General information

PPP-number	TKI-AF-16141
Title	Rapid at-line detection of environmental Listeria in the food industry
Theme	Food safety
Implementing institute	Wageningen Food & Biobased Research (WFBR)
Project leader research (name + e-mail address)	Heleen van den Bosch Heleen.vandenbosch@wur.nl
Coordinator (on behalf of private partners)	Gerold de Valk (BiosparQ) devalk@biosparq.nl
Project-website address	**https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksprojecten-LNV/Expertisegebieden/kennisonline/AF16141-Rapid-at-line-detection-of-environmental-Listeria-1.htm**
Start date	01-01-2017
Final date	31-03-2020

Approval by the coordinator of the consortium

The annual report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the annual report.

Assessment of the report by the coordinator on behalf of the consortium:	X Approved ☐ Not approved
Additional comments concerning the annual report:	In the course of 2019 Heijs Food Products decided to withdraw from the consortium. Their task, validating DigiTOF data obtained by the food companies (Nestlé, Cargill, Arla Foods) against certified qPCR data, could not be fulfilled due to the delay of delivery of DigiTOF apparatus from BiosparQ to the food companies.

Summary of the project

Problem definition	Suitable diagnostic tests for rapid, at-line detection, identification and typing of microorganisms are hardly available to the food industry. In this project BiosparQ's technology that is based on 'single cell' analysis of bacteria by means of MALDI TOF MS will be used for the detection of microorganisms. The ultimate goal is the assessment of the full bacterial composition of a sample in a couple of minutes. Enrichment and culturing of samples would not be necessary, since each cell is detected and identified and typified separately. The primary focus in the project will be on the detection of environmental *Listeria* which is a serious risk in food processing plants. A successful introduction of the technology would require the development of rapid and dedicated sample pretreatment protocols to concentrate sufficient microorganisms to a small volume. The participants will explore new ways to concentrate bacterial cells from food products.
Project goals	In global lines the project will deliver the following products/results: • Specific sample pretreatment protocols for environmental *Listeria* swabs and for a number of food samples to be chosen by the participating food industries. These protocols will be focused on the concentration of the population of bacterial cells from swabs

	or food samples to a small volume (50 to 100 μL) that will be used for subsequent analysis by single cell MALDI TOF MS.
	• A single cell MALDI TOF MS apparatus (BiosparQ) that is suited for the rapid characterisation (some minutes) and typing of populations of bacterial cells.
	• MALDI TOF MS database information specific for the typing of individual bacterial cells such as *Listeria* (*monocytogenes*) and other pathogenic and food spoilage microorganisms as chosen by the participating food companies.
	• Validated procedures to detect *Listeria* (*monocytogenes*) in environmental samples by BiosparQ technology. Validated procedures to detect populations of pathogenic and/or spoilage microorganisms by BiosparQ technology.

Results	
Planned results 2019	- Experiments will be performed to check whether *Listeria innocua* (as a model to *Listeria monocytogenes*) can be bound to the expressed protein polymers in the supernatant of *Pichia pastoris* fermentations. Fermentations have been performed in 2018. Hereto bacteriophage Ply-domains that are able to bind *Listeria* cells, were cloned in *Pichia*. Ply-domains are cell wall binding domains (CBDs) from bacteriophages. - New fermentations in *Pichia pastoris* - Experiments with WGA beads at Arla Food's facility - Experiments with metal membranes in combination with concentration by WGA beads. - Construct a (*Listeria*) database for BioSparq's DigiTOF apparatus. - Place prototypes of the DigiTOF at different partners to do experiments.

Achieved results 2019	- Different methods have been used to check whether *Listeria innocua* can be bound to the expressed protein polymers in the supernatant of the *Pichia* fermentation (wildtype and protease resitant strain). To choose the best fermentation supernatant, a MALDI TOF experiment was performed to analyze the proteins in the supernatants of the different fermentations.
	   - Experiments with Strep-Tactin beads did not show binding of *Listeria* to proteins from the fermentation supernatant that, by design, include a Strep-tag.
	   - Microscopical analysis with fluorescent Strep-Tactin: A very weak fluorescent signal visible. A clear signal could not be produced. In an attempt to get a better interaction between the polymer and the *Listeria* bacteria, the cells were fixated using trichloroacetic acid (TCA). However no positive result could be obtained.
	So, unfortunately, none of these methods showed binding of the supernatant to *Listeria innocua*.
	
	- Two new fermentations were performed in the wildtype strain and in the mutant strainy. In these fermentations more amino acids were added to reduce proteolysis and for the same purpose the fermentations were performed at a lower temperature. The fermentation supernatants have been analyzed by SDS-PAGE gel and did not show better results than the most optimal fermentation of 2018.
	
	- At Arla's facility the WGA beads protocol could be reproduced. Arla also tried to concentrate spiked *Listeria innocua* from milk, however, that appeared not to be possible. Most probably the salt concentration and/or the pH of milk was not optimal. It was decided to go back to

	the original plan: swab dried *Listeria innocua* cells from a surface and concentrate these swabbed cells using WGA beads. - First experiments have been performed with metal membranes with a pore size of 450 nm. Unfortunately, the *Listeria* cells appeared to be able to go through the pores of the membrane because the bacteria are long and thin (0.4–0.5 µm in diameter by 1–2 µm long). This effect was shown in other filters as well. (Ref: Nakazawa et al. Applied and environmental Microbiology, 2005 Nov; 71(11); 7571-7574). In this reference it was shown that *Listeria monocytogenes* infiltrated the reticulate structure of a membrane filter and passed through a filter with pore sizes of 0.45 µm and 0.2 µm in 6 to 24 h and 5 to 6 days, respectively. In our case, pressure was created using a syringe, most probablty speeding up this process. New metal membranes with 150 nm pores showed better results. However, the equipment that had to be used was not optimal for the size of the metal membranes used; the metal membranes had a smaller diameter. New metal membranes were ordered at the company Metalmembranes (one of the partners in the project). However in that period the company was liquidated and the metal membranes could not be delivered anymore. - WorldBioproducts developed protocols for swabbing (*Listeria*) bacteria from surfaces. The results were documented in 8 documents, that were shared with the project team. Subjects of these documents involved surface sampling, *Listeria* viability, device comparison and broth comparison. - Building a database that can be used to classify individual spectra from the single cell MALDI TOF MS apparatus appeared to be more difficult than anticipated by BiosparQ. As Listeria is a hazardous microorganism to work with it was decided to develop the data analysis subsystem with less virulent bacteria. A prototype database with *E. coli*, *E. faecalis*, *S. aureus*, *S. epidermidis* and *K. pneumonia* was constructed and tested, in close co-operation with Ghent University. Using their advanced AI technologies, a Single Cell Confusion Matrix was extracted, showing promising results on the single particle spectra level to discriminate between these different species, prior to accumulation and final identification. - Building a Beta prototype instrument was also more challenging than expected. Only recently, working prototypes were built (3) that complied with BiosparQ's stringent specifications regarding limited footprint, automation, ease of use and safety. BiosparQ has now set up a supply chain that can built more of such prototypes to test in a relevant environment. Based on the feedback of such tests, the design will be optimized and frozen.
Planned results 2020	- Limit of detection experiments (*Listeria innocua*) using WGA beads - Swab experiments at WFBR and Arla Food :swab dried *Listeria innocua* cells from a surface and concentrate these swabbed cells using WGA beads. Swabs and protocols will be provided by WorldBioproducts

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Deliverables/products in 2019 (provide the titles and /or a brief description of the products/deliverables or a link to a website.

<u>Scientific articles:</u>

Fast pathogen identification using single-cell MALDI-TOF mass spectrum data and machine learning techniques, Christina Papagiannopoulou, René Parchen, Willem Waegeman (Department of Data Analysis and Mathematical Modelling, Ghent University, Belgium, BiosparQ B.V., Leiden, the Netherlands)

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External reports:

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Articles in professional journals/magazines:

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(Poster) presentations at workshops, seminars, or symposia.

Point of care testing with the Cirrus D20 for Waddlia chondrophila and Chlamydia trachomatis, presentation by BiosparQ for the 14th AACM congress Amsterdam.Technology update 3, Cirrus® D20, Presentation by BiosparQ for the Emerging Antimicrobials and Diagnostics in AMR meeting 2019,19-20 November, Amsterdam

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TV/ radio / social media / newspaper:

BiosparQ was nominated as finalist for the election for the Dutch National Icon. These National Icons are Dutch solutions for global issues in the areas of health, energy, digitisation, circular materials and mobility, several items in (local) newspapers, government websites atc.

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Remaining deliverables (techniques, devices, methods, etc.):

Titles of Worldbioproducts' documents:
- Poster: Development of a Laboratory Method Using Stainless Steel Coupons for Determining the Efficacy of Surface Sampling
- Study: Comparative Evaluation of PUR-Blue™ Swab Collection Devices and EZ Reach™ Sponge Collection Devices for Recovery of Listeria from 1 ft2 Stainless Steel Surfaces
- Study: Comparison of HiCap Neutralizing Broth™ and D/E Neutralizing Broth to Maintain Listeria monocytogenes After Sampling of Inoculated Stainless-Steel Surfaces
- Study: Comparison of HiCap Neutralizing Broth™ and Letheen Broth to Maintain Listeria monocytogenes After Sampling of Inoculated Stainless-Steel Surfaces
- Study: Comparison of HiCap Neutralizing Broth™ and Neutralizing buffer to Maintain Listeria monocytogenes After Sampling of Inoculated Stainless-Steel Surfaces
- Study - World Bioproducts Device Comparison
- Study - World Bioproducts HiCap - Phase 1 Final Report
- Study - World Bioproducts HiCap - Phase 2 Final Report

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https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksprojecten-LNV/Expertisegebieden/kennisonline/AF16141-Rapid-at-line-detection-of-environmental-Listeria-1.htm

https://research.wur.nl/en/projects/af16141-rapid-at-line-detection-of-environmental-listeria-bo-46-0