| General data | |
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| 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 | 1-12-2016 |
| Final date | 30-06-2021 |

**Met opmerkingen [WH(1]:** Aafke kunnen jullie dit checken

**Met opmerkingen [WH(2]:** Aafke kunnen jullie dit checken

| Approval coordinator/consortium | |
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| 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<br>□ not approved |
| Potential comments regarding the final report | The project is well at scheme |

| Description content/aim PPP | |
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| 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.<br>▪ 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.<br>▪ 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.<br>▪ Proof of concept of a novel processing treatment that prevents fungal spoilage making use of a combination of mild interventions. |

| Results |
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| Expected results 2020 | ▪ Description of the impact of the genetic background on variability in stress resistance between strains of model fungi.<br>▪ Description of the impact of environmental growth conditions on variability in stress resistance between strains of model fungi.<br>▪ Description of the impact of the developmental state of the mycelium and the spores on variability in stress resistance between strains of model fungi.<br>▪ Molecules triggering germination of a (sub)population of spores of model fungi.<br>▪ Mechanism(s) underlying spore heterogeneity with respect to stress resistance.<br>▪ Generic models describing growth/no growth boundaries and/or germination and outgrowth kinetics. |
| Achieved results 2020 | **Description of the impact of the genetic background on variability in stress resistance between strains of the model fungi**<br>Aspergillus niger<br>▪ The effect of compatible solutes on heat resistance of conidia was quantified by comparing compatible solute profiles and heat resistance of 20 knockout strains. Mannitol and trehalose concentrations have impact on heat resistance of conidia, arabitol does not.<br>Penicillium roqueforti<br>▪ $D_{56}$ values of conidia of 20 strains vary between 1.6 and 13.6 min.<br>Paecilomyces variotii<br>▪ D60 values of conidia of 20 strains vary between 3.5 and 26.7 min.<br>▪ Sorbate MIC values (after 28 days) in MEB of 20 strains varied between 3.2 and 10.1 mM undissociated sorbate.<br>▪ Benzoate MIC values (after 28 days) in MEB of 20 strains varied between 3.3 and 5.9 mM undissociated benzoate.<br><br>**Description of the impact of environmental growth conditions on variability in stress resistance between strains of the model fungi used in this project**<br>Aspergillus niger<br>▪ Proteome and transcriptome of dormant conidia harvested at different temperatures has been obtained.<br><br>**Description of the impact of developmental state on variability in stress resistance between strains of the model fungi used in this project**<br><br>**Molecules triggering germination of a (sub)population of spores of the model fungi used in this project**<br>Aspergillus niger<br>▪ Amino acids trigger different germination responses in A. niger.<br>Penicillium roqueforti<br>▪ Amino acids trigger different germination responses in P. roqueforti.<br><br>**Mechanism(s) underlying spore heterogeneity with respect to stress resistance**<br>Saccharomyces diastaticus.<br>▪ Gene inactivation studies revealed that srd1 functions in initiation of sporulation, while osw1 is involved in spore maturation. On the other hand, genes cwp1 and cwp2 function in heat resistance of matured ascospores.<br>Paecilomyces variotii<br>▪ GWAS and RNAseq study revealed loci that are possibly involved in heat resistance<br><br>**Generic models describing growth/no growth boundaries and/or germination and outgrowth kinetics. Proof of concept of a novel processing treatment that prevents fungal spoilage making use of a combination of mild interventions.**<br>Aspergillus niger, Penicillium roqueforti, Paecilomyces variotii |

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| | ▪ Genotypic, biological and experimental variation of heat resistance has been modelled for A. niger, P. variotii, and P. roqueforti.<br>Aspergillus niger<br>• Germination percentage and time needed to initiate germination has been modelled for A. niger. |
| Expected results 2021 | Aspergillus niger<br>▪ Understanding which compatible solutes contribute to germination<br>▪ Description of the dormant spore content and its differences with the mycelium.<br>▪ Understanding the role of heat shock proteins on conidial heat resistance<br>▪ An overview of heterogeneity in sorbic acid resistance of A. niger isolates.<br>Penicillium roqueforti<br>▪ Impact of warB on MIC of sorbic acid and on heterogeneity.<br>▪ Impact of strain heterogeneity on growth on yoghurt either or not containing preservatives.<br>Paecilomyces variotii<br>▪ Mechanism(s) underlying spore stress resistance heterogeneity.<br>▪ Factor effects and interactions on growth kinetics<br>Saccharomyces diastaticus<br>▪ Project was finished early 2020 |
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| **Delivered products in 2020** (give titles and/or description of products, or a link to the products on the project website, or other public websites). |
| Scientific articles:<br>• van den Brule T, Punt M, Teertstra W, Houbraken J, Wösten H, Dijksterhuis J (2020). The most heat-resistant conidia observed to date are formed by distinct strains of Paecilomyces variotii. Environ Microbiol. 2020 Mar;22(3):986-999. doi: 10.1111/1462-2920.14791.<br>• van den Brule T, Lee CLS, Houbraken J, Haas PJ, Wösten H, Dijksterhuis J. Conidial heat resistance of various strains of the food spoilage fungus Paecilomyces variotii correlates with mean spore size, spore shape and size distribution. Food Res Int. 137:109514. doi: 10.1016/j.foodres.2020.109514.<br>• Punt M, van den Brule T, Teertstra WR, Dijksterhuis J, den Besten HMW, Ohm RA, Wösten HAB (2020). Impact of maturation and growth temperature on cell-size distribution, heat-resistance, compatible solute composition and transcription profiles of Penicillium roqueforti conidia. Food Res Int. 136:109287. doi: 10.1016/j.foodres.2020.109287.<br>• Ijadpanahsaravia M, Punt M, Wösten HAB, Teertstra WR (2020) Minimal nutrient requirements for induction of germination of Aspergillus niger conidia. Fungal Biology (in press). |
| External reports: |
| Professional articles in journals: |
| Lectures/posters during workshops, conferences and symposia:<br>• Seekles, S.J. et al. "The effect of cultivation temperature on the heat resistance of Aspergillus niger conidia." Oral & Poster presentation at European Conference of Fungal Genomics (ECFG15), Rome, Italy. As part of Asperfest. 2020<br>• van den Brule, T., et al., "A Genome Wide Association Study reveals genomic insights in conidial heat resistance of *Paecilomyces variotii*". Poster presented at European Conference of Fungal Genomics (ECFG15) Rome, Italy. February 19, 2020 |
| TV/radio/social media/newspaper: |
| Others (techniques, machines, methods, etc.): |

| **Brief description content/aim PPP**<br>What is the matter and what does the project contribute?<br>What does the project deliver and what are the effects of its delivery? |
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| 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.<br><br>In this project we study the impact of the<br>▪ genetic background of the model fungi used in this project by using strains of different geographic origin and originating from contaminated food and beverages.<br>▪ 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.<br>▪ 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.<br><br>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 2020**<br>Give a brief description of the high-lights in 2020. |
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| ▪ We have quantified variability in conidial heat resistance in A. niger, P. variotii and P. roqueforti at strain level, reproduction level and experimental leve.l The impact of each of the variables is similar to that found in bacteria<br>▪ We have shown that heat resistance can be rapidly acquired during stress conditions by inbreeding. This may also occur in breweries<br>▪ We have identified genes in S. diastaticus that are involved in ascospore formation, ascoposre maturation and ascospore heat resistance<br>▪ We have shown that trehalose and mannitol, but not arabitol, are involved in heat resistance of spores of A. niger |

| **Number of delivered products in 2020** (give titles and/or description of products, or a link to the products on the project website, or other public websites). | | | |
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| Scientific articles | Reports | Articles professional in journals | Lectures/workshops |
| 4 | | | 1 |
| **Titles/descriptions of prominent products in 2020 (max. 5) and their targets groups** | | | |
| Scientific audience:<br>▪ van den Brule T, Punt M, Teertstra W, Houbraken J, Wösten H, Dijksterhuis J. The most heat-resistant conidia observed to date are formed by distinct strains of Paecilomyces variotii. Environ Microbiol. 2020 Mar;22(3):986-999. doi: 10.1111/1462-2920.14791.<br>▪ van den Brule T, Lee CLS, Houbraken J, Haas PJ, Wösten H, Dijksterhuis J. Conidial heat resistance of various strains of the food spoilage fungus Paecilomyces variotii correlates with mean spore size, spore shape and size distribution. Food Res Int. 2020 Nov;137:109514. doi: 10.1016/j.foodres.2020.109514.<br>▪ Punt M, van den Brule T, Teertstra WR, Dijksterhuis J, den Besten HMW, Ohm RA, Wösten HAB. Impact of maturation and growth temperature on cell-size distribution, heat-resistance, | | | |

compatible solute composition and transcription profiles of Penicillium roqueforti conidia. Food Res Int. 2020 Oct;136:109287. doi: 10.1016/j.foodres.2020.109287.

- Ijadpanahsaravia M, Punt M, Wösten HAB, Teertstra WR (2020) Minimal nutrient requirements for induction of germination of Aspergillus niger conidia. Fungal Biology (in press).

Participating industry:

- Impact of genotypic, biological and experimental variation on heat resistance, which is important to predict shelf life.