General data	
PPP number	**AF-15507**
Title	**Heterogeneity in spores of food spoilage fungi**
Theme	**Gezond & Veilig**
Executing research organisation(s)	**Universiteit Utrecht, Universiteit Leiden, Wageningen Universiteit, Westerdijk Instituut-KNAW**
Project leader research (name + email address)	**Prof dr HAB Wösten, Universiteit Utrecht, h.a.b.wosten@uu.nl**
Coordinator (on behalf of private parties)	**Dr J-W Sanders, Unilever, jan-willem.sanders@unilever.com**
Contact person of government	
Total project budget (k€)	**2.560.000 €**
Project website address	
Starting date	**1-6-2016**
Final date	**1-6-2020 (with a possibility to extend to 1-12-2021)**

Approval coordinator/consortium The annual report has to be discussed with the coordinator/consortium. The TKI(s) like to be informed regarding potential comments on the annual report.	
The annual report is ……… by the coordinator on behalf of the consortium	X approved ☐ not approved
Potential comments regarding the final report	**The project is well at scheme**

Description content/aim PPP	
Description of problem	Food availability should increase by 70% to feed the human world population in 2050. Reducing food spoilage could significantly contribute to this challenge. At the moment, 25% of food is spoiled, a significant part due to fungal contamination. Fungal spoilage not only affects visual and organoleptic properties but may also result in the production of toxins. Food preservation methods like sterilization and addition of salt reduce spoilage enormously. However, consumers prefer minimal processing to maintain food quality. This, however, leads to increased risk of fungal spoilage and therefore new mild food processing protocols are needed. Food spoilage often starts with contamination with fungal spores. These reproductive structures are abundant in the environment such as in the air. Experimental data indicate the existence of subpopulations of spores with different levels of resistance to preservation methods.
Goals of the project	▪ Study the impact of the genetic background of model fungi by using strains of different geographic origin and originating from contaminated food and beverages. ▪ Study the impact of environmental growth conditions by isolating spores from colonies of model fungi that have been grown at different substrates either or not in the presence of sub-lethal stress conditions. ▪ Study the impact of the developmental stage of colonies and spores by isolating spores of different age from different zones of colonies of model fungi. ▪ Proof of concept of a novel processing treatment that prevents fungal spoilage making use of a combination of mild interventions.

Results	
Expected results 2019	▪ Description of the impact of the genetic background on variability in stress resistance between strains of model fungi. ▪ Description of the impact of environmental growth conditions on variability in stress resistance between strains of model fungi. ▪ Description of the impact of the developmental state of the mycelium and the spores on variability in stress resistance between strains of model fungi. ▪ Molecules triggering germination of a (sub)population of spores of model fungi. ▪ Mechanism(s) underlying spore heterogeneity with respect to stress resistance. ▪ Generic models describing growth/no growth boundaries and/or germination and outgrowth kinetics.
Achieved results 2019	**Description of the impact of the genetic background on variability in stress resistance between strains of the model fungi** Aspergillus niger ▪ 7 transcription factor genes have been identified that are involved in weak acid resistance, among which *wasB*. ▪ The *mtdB* gene has been inactivated. HPLC confirmed that *mtdB* is the mannitol dehydrogenase gene and not *mtdA* described in the literature. ▪ Melanin deficient deletion strains were made. Their conidia are sensitive to UV radiation, but not altered in their heat resistance. Penicillium roqueforti ▪ Genome wide association studies have revealed a gene cluster containing wasB that is absent in sorbic acid sensitive strains and present in sorbic acid resistant strains. ▪ D56 values of conidia of 12 strains vary between 1.5 and 14.5 min. ▪ Melanin deficient deletion strains were made. Their conidia are sensitive to UV radiation, but not altered in their heat resistance. Paecilomyces variotii ▪ Genome wide association studies have revealed a gene cluster of 60 kb and some other loci that are absent in temperature sensitive strains and present in temperature resistant strains. ▪ D60 values of conidia of 20 strains vary between 3.5 and 26.7 min. ▪ Melanin deficient deletion strains were made. Their conidia are sensitive to UV radiation, but not altered in their heat resistance. Saccharomyces diastaticus ▪ Genome wide association studies have revealed loci that are absent in temperature sensitive strains and present in temperature resistant strains. ▪ Decimal reduction after 10 minutes at 60 °C varies between 0.8 and 4.8. **Description of the impact of environmental growth conditions on variability in stress resistance between strains of the model fungi used in this project** Aspergillus niger ▪ Strains grown at higher temperature are more resistant to heat stress. Conidia formed at higher cultivation temperatures contain more trehalose and less mannitol. Trehalose deficient mutants still show an increase in heat resistance at higher cultivation temperatures. Thus, this heat resistance cannot be explained by this compatible solute. Penicillium roqueforti ▪ Strains grown at higher temperature are more resistant to heat stress. Paecilomyces variotii ▪ Strains grown at higher temperature are more resistant to heat stress. ▪ Strains grown at lower water activity are more sensitive to heat stress. Saccharomyces diastaticus ▪ Heat resistant strains can be easily obtained by experimental evolution. Their D60 value increase from 11 to 22 min within 8 selection cycles. **Description of the impact of developmental state on variability in stress resistance between strains of the model fungi used in this project**

	Aspergillus niger ▪ Spores harvested 39 hours after inoculation are more heat sensitive than after 72 hours, which correlates withcompatible solute concentration. Penicillium roqueforti ▪ Spores formed by 3 day old cultures are more heat sensitive than spores formed by 10-day-old cultures. Paecilomyces variotii ▪ Spores formed by 3 day old cultures are more heat sensitive than spores formed by 10-day-old cultures. Saccharomyces diastaticus ▪ 20 h old vegetative cells are more heat sensitive than 40 h old vegetative cells. ▪ Spores formed by 2 day old cultures are more heat sensitive than spores formed by 5-day-old cultures. **Molecules triggering germination of a (sub)population of spores of the model fungi used in this project** Aspergillus niger ▪ Amino acids trigger different germination responses in A. niger. **Mechanism(s) underlying spore heterogeneity with respect to stress resistance** Aspergillus niger ▪ Transcription factors involved in weak acid degradation and in primary metabolism are involved in weak acid resistance. Penicillium roqueforti ▪ Spore germination is more heterogeneous during increased stress conditions Saccharomyces diastaticus. ▪ The cell wall proteins CWP1 and CWP2 are involved in heat resistance.
Expected results 2020	Aspergillus niger ▪ Mechanisms underlying increased heat resistance when spores are older and/or cultivated at higher temperatures. ▪ Impact of trehalose and mannitol on conidial heat resitance. ▪ Description of the dormant spore content. Penicillium roqueforti ▪ Impact of wasB on MIC of sorbic acid and on heterogeneity. ▪ Isolates with enhanced growth on yoghurt linked with WGS analysis. Paecilomyces variotii ▪ Mechanism(s) underlying spore stress resistance heterogeneity. ▪ Factor effects and interactions on growth kinetics Saccharomyces diastaticus ▪ Additional heat resistance data to improve the GWAS analysis ▪ Improved range of D60 values for 10 minutes that is now based on 18 strains ▪ Study quantitative trait loci involved in heat resistance.

Delivered products in 2019 (give titles and/or description of products, or a link to the products on the project website, or other public websites).
<u>Scientific articles</u>: van den Brule, T., et al. (2019). "The most heat-resistant conidia observed to date are formed by distinct strains of Paecilomyces variotii." Environ Microbiol. doi: 10.1111/1462-2920.14791
<u>External reports</u>:
<u>Professional articles in journals</u>:

Lectures/posters during workshops, conferences and symposia: van den Brule, T., et al., "Strain variability in three strains of the food spoilage fungus *Paecilomyces variotii*". Poster presented at European symposium International Association of Food Protection Nantes. April 24-26, 2019. van den Brule, T., et al., "Strain variability in in conidial heat resistance of food spoilage fungi". Oral presentation at European symposium International Association of Food Protection Nantes. April 24-26, 2019. van den Brule, T., et al., "Strain variability in three strains of the food spoilage fungus *Paecilomyces variotii*". Oral presentation at International Commission on Food Mycology workshop Freising. June 3-5, 2019. Punt., et al., "Heterogeneity in conidia of the spoilage fungi Penicillium roqueforti" Presented at International Commission on Food Mycology workshop Freising. June 3-5, 2019. Punt et al., "Heterogeneity in heat-resistance of Penicillium roqueforti conidia caused by cultivation time and temperature" Poster presentation at TiFN retreat 2019. Seekles et al., "The effect of cultivation temperature on the heat resistance of *Aspergillus niger* conidia". Poster presentation at TiFN retreat 2019 Seekles et al. "Heterogeneity in food spoiler Aspergillus niger: The effect of spore age on conidial heat resistance" Oral presentation at International Commission on Food Mycology workshop Freising 2019 Seekles et al. "Spore heterogeneity of food spoilage fungi; *Aspergillus niger*" Poster presentation at International Commission on Food Mycology workshop Freising 2019 Suiker et al., "Presence of *Saccharomyces cerevisiae* subsp. *diastaticus* in industry and in nature" Poster presentation at TiFN retreat 2019.
TV/radio/social media/newspaper:
Others (techniques, machines, methods, etc.):

Brief description content/aim PPP What is the matter and what does the project contribute? What does the project deliver and what are the effects of its delivery?
Food availability should increase by 70% to feed the human world population in 2050. Reducing food spoilage could significantly contribute to this challenge. At the moment, 25% of food is spoiled, a significant part due to fungal contamination. Fungal spoilage not only affects visual and organoleptic properties but may also result in the production of toxins. Food preservation methods like sterilization and addition of salt reduce spoilage enormously. However, consumers prefer minimal processing to maintain food quality. This, however, leads to increased risk of fungal

spoilage and therefore new mild food processing protocols are needed. Food spoilage often starts with contamination with fungal spores. These reproductive structures are abundant in the environment such as in the air. Experimental data indicate the existence of subpopulations of spores with different levels of resistance to preservation methods.

In this project we study the impact of the

- genetic background of the model fungi used in this project by using strains of different geographic origin and originating from contaminated food and beverages.
- environmental growth conditions by isolating spores from colonies of the model fungi used in this project that have been grown at different substrates either or not in the presence of sub-lethal stress conditions.
- developmental stage of colonies and spores by isolating spores of different age from different zones of colonies of the model fungi used in this project.

This should reveal a proof of concept of a novel processing treatment that prevents fungal spoilage making use of a combination of mild interventions.

Results 2019

Give a brief description of the high-lights in 2019.

- We are well on the way to quantify the range in temperature and weak acid preservative resistance of spores of strains of the different model fungi.
- We have shown that environmental growth conditions impact temperature resistance of spores of the different model fungi.
- We have identified genes involved in weak acid and temperature resistance o.f spores of strains of the different model fungi.

Number of delivered products in 2019 (give titles and/or description of products, or a link to the products on the project website, or other public websites).

Scientific articles	Reports	Articles professional in journals	Lectures/workshops
1			4

Titles/descriptions of prominent products in 2019 (max. 5) and their targets groups

Scientific audience: van den Brule, T., et al. (2019). "The most heat-resistant conidia observed to date are formed by distinct strains of Paecilomyces variotii." Environ Microbiol. doi: 10.1111/1462-2920.14791