



**PPP Project Annual Report 2018**

The PPP-projects that have been established under the direction of the top sectors must submit an annual report on their technical and financial progress. This format is to be used for reporting the technical progress. A separate format ('PPP final report') is available for PPP-projects that have been completed in 2018.

**The annual reports will be published in full on the websites of the TKIs/top sector, excluding the blocks 'Approval coordinator/consortium' and 'Planning and progress' . Please ensure that no confidential matters are left in the remaining blocks.**

The PPP Project Annual Reports must be submitted by 15 February 2019 to Hans van der Kolk

<b>General information</b>	
PPP number	TKI-AF-16141
Title	Rapid at-line detection of environmental Listeria in the food industry
Theme	Food safety (gezond en veilig)
Executive knowledge institution(s)	Wageningen Food & Biobased Research (WFBR)
Research project leader (name + e-mail address)	Heleen van den Bosch Heleen.vandenbosch@wur.nl
Coordinator (on behalf of private parties)	Gerold de Valk (BiosparQ) devalk@biosparq.nl
Government contact person	M.G.M. van Creij
Total project size (k€)	960 k€
Address project website	<a href="https://topsectoragrifood.nl/project/rapid-at-line-detection-of-environmental-listeria-in-the-food-industry/">https://topsectoragrifood.nl/project/rapid-at-line-detection-of-environmental-listeria-in-the-food-industry/</a>
Start date	01-01-2017
End date	31-12-2019

**Approval coordinator/consortium**

The annual report should be discussed with the coordinator/the consortium. The TKIs appreciate being informed of possible feedback on the annual report.

The coordinator has assessed the annual report on behalf of the consortium:	<input checked="" type="checkbox"/> approved <input type="checkbox"/> rejected
Possible feedback on the annual report:	

**Short content description/aim PPS**

What is going on and how is this project involved?

What will be delivered by the project and what is the effect of this?

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.

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.

**Planning and progress** (if there are changes to the project plan, please explain)

Is the PPP going according to plan?	Yes
Have there been changes in the consortium/project partners?	No
Is there a delay and/or deferred delivery date?	Yes. The <i>Listeria</i> classifier database has not been fully developed yet. Furthermore, as the first year was used to search for alternative pretreatment methods to filtration, the development of a pretreatment protocol could not start before the second year. The detection of environmental <i>Listeria</i> with the BiosparQ single cell MALDI TOF MS apparatus has been planned for the third year.
Are there any substantive bottlenecks?	The SDS page protein gels of the fermentations' supernatant show several bands. The main band is a band of the expected size, however degradation products may occur. Therefore besides the wildtype <i>Pichia pastoris</i> strain, a mutant strain has been used that has less protease activity. Furthermore casamino acids were added to reduce proteolysis. In 2019 experiments will be performed to check whether <i>Listeria innocua</i> (as a model to <i>Listeria monocytogenes</i> ) can be bound to the expressed protein polymers in the supernatant of these fermentations.
Are there any deviations from the projected budget?	No

**Results in 2018/ so far**

Give a short description of the high-lights and (most important) project deliverable in 2018 / so far and their target group

- A method has been set up to collect *Listeria innocua* from medium. In this protocol commercially available wheat germ agglutinin coated magnetic beads (WGA beads) have been used.
- Last year Ply domains that are able to bind *Listeria* cells, were cloned in *Pichia pastoris*. Ply domains are cell wall binding domains (CBDs) from bacteriophages. Three different domains have been cloned: Ply 500, P35 and P40. To be able to purify the expressed CBD after the fermentation, streptavidin and histidine tags have been included in the construct in front of the Ply domains.
- *Pichia pastoris* transformants of all three Ply domains have been fermented.
- The BiosparQ apparatus (DigiTof) was miniaturised. The prototype that is available at the moment can be transported in a car.

<b>Number of delivered products in 2018 / so far</b> (in an appendix, please provide the titles and/or description of the products or a link to the products on public websites)			
Academic articles	Reports	Articles in journals	Introductions/workshops
x	x	x	A poster entitled "Rapid <i>Listeria</i> capture to enable single cell MALDI-TOF MS" was presented during the 12th Rapid Methods Europe (RME) conference from 5-7 November 2018, Amsterdam, The Netherlands. <a href="https://www.rapidmethods.eu/programme.php">https://www.rapidmethods.eu/programme.php</a>

**Appendix: Names of the products or a link to the products on a public website**

<https://topsectoragrifood.nl/project/rapid-at-line-detection-of-environmental-listeria-in-the-food-industry/>

<https://www.wur.nl/en/Research-Results/show-2/Rapid-at-line-detection-of-environmental-Listeria.htm>