Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Food Science

Germination strategies of stress-resistant *Aspergillus* conidia

Maryam Ijadpanahsaravi and Han A.B. Wösten

Aspergilli are highly relevant for the food industry. They are pathogens of crops and spoil food at different postharvest stages. On the other hand, aspergilli and their products are used to produce food. Crops and food are contaminated with stress-resistant conidia, while these same spores are used to start food fermentation and the production of food ingredients. This review discusses recent insights into the stress resistance and germination of *Aspergillus* conidia and how this knowledge can be used in agriculture and the food industry.

Address
Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, the Netherlands

Corresponding author: Wösten, Han AB (h.a.b.wosten@uu.nl)

Current Opinion in Food Science 2024, **57**:101169

This review comes from a themed issue on **Food Mycology**

Edited by **Alicia Rodríguez, Alejandro Hernández León** and **Santiago Ruiz-Moyano Seco de Herrera**

For complete overview of the section, please refer to the article collection, "Food Mycology 2024"

Available online 3 May 2024

https://doi.org/10.1016/j.cofs.2024.101169

Introduction

The genus *Aspergillus* comprises 837 species [1], some of which are among the most abundant fungi in the world. Aspergilli grow on dead organic material but they can also be endophytes or pathogens of plants, animals, and humans [2,3]. Aspergilli such as *Aspergillus flavus* and *Aspergillus niger* are also found on a wide range of food products and can thereby cause food spoilage, which may include the formation of mycotoxins [4]. In fact, climate change may increase the incidence of mycotoxin production in crops and food [5]. On the other hand, aspergilli such as *Aspergillus oryzae* and *Aspergillus sojae* are used in fermentation to produce for instance soy sauce and miso [6]. Also, *Aspergillus* species such as *A. niger* and *A. oryzae* are widely used as cell factories for the production of enzymes (e.g. amylases) and small molecules (e.g. citric acid) that are used in food production [7].

Conidia are the dispersal vectors of aspergilli and, as such, are at the start of infections and spoilage, and, on the other hand, the production of food and food ingredients. This review discusses recent findings in stress resistance and germination of conidia of aspergilli. These findings may contribute to the prevention of crop infection and food spoilage by aspergilli and possibly other fungi as well. Also, these findings may be used to optimize the production of food and food products with the help of aspergilli.

Conidia as stress-resistant dispersal structures

Conidia of aspergilli can be produced in massive amounts illustrated by the fact that a colony can produce up to a billion spores per cm^2 [8] with a single conidiophore producing more than 10 000 conidia [3]. These conidia are much more stress-resistant than vegetative hyphae and are thereby not only dispersal but also survival structures. *A. niger* conidia show inter-species and inter-strain variability in stress resistance [9,10]. In fact, strain variability was similar to that of bacteria, indicating the existence of a microbial signature of variation that exceeds kingdom boundaries [9]. Intra-strain variability of stress resistance is also observed, which can be caused by environmental conditions. Transcription in the conidia of *Aspergillus nidulans*, *Aspergillus fumigatus*, and *Talaromyces marneffei* is modulated by changes in the environment as long as these spores are part of the conidiophore [11]. This modulation may differ between different parts of the colony, for instance, in regions with low and high nutrient availability. Modulation results in differences in the composition of mRNA, proteins, compatible solutes, and secondary metabolites and also impacts gene expression when germination is initiated. As a result, conidia are prepared for growth in the environment in which they are formed. This would improve their fitness because they are conditioned to grow under the local environmental stresses. Indeed, cultivation of *A. niger* at elevated temperature results in conidia with increased heat resistance [12]. Since conditions within the colony differ, environmental conditions also impact germination heterogeneity [13] (see below).

Resting conidia harvested from agar media are not completely dormant but have low metabolic activity

[8,14]. They are much more resistant to, for instance, drought, temperature, UV, salt, and reactive oxygen stress than the vegetative hyphae. Stress resistance of *Aspergillus* conidia is mediated by compatible solutes, heat shock proteins, melanin, and other compounds as well [15]. Trehalose and mannitol are the most abundant compatible solutes in conidia of *A. niger* making up 4–5% and 10–15% of the dry weight of these spores, respectively [16,17]. These compatible solutes accumulate during maturation of the conidia on the conidiophore, which coincides with increased heat resistance [12]. The causal role of trehalose and mannitol in heat resistance was demonstrated by a deletion strain that produces very low amounts of these molecules and that shows a fifteen-order-magnitude lower heat resistance. In contrast, conidia lacking only trehalose or mannitol are still quite heat-resistant when compared with the strain lacking both compatible solutes. The fact that two heat shock protein genes [12] and a melanin synthesis gene [18] do not impact heat resistance of *A. niger* conidia illustrates that stress resistance genes can function under specific stresses.

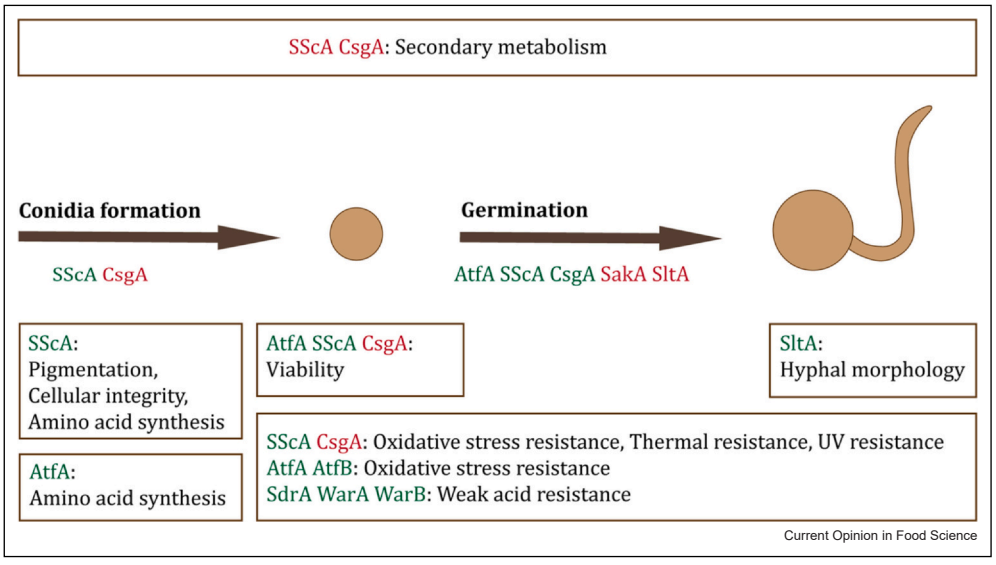
Transcription factors (TFs) have been identified that are involved in regulation of stress resistance in aspergilli (Figure 1). AtfA and AtfB are involved in oxidative stress resistance of conidia and vegetative hyphae [19], while AtfA of *A. oryzae* is also involved in conidial storage stability [20]. On the other hand, SdrA, WarA, and WarB are involved in weak-acid resistance of *A. niger*, showing an additive effect on the sorbic acid stress response [10].

Germination of conidia

The decision of conidia to germinate is crucial since the spore will exchange its stress resistance for the ability to colonize a substrate. Germination of conidia in the substrate typically includes the stages of activation, swelling, and formation of a germ tube [21]. The fact that germination of conidia of *Penicillium roqueforti* can take place without detectable swelling [22] raises the question of whether this is a prerequisite for germination of fungal conidia. Swelling and germ tube formation of *Aspergillus* spores are typified by an increase in volume and a decrease in circularity, respectively. The activation stage is not characterized by a morphological change, which hampers pinpointing its start. All three germination stages are characterized by changes in the composition of the conidia [15]. For instance, genes related to respiration, translation, replication, and transcription are being upregulated during the activation stage of *A. niger* conidia, while trehalose is being degraded [8]. There has been a debate about the role of compatible solutes as a carbon source during germination. The fact that the conidia of an *A. niger* strain that hardly produces trehalose and mannitol still germinate [12] indicates that these compounds do not serve such a role. Also, it is not yet clearly established whether carbon sources are taken up from the medium during the activation stage but it does happen during swelling [8].

TFs have been identified that control various processes in (germinating) *Aspergillus* conidia [23] (Figure 1). For instance, SscA of *A. nidulans* and other aspergilli is

Figure 1



TFs are involved in the formation, germination, and viability of conidia, their stress resistance, pigmentation, and amino acid synthesis, as well as hyphal morphology. The TFs indicated in green and red are activators and repressors, respectively.

involved in the formation, germination, integrity, and long-term viability of conidia as well as in stress resistance, pigmentation, amino acid production, and secondary metabolism [24]. CsgA of *A. nidulans*, SltA of *A. fumigatus*, AtfA of *A. oryzae*, and the high-osmolarity glycerol (HOG) pathway in *A. fumigatus* also have pleiotropic phenotypes. CsgA regulates the formation of asexual and sexual spores, has a positive effect on germination, and negatively regulates trehalose content, spore viability, sterigmatocystin production, tolerance to thermal and oxidative stresses, and normal germ tube length [25]. SltA of *A. fumigatus* regulates timing of swelling and, as a consequence, timing of germ tube formation [26]. Additionally, it regulates hyphal morphology. AtfA of *A. oryzae* is not only involved in oxidation stress resistance and storage stability (see above), it is also involved in germination. It regulates carbon and nitrogen source metabolism via the synthesis of glutamate, which is the most abundant amino acid in the *A. oryzae* spores [20]. The HOG pathway, known to be involved in mitigating various stresses, including high osmolarity and oxidative stress, is involved in the germination of *A. fumigatus* spores by inhibiting germination at low-glucose conditions [27]. By contrast, spores do germinate under these conditions when components of the HOG pathway such as SakA are mutated.

Germination of *Aspergillus* spores is influenced by the conditions during which the spores are produced [13] as well as environmental factors during germination, including temperature, light, pH, CO_2, and water either or not combined with the presence of nutrients [28]. Recently, it was shown that aspergilli respond differently to the absence of nutrients. *A. niger*, *A. terreus*, and *A. oryzae* need nutrients to initiate germination, while *A. clavatus* and *A. nidulans* spores can germinate in pure water [29,30]. The latter implies that *Aspergillus* spores actually do not need nutrients per se to initiate germination and to form germ tubes(Figure 2).

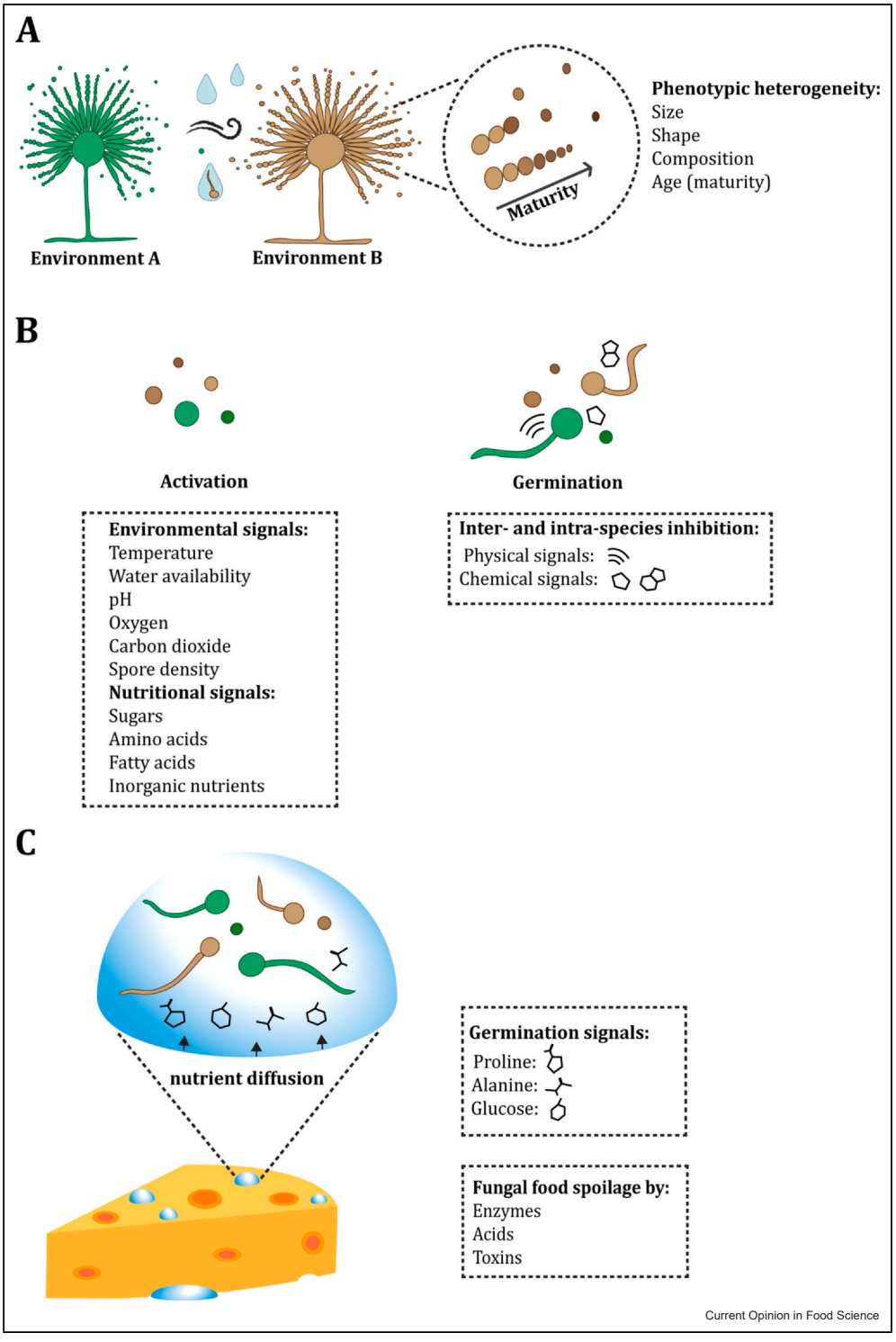
Clearly, nutrients are needed to sustain the growth of the germlings. Therefore, *A. clavatus* and *A. nidulans* take the risk of aborted growth by initiating germination without sensing the nutrients that are present in the environment. Yet, this strategy may increase competitiveness by being faster at colonizing the substrate when nutrients become available at a later stage. Such a scenario may take place when spores are dispersed in water droplets and end up at a substrate (e.g. a food product), from which nutrients such as minerals and the carbon and nitrogen source (slowly) diffuse (Figure 2). It should be noted that although *A. niger*, *A. terreus*, and *A. oryzae* do not initiate germination in pure water, they do so in media that do not consist of all the nutrients needed to support full growth. For instance, part of the *A. niger* spores starts to swell when exposed to glucose combined with $NaNO_3$, KH_2PO_4, or $MgSO_4$ [29]. Aspergilli can take the risk of initiating germination in conditions not able to support growth because of the enormous numbers of conidia that are dispersed [8].

Every conidium of *A. niger* is viable and can germinate in a complete medium [12]. Yet, only 25% and 40% of the *A. clavatus* and *A. nidulans* spores germinate in water, while about 5% of the *A. niger* spores germinate in a nutrient-restricted solution consisting of glucose with $NaNO_3$, KH_2PO_4, or $MgSO_4$. This illustrates that the germination response is heterogeneous [29,30]. In general, the percentage of spores remaining dormant decreases when the diet is more diverse. The results indicate that different sensors are involved in determining the nutrient status of the medium and that their collective input generates a signal that can activate germination. Such sensors may not be homogenously distributed between the conidia of the population, thereby creating heterogeneity in the germination response. Sensor distribution may also explain why the fraction of *A. niger*, *A. clavatus*, *A. nidulans*, and *A. oryzae* spores that are larger in size is more responsive to inducers and thereby show higher germination incidence [30].

Partial germination of the clonal spore population would provide a bet-hedging strategy to ensure colonization of a substrate while preventing all individuals from dying because of a lack of nutrients or when other environmental conditions become unfavorable. For instance, swollen spores and germlings, but not the dormant conidia, may be killed when the temperature increases during the day and exceeds the cardinal temperature of the fungus [29]. Aggregation of spores may be part of the bet-hedging strategy. Spore aggregates result in larger colonies in liquid cultures than individual spores, and these large microcolonies are more resistant to heat and oxidative stress [31]. This higher resistance is not only explained by the shielding of the core of the colony by the outer part of the mycelium but also by the fact that only part of the aggregated spores germinate.

Carbon and nitrogen sources can be distinguished based on their (in)ability to activate and/or support the outgrowth of conidia [29,30,32,33]. For instance, proline and alanine are highly inducing amino acids in the cases of *A. niger*, *A. clavatus*, *A. oryzae*, and *A. nidulans* (but not in *A. terreus*) [30]. Their high inducing activity may have evolved because these amino acids accumulate in stressed plants, which are easier to colonize than healthy plants. Moreover, proline can be abundant in plants both as a free amino acid and as part of the abundant proline-rich cell wall proteins, thereby providing a carbon and nitrogen source [34]. On the other hand, cysteine and methionine weakly induce germination in *A. niger*, *A. clavatus*, *A. oryzae*, *A. nidulans*, and *A. terreus* [29,30]. Other amino acids show different germination responses

Figure 2



Germination of conidia of *Aspergillus.* **(a)** Aspergilli produce conidia on conidiophores. The environmental conditions during the formation of these spores impact their properties. Still, the properties of the conidia of a single conidiophore can be heterogeneous despite the fact that they are exposed to identical environmental conditions. **(b)** Environmental conditions determine whether conidia are activated to germinate. For instance, the conidia of some aspergilli can germinate in pure water, while others cannot. Notably, in many conditions, not all conidia will germinate. As a result, some of the spores will remain in their stress-resistant state, while others will colonize the substrate. Apart from activation, inhibition of germination of *Aspergillus* spores can occur. Both inter- and intra-species inhibition is observed, which may be caused by chemicals released by the spores or by physical signals (e.g. vibration or oscillation). **(c)** Nutrients such as amino acids and sugars will diffuse from the substrate into the moist environment of the spores. This facilitates the germination of *Aspergillus* spores that cannot do so in pure water and also sustains hyphal growth. The enzymes, acids, and toxins that are secreted by the hyphae can spoil food.

between the aspergilli. Clustering of the germination response on each of the 19 amino acids tested [30] does not follow phylogeny [35]. This implies that these responses have evolved relatively late in evolution, despite the fact that they may impact the competitive potential of different substrates.

Inhibition of germination

The incidence of swelling and germ tube formation induced by the presence of alanine or proline decreases at a higher density of *A. niger* conidia [28]. This phenomenon could be caused by a quorum sensing-like mechanism due to the release of self-inhibitors (Figure 2). The concentration of such inhibitors in the culture medium would be higher at high spore density. For instance, the self-inhibitor 1-octen-3-ol is released by *Penicillium paneum* spores [36], while the self-inhibitors 1-octen-3-ol, 3-octanone, and 3-octanol are released by the spores of *A. nidulans* [37]. Notably, 1-octen-3-ol can shape inter-species *Aspergillus* interactions by eliciting an attenuated developmental response [38]. Other mechanisms of germination inhibition may be involved as well. This is based on the fact that the study of Ijadpanahsaravi et al. [28] used spores that had been washed before inoculation, which would have removed the self-inhibitors. Also, culture media of high-density spore *A. niger* cultures do not inhibit germination when added to low-density conidia. It was therefore proposed that physical signaling may also play a role in inhibiting germination of conidia when present at high density [28] (Figure 2).

Conidia of other aspergilli have in general a stronger negative effect on spore germination than *A. niger* conidia themselves [28]. This is not due to the strong uptake of the carbon or nitrogen source by the competing aspergilli. Germination inhibition may therefore be caused by the release of primary and/or secondary metabolites. Evidence indicates that such compounds are released by dormant conidia, for instance, from the cell wall. This does not exclude the possibility that germ tubes and vegetative hyphae also release inhibitors of germination. This hypothesis is strengthened by the fact that the density effect is stronger on germ tube formation than on swelling [28].

Outlook

Fungal conidia are more stress-resistant than vegetative cells, for instance, to antifungal compounds. Therefore, germination is an interesting target to control infection and spoilage [39–41]. Several targets for novel antifungal compounds have been proposed, including AtfA [40], the hydrolysis of trehalose [39,40], mannitol, and glycerol [39], while nutrient signaling and nutrient transport could be targets as well. Instead of preventing germination, one could also deliberately induce germination combined with a stress (e.g. heat or an antifungal compound) to prevent fungal infections or food spoilage. However, conidia show inter-species, inter-strain, and intra-strain variation in stress resistance and germination responses. This variation hampers control of spore germination to prevent infection of crops and spoilage of food on the one hand and to promote production of food and food products on the other hand. One can deal with inter-species and inter-strain variation by focusing on what is conserved between the species and strains. Baltussen et al. [42] identified four conserved co-expression modules during the germination of *A. fumigatus* and *A. niger* conidia. Genes within these modules were proposed to be potential antifungal targets. Still, this does not take away the heterogeneity in the germination response within a clonal population of conidia. By studying the mechanisms underlying these different germination responses, we may be able to synchronize the induction or inhibition of spore germination. However, the heterogeneity in germination responses itself hampers studying this phenomenon. Yet, with the recent availability of protocols to isolate RNA from single spores [43], these studies can now be done.

Heterogeneity in stress resistance is not particularly relevant as long as the treatment effectively kills or inactivates the most resistant spores. Self-inhibitors or inter-species or inter-genus inhibitors could be a natural class of molecules that can be used to prevent infection or spoilage. The fact that the self-inhibitor 1-octen-3-ol is effective in preventing dry bubble disease in white button mushroom production [44] shows this potential.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hawksworth DL: **Naming Aspergillus species: progress towards one name for each species**. *Med Mycol* 2011, **49**:S70-S76, https://doi.org/10.3109/13693786.2010.504753

2. Bennett JW: **An overview of the genus Aspergillus**. In *Aspergillus: Molecular Biology and Genomics*. Edited by Machida M, Gomi KCaiser. Academic Press; 2010:1-17, , https://doi.org/10.21775/9781910190159

3. Krijgsheld P, Bleichrodt R, van Veluw G, Wang F, Müller W, Dijksterhuis J, Wösten HAB: **Development in *Aspergillus***. *Stud Mycol* 2013, **74**:1-29, https://doi.org/10.3114/sim0006

4. In *Fungi and Food Spoilage*. Edited by Pitt JI, Hocking AD. Blackie Academic & Professional; 1997, , https://doi.org/10.1007/978-0-387-92207-2

5. Medina A: **Emerging mycotoxin risks due to climate change. What to expect in the coming decade?** In *Present Knowledge in Food Safety*. Edited by Knowles ME, Anelich LE, Boobis AR, Popping B. Academic Press; 2023:309-314, , https://doi.org/10.1016/B978-0-12-819470-6.00053-6

6. Kusumoto KI, Yamagata Y, Tazawa R, Kitagawa M, Kato T, Isobe K, Kashiwagi Y: **Japanese traditional miso and koji making**. *J Fungi* 2021, **7**:579, https://doi.org/10.3390/jof7070579

7. Wösten HAB: **Filamentous fungi for the production of enzymes, chemicals and materials**. *Curr Opin Biotechnol* 2019, **59**:65-70, https://doi.org/10.1016/j.copbio.2019.02.010

8. Novodvorska M, Stratford M, Blythe MJ, Wilson R, Beniston RG, Archer DB: **Metabolic activity in dormant conidia of Aspergillus niger and developmental changes during conidial outgrowth**. *Fungal Genet Biol* 2016, **94**:23-31, https://doi.org/10.1016/j.fgb.2016.07.002

9. • van den Brule T, Punt M, Seekles SJ, Segers FJJ, Houbraken J, Hazeleger WC, Ram AFJ, Wösten HAB, Zwietering MH, Dijksterhuis J, den Besten HMW: **Intraspecific variability in heat resistance of fungal conidia**. *Food Res Int* 2022, **156**:111302, https://doi.org/10.1016/j.foodres.2022.111302.

Variability of microbial species is reflected in intraspecies genotypic and phenotypic differences. This variation causes differences in stress resistance and germination responses. This paper shows that intra-species variability is in the same order of magnitude between fungi and bacteria, suggesting the existence of an inter-kingdom microbial signature of variation.

10. Seekles SJ, van Dam J, Arentshorst M, Ram AFJ: **Natural variation and the role of Zn2Cys6 transcription factors SdrA, WarA and WarB in sorbic acid resistance of *Aspergillus niger***. *Microorganisms* 2022, **10**:221, https://doi.org/10.3390/microorganisms10020221

11. •• Wang F, Sethiya P, Hu X, Guo S, Chen Y, Li A, Tan K, Wong KH: **Transcription in fungal conidia before dormancy produces phenotypically variable conidia that maximize survival in different environments**. *Nat Microbiol* 2021, **6**:1066-1081, https://doi.org/10.1038/s41564-021-00922-y.

This paper describes that environmental conditions modulate transcription in conidia on the conidiophore. As a consequence, not only the composition (e.g. mRNA, protein and secondary metabolites) of the conidia changes but also the gene expression once dormancy is broken. This modulation of transcription affects the fitness and capabilities of the germinated conidia for instance by an elevated stress and antifungal drug resistance.

12. Seekles SJ, van den Brule T, Punt M, Dijksterhuis J, Arentshorst M, Ijadpanahsaravi M, Roseboom W, Meuken G, Ongenae V, Zwerus J, *et al.*: **Compatible solutes determine the heat resistance of conidia**. *Fungal Biol Biotechnol* 2023, **10**:21, https://doi.org/10.1186/s40694-023-00168-9

13. Kang SE, Celia BN, Bensasson D, Momany M: **Sporulation environment drives phenotypic variation in the pathogen *Aspergillus fumigatus***. *G3 Genes Genome Genet* 2021, **11**:jkab208, https://doi.org/10.1093/g3journal/jkab208

14. Teertstra WR, Tegelaar M, Dijksterhuis J, Golovina EA, Ohm RA, Wösten HAB: **Maturation of conidia on conidiophores of Aspergillus niger**. *Fungal Genet Biol* 2017, **98**:61-70, https://doi.org/10.1016/j.fgb.2016.12.005

15. Wyatt TT, Wösten HA, Dijksterhuis J: **Fungal spores for dispersion in space and time**. *Adv Appl Microbiol* 2013, **85**:43-91, https://doi.org/10.1016/B978-0-12-407672-3.00002-2

16. Svanström Å, Melin P: **Intracellular trehalase activity is required for development, germination and heat-stress resistance of *Aspergillus niger* conidia**. *Res Microbiol* 2013, **164**:91-99, https://doi.org/10.1016/j.resmic.2012.10.018

17. Witteveen CFB, Visser J: **Polyol pools in *Aspergillus niger***. *FEMS Microbiol Lett* 1995, **134**:57-62, https://doi.org/10.1111/j.1574-6968.1995.tb07914.x

18. Seekles SJ, Teunisse PPP, Punt M, van den Brule T, Dijksterhuis J, Houbraken J, Wösten HAB, Ram AFJ: **Preservation stress resistance of melanin deficient conidia from Paecilomyces variotii and Penicillium roqueforti mutants generated via CRISPR/Cas9 genome editing**. *Fungal Biol Biotechnol* 2021, **8**:4, https://doi.org/10.1186/s40694-021-00111-w

19. Kocsis B, Lee MK, Antal K, Yu JH, Pócsi I, Leiter É, Emri T: **Genome-wide gene expression analyses of the AtfA/AtfB-mediated menadione stress response in *Aspergillus nidulans***. *Cells* 2023, **12**:463, https://doi.org/10.3390/cells12030463

20. Sakamoto K, Iwashita K, Yamada O, Kobayashi K, Mizuno A, Akita O, Mikami S, Shimoi H, Gomi K: ***Aspergillus oryzae atfA* controls conidial germination and stress tolerance**. *Fungal Genet Biol* 2009, **46**:887-897, https://doi.org/10.1016/j.fgb.2009.09.004

21. d'Enfert C: **Fungal spore germination: insights from the molecular genetics of Aspergillus nidulans and Neurospora crassa**. *Fungal Genet Biol* 1997, **21**:163-172, https://doi.org/10.1006/fgbi.1997.0975

22. Punt M, Teertstra WR, Wösten HAB: ***Penicillium roqueforti* conidia induced by L-amino acids can germinate without detectable swelling**. *Antonie Van Leeuwenhoek* 2022, **115**:103-110, https://doi.org/10.1007/s10482-021-01686-5

23. Son YE, Yu JH, Park HS: **Regulators of the asexual life cycle of *Aspergillus nidulans***. *Cells* 2023, **12**:1544, https://doi.org/10.3390/cells12111544

24. Son Y-E, Yu J-H, Park H: **The novel spore-specific regulator SscA controls *Aspergillus* conidiogenesis**. *mBio* 2023, **14**:e0184023, https://doi.org/10.1128/mbio.01840-23

25. Cho HJ, Park HS: **The function of a conidia specific transcription factor CsgA in *Aspergillus nidulans***. *Sci Rep* 2022, **12**:15588, https://doi.org/10.1038/s41598-022-19749-6

26. Baltussen TJH, van Rhijn N, Coolen JPM, Dijksterhuis J, Verweij PE, Bromley MJ, Melchers WJG: **The C2H2 transcription factor SltA is required for germination and hyphal development in *Aspergillus fumigatus***. *mSphere* 2023, **8**:e0007623, https://doi.org/10.1128/msphere.00076-23

27. • Kirkland ME, Stannard M, Kowalski CH, Mould D, Caffrey-Carr A, Temple RM, Ross BS, Lofgren LA, Stajich JE, Cramer RA, Obar JJ: **Host lung environment limits *Aspergillus fumigatus* germination through an SskA-dependent signaling response**. *mSphere* 2021, **6**:e0092221, https://doi.org/10.1128/msphere.00922-21.

This paper describes that the HOG pathway is involved in germination of *A. fumigatus conidia*. Wild-type conidia do not germinate when glucose concentrations are low, while strains with an impaired HOG pathway do germinate. This finding shows that a stress signaling pathway can also contribute to decision making whether or not to germinate.

28. • Ijadpanahsaravi M, Snoek LB, Teertstra WR, Wösten HAB: **The impact of inter- and intra-species spore density on germination of the food spoilage fungus Aspergillus niger**. *Int J Food Microbiol* 2024, **410**:110495, https://doi.org/10.1016/j.ijfoodmicro.2023.110495.

This paper describes a method to monitor germination of conidia of mixed *Aspergillus* spores. It shows that dormant spores of aspergilli repress germination of *A. niger*. This inhibition is stronger than that observed when the density of *A. niger* spores itself is increased.

29. • Ijadpanahsaravi M, Punt M, Wösten HAB, Teertstra WR: **Minimal nutrient requirements for induction of germination of *Aspergillus niger* conidia**. *Fungal Biol* 2021, **125**:231-238, https://doi.org/10.1016/j.funbio.2020.11.004.

This paper describes the heterogeneity in the germination response of stress resistant *A. niger* conidia under low nutrient conditions. A bet hedging strategy is proposed that mitigates the risk that all germlings, which are inherently stress sensitive, die when environmental conditions become unfavorable.

30. Ijadpanahsaravi M, Teertstra WR, Wösten HAB: **Inter- and intra-species heterogeneity in germination of Aspergillus conidia**. *Antonie Van Leeuwenhoek* 2022, **115**:1151-1164, https://doi.org/10.1007/s10482-022-01762-4

31. Lyu J, Tegelaar M, Post H, Moran Torres J, Torchia C, Altelaar AFM, Bleichrodt RJ, de Cock H, Lugones LG, Wösten HAB: **Heterogeneity in Spore aggregation and germination results in different sized, cooperative microcolonies in an *Aspergillus niger* culture**. *mBio* 2023, **14**:e0087022, https://doi.org/10.1128/mbio.00870-22

32. Hayer K, Stratford M, Archer DB: **Structural features of sugars that trigger or support conidial germination in the filamentous fungus *Aspergillus niger***. *Appl Environ Microbiol* 2013, **79**:6924-6931, https://doi.org/10.1128/AEM.02061-13

33. Hayer K, Stratford M, Archer DB: **Germination of *Aspergillus niger* conidia is triggered by nitrogen compounds related to L-amino acids**. *Appl Environ Microbiol* 2014, **80**:6046-6053, https://doi.org/10.1128/AEM.01078-14

34. Tarchevsky IA, Egorova AM: **Participation of proline in plant adaptation to stress factors and its application in agrobiotechnology**. *Appl Biochem Microbiol* 2022, **58**:347-360, https://doi.org/10.1134/S0003683822040160

35. Houbraken J, Kocsubé S, Visagie CM, Yilmaz N, Wang XC, Meijer M, Kraak B, Hubka V, Bensch K, Samson RA, *et al*.: **Classification of Aspergillus, Penicillium, Talaromyces and related genera (Eurotiales): an overview of families, genera, subgenera, sections, series and species**. *Stud Mycol* 2020, **95**:5-169, https://doi.org/10.1016/j.simyco.2020.05.002

36. Chitarra GS, Abee T, Rombouts FM, Posthumus MA, Dijksterhuis J: **Germination of *Penicillium paneum* conidia is regulated by 1-octen-3-ol, a volatile self-inhibitor**. *Appl Environ Microbiol* 2004, **70**:2823-2829, https://doi.org/10.1128/AEM.70.5.2823-2829.2004

37. Herrero-Garcia E, Garzia A, Cordobés S, Espeso EA, Ugalde U: **8-Carbon oxylipins inhibit germination and growth, and stimulate aerial conidiation in *Aspergillus nidulans***. *Fungal Biol* 2011, **115**:393-400, https://doi.org/10.1016/j.funbio.2011.02.005

38. Singh D, Son SY, Lee CH: **Critical thresholds of 1-octen-3-ol shape inter-species *Aspergillus* interactions modulating the growth and secondary metabolism**. *Sci Rep* 2020, **10**:11116, https://doi.org/10.1038/s41598-020-68096-x

39. Kalampokis IF, Erban A, Amillis S, Diallinas G, Kopka J, Aliferis KA: **Untargeted metabolomics as a hypothesis-generation tool in plant protection product discovery: highlighting the potential of trehalose and glycerol metabolism of fungal conidiospores as novel targets**. *Metabolomics* 2020, **16**:79, https://doi.org/10.1007/s11306-020-01699-7

40. Verburg K, van Neer J, Duca M, de Cock H: **Novel treatment approach for aspergilloses by targeting germination**. *J Fungi* 2022, **8**:758, https://doi.org/10.3390/jof8080758

41. Ortiz SC, Huang M, Hull CM: **Discovery of fungus-specific targets and inhibitors using chemical phenotyping of pathogenic spore germination**. *mBio* 2021, **12**:e0167221, https://doi.org/10.1128/mBio.01672-21

42. Baltussen TJH, Coolen JPM, Verweij PE, Dijksterhuis J, Melchers WJG: **Identifying conserved generic *Aspergillus* spp. co-expressed gene modules associated with germination using cross-platform and cross-species transcriptomics**. *J Fungi* 2021, **7**:270, https://doi.org/10.3390/jof7040270

43. Geoghegan IA, Emes RD, Archer DB, Avery SV: **Method for RNA extraction and transcriptomic analysis of single fungal spores**. *MethodsX* 2019, **7**:50-55, https://doi.org/10.1016/j.mex.2019.12.002

44. Berendsen RL, Kalkhove SIC, Lugones LG, Baars JJP, Wösten HAB, Bakker PAHM: **Effects of the mushroom-volatile 1-octen-3-ol on dry bubble disease**. *Appl Microbiol Biotechnol* 2013, **97**:5535-5543, https://doi.org/10.1007/s00253-013-4793-1