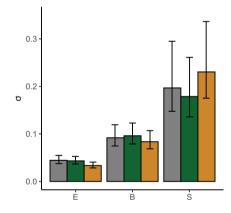

Figure 3.3. Quantification of variability. Experimental variability (E), biological variability (B) and strain variability (S) of *A. niger* (grey), *P. roqueforti* (green) and *Pae. variotii* (orange). For strain variability 18 *A. niger*, 20 *P. roqueforti* and 20 *Pae. variotii* strains were used to determine σ$_s$ values. Error bars represent the 95% confidence interval of the σ values.

Meta-analysis

The conidial heat resistance of *A. niger* and *P. roqueforti* strains presented in this study was compared with available data from the literature. Only recently, two studies de-scribed the heat resistance for *Pae. variotii* conidia [23,30] and therefore this fungus was

excluded for the meta-analysis. The D-values from literature for *A. niger* and *P. roque-forti* and the D-values collected in the current study are shown in Figure 3.4a and 3.4b, respectively. The linear correlation between the $\log_{10} D$-values and temperature allowed to calculate the z-value, indicating the temperature increase needed to decrease D-values 10-fold. The z-values were 8.9°C for *A. niger* and 7.8°C for *P. roqueforti*, which is comparable to z-values found for multiple bacterial species [31]. The deviation of each data point to the linear regression between $\log_{10} D$-value and temperature was used to quantify the overall variability σ_T, which was 0.432 for *A. niger* and 0.413 for *P. roqueforti*. With σ_s values 46% and 43% of the σ_T values for *A. niger* and *P. roqueforti*, respectively, these results indicate that strain variability is a substantial source of variability in the overall variability found.

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Figure 3.4. Meta-analysis of *A. niger* and *P. roqueforti* D-values. $\log_{10} D$-values from literature (○) and mean $\log_{10} D$-values per strain presented in this study (◇) were combined to determine z-values and overall variability for *A. niger* (a) and *P. roqueforti* (b). Mean $\log_{10} D$-values of *Pae. variotii* strains presented in this study are depicted as orange ◇ in both panels. The 95% prediction intervals of the linear regression analysis are depicted as dashed lines in both panels.

Interkingdom comparison

It is well known that heat resistance of bacterial spores and vegetative cells differs enormously among species, and consequently the D-values are very different when determined at the same temperature. Interestingly, contrary to the magnitude, the intraspecies

variability of bacterial species inactivation rates were in the same order of magnitude when five different bacterial species were compared [11], including 3 spore-forming bacteria, *B. subtilis*, *B. cereus*, *G. stearothermophilus*, and 2 non-spore-forming bacteria, *L. monocytogenes* and *Lpb. plantarum*. Because the current study used a similar experimental set up to determine heat resistance between fungal strains, taking into account the variability between biologically independent replicates and variability between technical duplicates, we could compare the σ_e, σ_b and σ_s values of the three fungal species to those of the five bacterial species (Fig. 3.5). Note that the *B. subtilis* strains were grouped in a high-level heat resistant group and a low-level heat resistant group for quantification of strain variability. The *B. subtilis* strains that produced high-level heat resistant spores proved to harbour a mobile genetic element, *spoVA*[2mob], that confers high-level heat resistance to spores [32], giving genetic evidence for clustering the corresponding strains into two groups. Interestingly, for all microbial species, strain variability was larger than biological and experimental variabilities. Altogether, these data suggest that the different levels of variability in heat resistance of fungal conidia are very similar to those of bacterial spores and cells.

Discussion

Intraspecies variability is inherent in microbial species. We scrutinized conidial heat resistance of three fungal species and quantified variability at experimental, biological and strain level. In total, 18 *A. niger*, 20 *P. roqueforti* and 20 *Pae. variotii* strains were used to quantify strain variability. Although some reference strains were included in the strain selection it is of importance to select the strains randomly in order to represent variability found in nature. In mycological research, it can be challenging to identify fungal isolates to species level as some are cryptic species. For instance, *A. niger* is difficult to distinguish from *Aspergillus luchuensis* and *Aspergillus welwitschiae* based on morphology [33]. Even identification by sequencing of genetic marker genes can be puzzling since databases can contain sequences of previously misidentified isolates [34,35]. Identifica-

tion of the strains used in this study by phylogenetic analysis of marker genes sequences of reference strains provided a robust identification of the current species. Therefore, the selection of fungal strains and the genetic locus used for comparison indicate that the observed variation in conidial heat resistance is truly intraspecies.
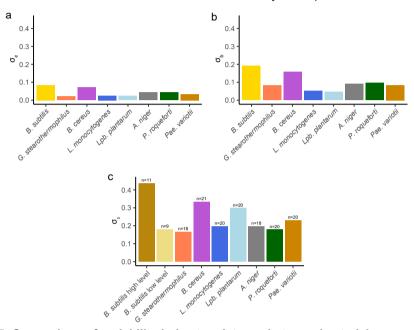

Figure 3.5. Comparison of variability in heat resistance between bacterial spores, vegetative cells and fungal conidia. The y-axes represent experimental σ_e (a), biological σ_b (b) and strain σ_s variability (c) in heat resistance of bacterial spores of *B. subtilis*, *B. cereus* and *G. stearothermophilus*, bacterial vegetative cells of *L. monocytogenes* and *Lpb. plantarum* and fungal conidia of *A. niger*, *P. roqueforti* and *Pae. variotii*. For strain variability, *n* represents the number of strains used to determine σ_s values. Data of bacterial species was adapted from den Besten *et al.*, 2018.

Differences in heat resistance are not only caused by variation in genetic background. Heterogeneity in genetically uniform cells can contribute to survival against environmental stress in yeast species [36]. Some strains of *Pae. variotii* can produce conidia populations that are heterogeneous in size [23]. The same study stated that strains producing conidia with a larger mean size tend to be more heat resistant; hinting that the larger conidia within spore populations could be more heat resistant compared to small size conidia. In addition to these examples of phenotypic heterogeneity, environmental growth conditions can have a significant effect on heat resistance. Besides cultivation

temperature [28,29], the maturation of conidia also plays a role in the development of heat resistance as conidia from older colonies of *A. niger* and *P. roqueforti* showed higher robustness to heat treatments [29,37]. On the other hand, environmental conditions during conidiation can also reduce heat resistance of fungal conidia. Growth at pH 4.6 resulted in sensitive conidia compared to the more optimal conditions at pH 8.0 of the insect-pathogenic fungus *Metarhizium robertsii* [38]. Intracellular compatible solutes and protective proteins are known to provide heat robustness to fungal species [16,39]. Conidia of *A. niger* contain large amounts of Hsp70 transcripts [40] and mannitol [37], while arabitol, the hydrophilins con-6 and con-10 and 17 predicted proteins with unknown function were implied to play a role in heat resistance of *P. roqueforti* conidia [29]. On the other hand, *Pae. variotii* conidia contain predominantly trehalose as compatible solute [25] and in higher amount than *A. niger* [37] and *P. roqueforti* [29], which might explain, at least in part, the higher heat resistance of this species.

Our experimental set up was aimed to reduce environmental variation as much as possible by spreading many conidia over one plate for inoculation. This way, we anticipated to differences due to colony age, which could be interpreted as environmental variation. In the meta-analysis, we compared our data with data available in literature, where different growth conditions and heating menstrua were applied, to visualize this overall variability. This demonstrated that strain variability is a large, if not the largest source of the overall variability. This is consistent with bacterial species, where σ_s values are typically 40% to 75% of the overall variability found in literature [11]. For two other bacterial species, *Escherichia coli* O157:H7 and *Staphylococcus aureus*, *D*-values have been for a large number of strains at one or two temperatures, allowing us also to quantify the strain variability for these two species using our approach (Eq. 4). This showed that the strain variability of *Escherichia coli* O157:H7 was 0.206 (n=17) as calculated by the $\log_{10}$ *D*-value at 55°C and 60°C, whereas for *S. aureus* the strain variability was 0.360 (n=15) as calculated by the $\log_{10}$ *D*-value at 58°C. Interestingly, these values are in the same order of magnitude as presented in Fig. 3.4c. To the best of our knowledge, for one species, namely *Salmonella* spp., reported differences in heat resistance among

strains isolated from various sources tend to be much smaller with σ_s values of 0.07 and 0.09 [6,11,41], and this supports the relevance to quantify the strain variability of this species in more detail. Quantified microbial variability is crucial information to be included in risk assessments to realistically predict microbial behaviour.

In conclusion, strain variability in conidial heat resistance of the three fungal species was in the same order of magnitude as for bacterial species, which hints to a natural diversity that stretches beyond kingdoms. In other fields of research, intraspecies variability also occurs in virulence, growth and biofilm formation [9], and an intriguing question is whether the impact of strain variability is also comparable for other microbial traits.

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Experimental Procedures

Strain selection and identification

All strains were selected and obtained from the CBS culture collection and the working collection of the Food and Indoor Mycology (DTO) group, both housed at the Westerdijk Fungal Biodiversity Institute (Table 3.S1). Strains included the previously studied *A. niger* N402 [42], *P. roqueforti* DTO 377-G3 [29] and *Pae. variotii* DTO 032-I3, DTO 212-C5, DTO 217-A2 and DTO 280-E5 (CBS 101075) [25,43]. The other strains were selected from both food and non-food sources. Identity of strains was confirmed by sequencing the partial calmodulin (*caM*) gene for *A. niger* and the partial beta-tubulin (*benA*) gene for *P. roqueforti* and *Pae. variotii* that can be used as identification marker [44–46]. After alignment using MUSCLE, a maximum likelihood tree of each species was computed with 1,000 bootstrap replications using MEGA7 [47]. Reference sequences of closely related species and the type strain were included in the phylograms [24]. For each tree, the model with the lowest Bayesian Information Criterion (BIC) score was used. The Kimura 2-parameter model including gamma distribution (K2+G) was used for *A. niger*, and the Jukes-Cantor (JC) model and the Kimura 2-parameter model including invariant sites (K2+I) model were used for *P. roqueforti* and *Pae. variotii*, respectively.

Growth conditions and harvesting conidia

Culturing and harvesting of conidia were performed as described [25]. In short, fungal strains stored in 30% (w/v) glycerol at -20°C were spot-inoculated on malt extract agar (MEA, Oxoid, Hampshire, UK) and incubated for 7 days at 25°C. *A. niger* strains DTO 028-I3 and DTO 058-I1 were cultured at 30°C because sporulation was not sufficient at 25°C. Freshly harvested conidia were used to spread-inoculate a new MEA plate to anticipate on differences in age within the conidia population. Conidia were harvested after 7 days of incubation in ACES buffer (10 mM N(2-acetamido)-2-aminoethanesulfonic acid, 0.02% Tween 80, pH 6.8) and filtered using either sterile glass wool in a syringe or sterilized Amplitude EcoCloth wipes (Contec Europe, Vannes, France). Subsequently,

conidia were washed two times in ACES buffer. The concentration of conidia in suspension was determined using a Coulter Counter Multisizer 3 (Beckman Coulter, Life Sciences, Indianapolis, USA) [23], a Bio-Rad TC20 Automated Cell Counter (Bio-Rad Laboratories, Lunteren, The Netherlands), or a Bürker-Türk heamocytometer (VWR, Amsterdam, The Netherlands) and the conidia suspension was set at $2 * 10^8$ conidia ml^{-1}.

Thermal treatments

A volume of 0.2 to 1 mL of conidia suspension was added to pre-heated ACES buffer to a total volume of 20 mL in Erlenmeyer flasks in a water bath. Conidia of *A. niger*, *P. roqueforti* and *Pae. variotii* were treated at 54°C, 56°C and 60°C, respectively. At various time points, 1 mL samples were taken, immediately chilled on ice, and decimally diluted. One hundred µL was surface-inoculated on MEA and plates were incubated at 25°C. The non-heated conidia suspension was also decimally diluted and subsequently plated to determine the initial viable concentration of conidia at *t*=0 minutes. Colonies of *Pae. variotii* were counted after three days of incubation, while *A. niger* and *P. roqueforti* colonies were counted after 7 days. The log_{10} colony forming units (CFU) mL^{-1} was calculated for each sampling time point.

Quantification of heat resistance

The reparameterised Weibull model (Eq. 1) [48] was fitted to the log_{10} CFU mL^{-1} data of each inactivation experiment with the R package Growthrates using the Levenberg-Marquardt algorithm [49]. The Weibull model allows fitting linear, concave, and convex inactivation curves and was able to fit the different thermal inactivation curves of the strains.

$$Log_{10}(N_t) = Log_{10}(N_0) - \Delta \cdot \left(\frac{t}{t_{\Delta D}}\right)^{\beta} \qquad (1)$$

where N_0 is the initial concentration of conidia (CFU mL^{-1}), N_t is the number of surviving conidia (CFU mL^{-1}) at time point t, Δ is the reference number of decimal reductions, $t_{\Delta D}$ represents the time needed to reduce the initial number of conidia with Δ decimals, and

the shape parameter where β > 1 gives a concave and β < 1 a convex behaviour. When β was significantly different from 1, the average D-value was estimated as $\frac{t_{\Delta D}}{\Delta}$. If not, the negative reciprocal of the linear regression slope, $\frac{-1}{slope}$, was used to estimate the D-value as described [25].

Quantification of variability

Experimental, biological and strain variabilities were quantified per species using the Aryani method [12]. For each strain, three biologically independent batches of conidial spores were prepared, and conidial heat resistance was tested in duplicate for each batch of conidial spores. Experimental variability (σ_e) was defined as the variability between parallel experimental replicates, and expressed by the root mean square error ($\sqrt{MSE_e}$) of Eq. 2

$$MSE_e = \frac{RSS_e}{DF_e} = \frac{\sum_{S=1}^{i}\sum_{B=1}^{3}\sum_{E=1}^{2}(X_{EBS}-X_{BS})^2}{n-p} \qquad (2)$$

where MSE_e is mean square error, RSS_e is Residual Sum of Squares, DF_e is Degrees of Freedom, X_{EBS} is the $\log_{10}$ D-value of each experiment 'E' of biological replicate 'B' and strain 'S', X_{BS} is the average of the $\log_{10}$ D-value of the experimental duplicates of each biological replicate 'B' of strain 'S', i is the number of strains used per species and is the number of data points ($n = 2 * 3 * i$) minus the number of parameters ($p = 3 * i$).

Biological variability (σ_b) was expressed by $\sqrt{MSE_b}$ of Eq. 3

$$MSE_b = \frac{RSS_b}{DF_b} = \frac{\sum_{S=1}^{i}\sum_{B=1}^{3}(X_{BS}-X_S)^2}{n-p} \qquad (3)$$

where X_s is the average of X_{BS} from the biological triplicates of strain 'S' and is the number of data points ($n = 3 * i$) minus the number of parameters ($p = 1 * i$).

Strain variability (σ_s) was expressed by $\sqrt{MSE_s}$ of Eq. 4

$$MSE_s = \frac{RSS_s}{DF_s} = \frac{\sum_{S=1}^{i}(X_S-X)^2}{n-p} \qquad (4)$$

where X is the average of X_s of all i strains and DF_s is the number of data points ($n = i$) minus the number of parameters ($p = i$).

The 95% confidence intervals of σ_e, σ_b and σ_s were calculated according to Eq. 5

$$\sqrt{\frac{RSS}{\chi^2_{DF;\,\alpha/2}}} \leq \sigma \leq \sqrt{\frac{RSS}{\chi^2_{DF;\,1-\alpha/2}}} \qquad (5)$$

where X^2 is the critical Chi-square value at $\alpha/2$ and $1 - \alpha/2$ with $\alpha = 0.05$, using the same *RSS* and *DF* definitions as in Eq. 2-4.

Meta-analysis

Data describing the inactivation kinetics of conidia of *A. niger* [50–57] and *P. roqueforti* [29,54,58–61] were collected from literature. The obtained *D*-values were $\log_{10}$ transformed and the mean $\log_{10}$ *D*-value of each strain tested in this study were added to the data set, resulting in 48 and 148 data points for *A. niger* and *P. roqueforti*, respectively. The $\log_{10}$*D*-values versus the temperature were used to calculated the *z*-value for each species, being negative reciprocal of the linear regression slope, $\frac{-1}{slope}$. Subsequently, the 95% prediction interval of the linear regression was calculated using Eq. 6

$$Log_{10}D_{ref} \pm t_{DF;\,1-0.5\alpha}\sqrt{\frac{RSS}{DF}} \qquad (6)$$

Where D_{ref} is the reference $\log_{10}$ *D*-value at the reference temperature, *t* is the Student *t*-value with degrees of freedom (*DF*) *n - 2* and $\alpha = 0.05$, *RSS* is the residual sum of squares calculated from the deviation of the data to the linear regression line. The overall variability (σ_t) was defined as the deviation of the data to the linear regression line, $\sqrt{\frac{RSS}{DF}}$ with *RSS* the residual sum of squares calculated from the deviation of the data to the linear regression line, and *n - 2*.

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Additional files

Table 3.S1. Overview of the strains used in this study

	Strain No.	Other collections	Substrate	Location	Genbank accession number[A]	Reference
Aspergillus niger	DTO 008-C3	CBS 113.50	Leather	Germany	MW148182	1
	DTO 028-I3	CBS 112.32	Unknown	Japan	MW148184	1
	DTO 029-B1	CBS 124.48	Unknown	Ghana	MW148185	
	DTO 058-I1	CBS 118.52	Unknown	Unknown	MW148186	
	DTO 096-A5	CBS 147371; IBT 20381	Green coffee bean	India	GU195635	2
	DTO 096-A7	CBS 147320; IBT 22937	Grape	Australia	MW148187	
	DTO 096-A9	CBS 147321; IBT 23366	Artic soil	Svalbard, Norway	MW148188	
	DTO 096-C6	CBS 147322; IBT 27294	Coffee	Brazil	MW148189	
	DTO 096-D7	CBS 147323; IBT 29331	Raisin	Turkey	MW148190	
	DTO 096-E1	CBS 147324; IBT 29884; NRRL 615			MW148191	3
	DTO 175-I5	CBS 147482	Surface Water	Portugal	MW148192	
	DTO 293-G7	CBS 147344	Coffee beans (Robusta)	Thailand	MW148193	
	DTO 316-E3	CBS 133816; IBT 24631	Black pepper	Denmark	GU195636	2
	DTO 316-E4	CBS 147345; IBT 26389; NRRL 599			MW148194	4
	DTO 321-E6	CBS 147346	CF patient material	the Netherlands	MW148195	
	DTO 326-A7	CBS 147347	Petridish from soft drink factory	the Netherlands	MW148196	
	DTO 367-D1	CBS 769.97	Leather	Germany	MW148197	
	DTO 367-D6	CBS 115989; NRRL 3122			MW148198	5
	DTO 368-I1	CBS 147352	Air next to bottle blower	Mexico	MW148199	
	DTO 368-I6	CBS 147353	Food factory	Italy	MW148200	
	N402	ATCC 64974			MW148183	6
Penicillium roqueforti	DTO 003-H1	CBS 147308	Environment dairy factory	the Netherlands	MW148162	
	DTO 012-A2	CBS 147309	Tortilla (flour)	California, USA	MW148163	
	DTO 012-A6	CBS 147310	Tortilla (corn)	California, USA	MW148164	
	DTO 013-F5	CBS 147311	Margarine	the Netherlands	MW148165	
	DTO 070-G2	CBS 147317	Wood	Unknown	MW148166	
	DTO 081-F9	CBS 147318	Air in cheese warehouse	the Netherlands	MW148167	
	DTO 101-D6	CBS 147325	Cheese surface	the Netherlands	MW148168	
	DTO 102-I9	CBS 147326	Drink	The Netherlands	MW148169	
	DTO 126-G2	CBS 147330	Air in bakery	USA	MW148170	
	DTO 127-F7	CBS 147331	Chicory root extract	the Netherlands	MW148171	
	DTO 127-F9	CBS 147332	Chicory root extract	the Netherlands	MW148172	
	DTO 130-C1	CBS 147333	Air of cheese factory	the Netherlands	MW148173	
	DTO 163-C3	CBS 147337	Single ascospore isolate of DTO 006-G1 and DTO 027-I6	the Netherlands	MW148174	
	DTO 163-F5	CBS 147338	Cheese, Garstang Blue	UK	MW148175	
	DTO 163-G4	CBS 147339	Barley	Denmark	MW148176	
	DTO 265-D5	CBS 147372	Edge of brine bath	the Netherlands	MW148177	
	DTO 369-A1	CBS 147354	From mayonnaise, containing K-sorbate	The Netherlands	MW148178	
	DTO 375-B1	CBS 147355	Cheese	Mexico	MW148179	
	DTO 377-G2	LCP 96.3914	Stewed fruit	France	MW148180	7
	DTO 377-G3	LCP 97.4111	Wood	France	MW148181	7
Paecilomyces variotii	DTO 021-C3	CBS 145656	Spoiled sports drink	USA	MN153215	8
	DTO 021-D3	CBS 145657	Heat shocked sucrose	USA	MN153219	8
	DTO 027-B3	CBS 121577	Spoiled sports drink	USA	EU037084	9
	DTO 027-B5	CBS 121579	Sucrose	USA	EU037082	9
	DTO 027-B6	CBS 121580	Spoiled apple juice	The Netherlands	EU037081	9
	DTO 027-B9	CBS 121583	Spoiled sports drink	USA	EU037078	9
	DTO 032-I3	CBS 121585	High Fructose Corn Syrup after heat shock	USA	EU037077	9
	DTO 045-G8	CBS 145658	Drink	USA	MN153224	8
	DTO 164-E3	CBS 145659	Blue berry ingredients	The Netherlands	MN153240	8
	DTO 166-G4	CBS 145660	Pectin, heat treated	The Netherlands	MN153245	8
	DTO 195-F2	CBS 145663	Margarine	Belgium	MN153269	8
	DTO 207-G8	CBS 145664	Fruit, ingredient	The Netherlands	MN153270	8
	DTO 212-C5	CBS 145665	Vanilla	The Netherlands	MN153272	8
	DTO 217-A2	CBS 145666	Ice pop, heat treated	The Netherlands	MN153275	8
	DTO 271-D3	CBS 145667	Industry environment	Guatamala	MN153286	8
	DTO 271-G3	CBS 145668	Ice tea	South Africa	MN153288	8
	DTO 280-E4	CBS 109073	Pectin	The Netherlands	EU037070	9
	DTO 280-E5	CBS 101075	Heat processed fruit beverage	Japan	EU037069	9
	DTO 282-E5	CBS 145669	Margarine	Italy	MN153294	8
	DTO 282-F9	CBS 145670	Wall covering, industry environment	UK	MN153297	8

[A]Genbank accession numbers of partial *caM* gene sequences for the studied *A. niger* strains and partial *benA* gene sequences for the *P. roqueforti* and *P. variotii* strains.

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Table 3.S2. D-values of experimental replicates (E) and biological replicates (B) of all strains used in this study. Shaping parameter β including 95% confidence interval (CI) of the Weibull model was used to check significance of the model.

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A. niger

Strain	B	E	D_{54}-value	β [95% CI]
DTO 008-C3	1	1	12.6	1.3 [0.82 - 1.78]
		2	11.6	1.16 [0.8 - 1.52]
	2	1	16.3	1.61 [0.55 - 2.67]
		2	15.5	1.51 [0.44 - 2.59]
	3	1	11.8	1.33 [0.63 - 2.02]
		2	11.7	1.48 [1.21 - 1.75]
DTO 028-I3	1	1	13.3	0.51 [0.32 - 0.7]
		2	13.1	0.47 [0.27 - 0.66]
	2	1	14.3	0.43 [0.23 - 0.63]
		2	13.9	0.42 [0.22 - 0.63]
	3	1	10.1	0.29 [0.15 - 0.44]
		2	11.0	0.3 [0.13 - 0.48]
DTO 029-B1	1	1	3.7	0.58 [0.17 - 0.99]
		2	4.7	0.83 [-0.54 - 2.2]
	2	1	3.4	1.01 [0.27 - 1.75]
		2	3.0	1.08 [0.41 - 1.75]
	3	1	3.7	1.08 [0.32 - 1.84]
		2	3.9	1.23 [-0.18 - 2.65]
DTO 058-I1	1	1	7.3	0.61 [0.32 - 0.9]
		2	6.8	0.6 [0.31 - 0.89]
	2	1	11.4	0.7 [0.5 - 0.9]
		2	11.5	0.7 [0.48 - 0.92]
	3	1	11.3	0.87 [0.53 - 1.21]
		2	11.2	1.11 [0.69 - 1.53]
DTO 096-A5	1	1	13.3	1.05 [0.62 - 1.47]
		2	12.7	1.09 [0.43 - 1.75]
	2	1	13.6	0.94 [0.12 - 1.76]
		2	14.9	0.87 [0.05 - 1.69]
	3	1	15.0	1.05 [0.63 - 1.48]
		2	15.3	1.31 [0.67 - 1.96]
DTO 096-A7	1	1	18.5	1.35 [1.14 - 1.57]
		2	17.4	1.2 [0.8 - 1.6]
	2	1	21.3	1.14 [0.77 - 1.5]
		2	22.0	1.03 [0.48 - 1.58]
	3	1	19.5	1.18 [0.97 - 1.39]
		2	19.8	1.24 [0.9 - 1.57]
DTO 096-A9	1	1	15.3	0.62 [-0.06 - 1.3]
		2	14.1	0.68 [-0.03 - 1.39]
	2	1	13.7	0.6 [0.16 - 1.04]
		2	10.6	0.58 [0.17 - 0.99]
	3	1	10.6	0.7 [0.2 - 1.2]
		2	10.6	0.7 [0.16 - 1.23]
DTO 096-C6	1	1	43.4	0.66 [0.02 - 1.3]
		2	46.4	1.78 [1.14 - 2.42]
	2	1	29.6	1.32 [0.74 - 1.89]
		2	30.3	1.32 [0.69 - 1.95]
	3	1	31.9	1.31 [0.66 - 1.96]
		2	31.1	1.54 [1.18 - 1.89]
DTO 096-D7	1	1	28.0	1.27 [0.73 - 1.81]
		2	31.2	1.43 [0.17 - 2.69]
	2	1	28.1	1.39 [1.32 - 1.46]
		2	25.5	1.99 [1.28 - 2.7]
	3	1	44.6	2.8 [-0.08 - 5.68]
		2	39.0	2 [1.29 - 2.71]
DTO 096-E1	1	1	26.2	1.19 [0.83 - 1.55]
		2	19.6	1.07 [0.7 - 1.44]
	2	1	32.5	0.66 [0.42 - 0.89]
		2	30.9	0.71 [0.55 - 0.88]
	3	1	21.8	0.6 [0.34 - 0.86]
		2	20.8	0.67 [0.4 - 0.93]
DTO 175-I5	1	1	14.2	1.57 [0.1 - 3.03]
		2	15.3	1.4 [0.33 - 2.48]
	2	1	21.9	0.74 [0.32 - 1.15]
		2	20.2	0.86 [0.45 - 1.26]
	3	1	19.5	0.67 [0.41 - 0.92]
		2	18.0	0.92 [0.56 - 1.28]
DTO 293-G7	1	1	30.5	0.62 [0.33 - 0.92]
		2	33.9	0.57 [0.29 - 0.85]
	2	1	16.1	0.96 [0.19 - 1.73]
		2	16.3	0.96 [0.23 - 1.68]
	3	1	28.6	0.79 [0.31 - 1.27]
		2	26.1	0.81 [0.29 - 1.32]
DTO 316-E3	1	1	47.5	2.43 [0.19 - 4.67]
		2	39.9	1.38 [1.01 - 1.74]
	2	1	37.7	0.97 [0.57 - 1.37]
		2	37.6	0.9 [0.6 - 1.21]
	3	1	18.9	1.15 [0.53 - 1.77]
		2	25.1	0.82 [0.33 - 1.32]
DTO 316-E4	1	1	15.8	1.2 [0.77 - 1.63]
		2	26.3	1.05 [0.63 - 1.46]
	2	1	18.0	0.96 [0.43 - 1.5]
		2	17.5	0.98 [0.5 - 1.47]
	3	1	14.5	0.54 [0.29 - 0.78]
		2	12.2	0.78 [0.58 - 0.97]
DTO 321-E6	1	1	47.1	0.41 [0.17 - 0.65]
		2	37.8	0.68 [0.35 - 1]
	2	1	28.3	0.74 [0.18 - 1.3]
		2	26.5	0.77 [0.25 - 1.29]
	3	1	23.8	0.71 [0.35 - 1.06]
		2	24.9	0.63 [0.22 - 1.04]
DTO 326-A7	1	1	59.8	1.03 [0.31 - 1.74]
		2	45.9	1.3 [1.04 - 1.56]
	2	1	50.9	1.32 [0.95 - 1.69]
		2	66.5	1.18 [0.79 - 1.56]
	3	1	47.5	0.9 [0.53 - 1.27]
		2	32.2	1.75 [0.8 - 2.7]
DTO 367-D1	1	1	10.8	1.06 [-0.3 - 2.43]
		2	8.9	1.05 [0.4 - 1.7]
	2	1	9.2	0.69 [0.52 - 0.86]
		2	9.8	0.77 [0.57 - 0.97]
	3	1	9.5	0.91 [0.48 - 1.34]
		2	8.3	0.72 [0.34 - 1.09]
DTO 367-D6	1	1	15.9	1.01 [0.41 - 1.61]
		2	15.1	0.9 [0.46 - 1.35]
	2	1	15.3	1.06 [0.57 - 1.55]
		2	15.0	0.95 [0.58 - 1.31]
	3	1	14.0	0.8 [0.48 - 1.12]
		2	14.6	0.71 [0.39 - 1.03]
DTO 368-I1	1	1	39.5	1.91 [1.36 - 2.45]
		2	40.1	1.92 [1.23 - 2.61]
	2	1	22.7	1.01 [0.57 - 1.46]
		2	20.5	1.29 [0.79 - 1.79]
	3	1	33.3	0.69 [0.37 - 1.02]
		2	34.8	0.65 [0.26 - 1.04]
DTO 368-I6	1	1	34.4	0.9 [0.6 - 1.2]
		2	31.4	1.32 [0.89 - 1.76]
	2	1	31.3	1.3 [0.35 - 2.26]
		2	37.0	1.87 [1.28 - 2.47]
	3	1	32.1	1.59 [1 - 2.18]
		2	31.2	1.27 [1.01 - 1.54]
N402	1	1	28.5	1.32 [0.54 - 2.09]
		2	26.7	1.24 [0.75 - 1.73]
	2	1	33.3	1.54 [0.92 - 2.16]
		2	32.2	1.6 [1.08 - 2.12]
	3	1	34.8	1.24 [0.88 - 1.59]
		2	33.4	1.15 [0.83 - 1.48]

P. roqueforti

Strain	B	E	D_{62}-value	β [95% CI]
DTO 003-H1	1	1	4.3	1.11 [0.65 - 1.58]
		2	4.4	1.06 [0.64 - 1.47]
	2	1	5.5	0.91 [0.53 - 1.3]
		2	5.7	0.82 [0.5 - 1.14]
	3	1	5.3	1.02 [0.28 - 1.77]
		2	7.7	0.7 [0.13 - 1.28]
DTO 012-A2	1	1	5.2	1.15 [0.81 - 1.5]
		2	5.0	1.26 [0.94 - 1.58]
	2	1	4.0	1.11 [0.82 - 1.39]
		2	3.9	1.11 [0.53 - 1.69]
	3	1	4.5	1.19 [0.53 - 1.86]
		2	4.6	1.28 [0.78 - 1.79]
DTO 012-A6	1	1	7.9	0.94 [0.2 - 1.67]
		2	8.0	0.88 [0.11 - 1.65]
	2	1	7.6	2.43 [1.96 - 2.9]
		2	9.2	1.27 [0.45 - 2.09]
	3	1	7.4	2.61 [2.06 - 3.16]
		2	7.7	2.51 [1.95 - 3.07]
DTO 013-F5	1	1	14.8	1.94 [1.6 - 2.28]
		2	15.6	1.6 [1.29 - 1.91]
	2	1	16.0	2 [1.88 - 2.13]
		2	15.8	2.26 [1.9 - 2.61]
	3	1	9.1	1.56 [1.1 - 2.02]
		2	10.5	1.86 [1.39 - 2.34]
DTO 070-G2	1	1	8.1	1.01 [0.4 - 1.62]
		2	7.5	1.7 [1.28 - 2.12]
	2	1	6.6	0.53 [0.18 - 0.89]
		2	4.5	1.02 [0.8 - 1.24]
	3	1	7.8	1.18 [0.38 - 1.97]
		2	7.2	1.97 [1.05 - 2.89]
DTO 081-F9	1	1	7.3	2.14 [1.74 - 2.54]
		2	7.5	2.04 [1.79 - 2.29]
	2	1	6.0	1.75 [0.72 - 2.77]
		2	7.8	5 [-0.71 - 10.71]
	3	1	2.5	1.8 [1.38 - 2.23]
		2	2.5	1.63 [1.22 - 2.04]
DTO 101-D6	1	1	6.8	0.9 [0.54 - 1.25]
		2	8.1	0.77 [0.47 - 1.06]
	2	1	5.9	1.11 [0.89 - 1.33]
		2	6.1	1.26 [1.14 - 1.39]
	3	1	6.5	0.95 [0.77 - 1.14]
		2	6.3	0.95 [0.88 - 1.02]
DTO 102-I9	1	1	7.5	1.09 [0.62 - 1.55]
		2	9.8	0.79 [0.51 - 1.07]
	2	1	7.8	0.64 [0.38 - 0.9]
		2	7.4	0.82 [0.37 - 1.27]
	3	1	5.7	0.58 [0.17 - 0.98]
		2	6.1	0.62 [0.26 - 0.98]
DTO 126-G2	1	1	3.9	1.38 [0.85 - 1.92]
		2	4.1	1.37 [1 - 1.74]
	2	1	3.7	1.43 [0.91 - 1.95]
		2	3.8	1.39 [0.91 - 1.87]
	3	1	4.2	1.61 [1.3 - 1.93]
		2	4.5	1.64 [-0.01 - 3.28]
DTO 127-F7	1	1	3.9	1.44 [1.14 - 1.75]
		2	3.9	1.82 [1.53 - 2.11]
	2	1	4.3	1.51 [0.66 - 2.37]
		2	5.2	0.83 [0.24 - 1.42]
	3	1	3.1	1.39 [1.08 - 1.71]
		2	3.0	1.67 [1.28 - 2.06]
DTO 127-F9	1	1	5.3	1.68 [1.2 - 2.12]
		2	4.9	1.86 [1.14 - 2.57]
	2	1	5.5	1.55 [0.76 - 2.35]
		2	5.6	1.16 [0.77 - 1.54]
	3	1	6.0	1.67 [1.28 - 2.06]
		2	6.0	1.65 [1.22 - 2.08]
DTO 130-C1	1	1	1.9	0.99 [0.1 - 1.87]
		2	1.7	1.02 [0.59 - 1.46]
	2	1	2.0	0.72 [0.14 - 1.31]
		2	1.6	0.93 [-0.36 - 2.23]
	3	1	1.1	NA
		2	1.1	NA
DTO 163-C3	1	1	9.0	1.54 [1.12 - 1.97]
		2	7.6	1.54 [0.89 - 2.19]
	2	1	6.1	1.62 [1.17 - 2.07]
		2	5.5	1.41 [0.83 - 2]
	3	1	5.4	1.79 [1.25 - 2.32]
		2	6.2	1.7 [-0.07 - 3.46]
DTO 163-F5	1	1	5.2	1.24 [1.03 - 1.44]
		2	5.6	1 [0.74 - 1.26]
	2	1	6.3	2.17 [1.15 - 3.2]
		2	7.3	2.32 [0.91 - 3.72]
	3	1	3.9	1.21 [0.87 - 1.55]
		2	4.6	1.04 [0.67 - 1.41]
DTO 163-G4	1	1	4.1	1.16 [0.92 - 1.39]
		2	4.4	0.91 [0.5 - 1.32]
	2	1	5.1	0.67 [0.29 - 1.05]
		2	4.5	0.86 [0.63 - 1.09]
	3	1	4.3	0.87 [0.68 - 1.06]
		2	3.7	0.83 [0.34 - 1.32]
DTO 265-D5	1	1	5.8	1.13 [0.63 - 1.64]
		2	5.3	0.81 [0.43 - 1.19]
	2	1	4.2	1.1 [0.86 - 1.35]
		2	4.2	1.17 [0.85 - 1.49]
	3	1	4.5	1.08 [0.5 - 1.66]
		2	3.9	1.06 [0.03 - 2.1]
DTO 369-A1	1	1	3.8	0.77 [0.25 - 1.3]
		2	5.5	0.57 [0.06 - 1.08]
	2	1	3.6	1 [0.53 - 1.47]
		2	4.0	0.99 [0.7 - 1.28]
	3	1	3.2	1.09 [0.46 - 1.73]
		2	3.7	0.82 [0.42 - 1.23]
DTO 375-B1	1	1	5.2	1.32 [0.76 - 1.89]
		2	4.3	1.69 [0.45 - 2.94]
	2	1	4.7	1.37 [0.63 - 2.11]
		2	3.9	1.36 [0.68 - 2.04]
	3	1	4.7	1.28 [0.91 - 1.66]
		2	4.4	1.17 [0.7 - 1.63]
DTO 377-G2	1	1	5.2	0.99 [0.77 - 1.22]
		2	6.2	0.88 [0.7 - 1.07]
	2	1	6.2	0.67 [0.3 - 1.03]
		2	5.9	0.63 [0.32 - 0.94]
	3	1	7.2	1.11 [0.86 - 1.36]
		2	7.5	0.99 [0.83 - 1.16]
DTO 377-G3	1	1	7.0	0.97 [0.35 - 1.59]
		2	7.5	0.99 [0.64 - 1.35]
	2	1	5.1	0.83 [0.63 - 1.02]
		2	3.9	1.19 [0.67 - 1.71]
	3	1	4.6	1.5 [1.09 - 1.92]
		2	4.1	1.69 [1.17 - 2.2]

Pae. variotii

Strain	B	E	D_{60}-value	β [95% CI]
DTO 021-C3	1	1	12.9	1.18 [1.04 - 1.31]
		2	13.5	1.05 [0.8 - 1.3]
	2	1	15.4	1.27 [0.68 - 1.85]
		2	15.5	1.37 [1.09 - 1.66]
	3	1	11.8	1.34 [0.86 - 1.83]
		2	12.1	1.17 [1.11 - 1.22]
DTO 021-D3	1	1	3.3	1.12 [0.47 - 1.77]
		2	3.5	1.27 [-0.98 - 3.51]
	2	1	4.6	1.2 [0.12 - 2.28]
		2	4.4	1.07 [-0.92 - 3.07]
	3	1	5.4	0.83 [0.28 - 1.39]
		2	5.2	0.83 [0.3 - 1.37]
DTO 027-B3	1	1	4.0	0.89 [0.76 - 1.02]
		2	4.1	1 [0.66 - 1.34]
	2	1	7.3	0.88 [0.49 - 1.28]
		2	7.4	0.94 [0.43 - 1.45]
	3	1	6.4	0.79 [0.39 - 1.19]
		2	6.0	0.8 [0.63 - 0.97]
DTO 027-B5	1	1	7.1	0.96 [0.36 - 1.56]
		2	6.7	1.45 [0.94 - 1.96]
	2	1	10.3	1.16 [0.54 - 1.77]
		2	11.1	0.99 [0.76 - 1.22]
	3	1	7.7	0.85 [0.47 - 1.22]
		2	7.1	0.98 [0.36 - 1.61]
DTO 027-B6	1	1	4.1	1.51 [0.57 - 2.44]
		2	4.0	1.19 [0.04 - 2.34]
	2	1	6.1	0.95 [0.46 - 1.43]
		2	6.1	1.05 [0.68 - 1.43]
	3	1	7.0	0.95 [0.44 - 1.45]
		2	6.8	0.99 [0.39 - 1.59]
DTO 027-B9	1	1	11.6	0.88 [0.62 - 1.14]
		2	9.4	1.05 [0.96 - 1.14]
	2	1	6.6	0.83 [0.57 - 1.08]
		2	6.2	0.82 [0.64 - 1]
	3	1	8.1	0.73 [0.39 - 1.06]
		2	8.4	0.74 [0.09 - 1.38]
DTO 032-I3	1	1	5.4	1.03 [0.64 - 1.42]
		2	4.8	0.8 [-0.02 - 1.63]
	2	1	6.8	0.94 [0.51 - 1.36]
		2	6.6	0.98 [0.5 - 1.45]
	3	1	5.9	1.03 [0.71 - 1.34]
		2	5.8	1.06 [0.91 - 1.2]
DTO 045-G8	1	1	7.3	1.21 [1.04 - 1.37]
		2	6.3	1.42 [1.2 - 1.65]
	2	1	6.4	0.92 [0.6 - 1.24]
		2	6.2	1.01 [0.82 - 1.2]
	3	1	10.0	0.75 [0.41 - 1.09]
		2	7.5	0.96 [0.67 - 1.25]
DTO 164-E3	1	1	5.1	0.81 [0.6 - 1.02]
		2	6.0	0.83 [0.4 - 1.26]
	2	1	6.5	0.89 [-0.07 - 1.84]
		2	5.1	0.88 [0.02 - 1.94]
	3	1	6.3	0.79 [0.28 - 1.3]
		2	6.7	0.8 [0.69 - 0.92]
DTO 166-G4	1	1	9.0	0.69 [0.23 - 1.16]
		2	8.4	0.86 [0.7 - 1.02]
	2	1	11.2	0.82 [0.41 - 1.23]
		2	10.4	0.92 [0.69 - 1.15]
	3	1	11.6	1.11 [0.9 - 1.32]
		2	11.4	1.2 [0.91 - 1.48]
DTO 195-F2	1	1	24.2	0.72 [0.32 - 1.12]
		2	21.3	0.71 [0.48 - 0.94]
	2	1	28.2	0.84 [0.67 - 1.02]
		2	26.4	1 [0.67 - 1.32]
	3	1	30.4	1.22 [0.42 - 2.02]
		2	29.2	0.79 [0.44 - 1.14]
DTO 207-G8	1	1	5.6	0.97 [0.27 - 1.67]
		2	5.8	1.07 [0.93 - 1.2]
	2	1	6.9	0.9 [0.26 - 1.53]
		2	5.8	0.94 [0.44 - 1.44]
	3	1	8.2	0.83 [0.61 - 1.06]
		2	7.8	0.89 [0.66 - 1.12]
DTO 212-C5	1	1	3.6	0.97 [-1.73 - 3.68]
		2	4.0	1.22 [-2.85 - 5.28]
	2	1	3.4	1.08 [-0.44 - 2.59]
		2	3.2	1.03 [-1.29 - 3.35]
	3	1	3.8	1.24 [0.48 - 2.01]
		2	3.2	1.24 [-2 - 4.49]
DTO 217-A2	1	1	24.5	0.79 [0.56 - 1.03]
		2	22.5	0.91 [0.54 - 1.29]
	2	1	19.2	1.02 [0.86 - 1.19]
		2	18.7	1.07 [0.98 - 1.16]
	3	1	16.3	1.28 [1.03 - 1.53]
		2	15.2	1.24 [0.95 - 1.53]
DTO 271-D3	1	1	7.2	0.83 [0.56 - 1.09]
		2	8.6	0.91 [0.61 - 1.21]
	2	1	8.4	1.03 [0.83 - 1.24]
		2	7.4	1.06 [0.94 - 1.18]
	3	1	7.8	1.26 [0.66 - 1.86]
		2	6.4	1.09 [0.88 - 1.3]
DTO 271-G3	1	1	5.9	0.85 [0.57 - 1.14]
		2	6.3	0.86 [0.29 - 1.43]
	2	1	3.7	1.41 [0.42 - 2.39]
		2	3.2	1.26 [0.7 - 1.81]
	3	1	3.2	1.37 [0.78 - 1.96]
		2	4.7	0.83 [-0.51 - 2.16]
DTO 280-E4	1	1	6.3	0.7 [0.58 - 0.82]
		2	6.5	0.85 [0.6 - 1.1]
	2	1	4.5	0.78 [-0.43 - 1.98]
		2	3.8	0.86 [-0.6 - 2.32]
	3	1	6.2	0.85 [0.47 - 1.23]
		2	6.4	0.84 [0.67 - 1.02]
DTO 280-E5	1	1	5.1	0.81 [0.6 - 1.02]
		2	6.0	0.85 [0.6 - 1.1]
	2	1	6.8	0.74 [0.34 - 1.15]
		2	6.5	0.75 [0.39 - 1.11]
	3	1	6.0	0.9 [0.66 - 1.13]
		2	5.8	0.94 [0.67 - 1.21]
DTO 282-E5	1	1	16.1	0.73 [0.36 - 1.1]
		2	13.7	0.72 [0.48 - 0.96]
	2	1	11.7	0.87 [0.76 - 0.97]
		2	11.5	1.0 [0.57 - 1.14]
	3	1	14.2	0.72 [0.49 - 0.96]
		2	13.5	0.67 [0.44 - 0.89]
DTO 282-F9	1	1	17.0	1.12 [0.84 - 1.39]
		2	15.4	1.2 [1.07 - 1.33]
	2	1	13.8	1.17 [0.9 - 1.44]
		2	14.2	1.26 [1.1 - 1.43]
	3	1	14.5	1.01 [0.89 - 1.13]
		2	14.5	1 [0.9 - 1.09]