PPP final report
PPPs that have been finalized need to deliver a factual and financial final report. For the financial report an overview of the project expenses on realisation and financing should be give in a separate format.
Final reports will be published in their entirety on the TKI/top-sector websites. Please make sure there are no confidential matters in the report.
PPP final reports have to be submitted - pooled for each research organisation - before 1 April 2019 to the TKIs at info@tkitu.nl, or at info@tki-agrifood.nl.

General data	
PPP number	**AF-15501**
Title	**A new pathway for transfer of genetic material in lactic acid bacteria. Demonstrating the role of tailless membrane lactococcal bacteriopahges in the transfer of DNA**
Theme	Gezond en veilig
Research Institute(s) involved	WUR
Project leader research (name + email address)	E. Smid (eddy.smid@wur.nl)
Coordinator (on behalf of private parties)	W. Meijer (CSK)
Contact person of government	--
Total project budget (k€)	69
Project website address	--
Starting date	1-9-2015
Final date	30-04-2016

Approval coordinator/consortium The final report has to be discussed with the coordinator/consortium. The TKI(s) like to be informed regarding potential comments on the final report.	
The annual report is …… by the coordinator on behalf of the consortium	v approved □ not approved
Potential comments regarding the final report	none

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?
Studies performed in the framework of TIFN project FF001 at the Laboratory of Food Microbiology (FHM) of Wageningen University on the microbial community in the starter culture named "Ur" revealed intriguing features about the interactions between (pro)phages and Lactococcus lactis. A hypothetical model was put forward, in which tailless phages are released from the hosts via a "budding"-like, non-lytic way; the phages enter the hosts by membrane fusion and meanwhile transfer genes among the hosts. The aim of this study was to collect experimental evidence for the hypothetical model about phage entry and release from the hosts. ProΦ1 from L. lactis TIFN1 was labelled with an antibiotic resistance marker (cat) or a green fluorescence protein (gfp) gene cassette. The strains were produced by constructing and transforming plasmids dedicated to DNA homologous recombination with TIFN1 chromosome at prophage site(s). To study the phage entry and DNA transfer, purified phage proΦ1 containing an inserted sequence encoding the cat cassette was used to infect various host strains. However, so far there has been no evidence showing that the host strains acquired the resistance marker from proΦ1. Phage particles were incubated with host strains and flow cytometry analysis

revealed phage adsorption to the host cells. To study phage replication and release, TIFN1 containing proΦ1 with an inserted sequence encoding the gfp cassette was used to perform phage induction. Flow cytometry analysis on the cells throughout the induction process revealed that 80% of the cells were positive for phage production, and 60% of the cells were highly active for phage production. This number, together with the observation of no detectable decrease in cell density during phage release, provided evidence for non-lytic phage release. Finally, more conditions have been tested for phage DNA transfer, but direct evidence for this process to be operational has not been found.

Mutations with respect to the original project plan and follow-up	
Have there been changes in the consortium/project partners? If so, which.	no
Have there been factual changes in the project?	no
Has a patent application been filed from this PPP (or a first-filing)?	no
Has a spin-off developed from this project (contract research, additional funding or spin-off activity)?	no
How many years will the private parties need in practice to use results from this project?	unknown
How did the project contribute to the development of the research organisation involved? (e.g. scientific track record, new technology, new collaboration?	Four scientific publications
Will there be a follow-up for the project such as a new project or a new collaboration? If so, please explain.	no

Results

What tangible results the project has yielded?
Four scientific publications (1 direct, 3 spin-off)

What are the effects of these results and for whom?
New insights into the use of food grade Gram-positive food for delivery of hydrophobic cargo to the human host.

W**hat has not been delivered according to the original project plan and for what reason(s)?**
A patent application has not been delivered. Although more conditions have been tested, direct evidence for phage DNA transfer via membrane engulfed phage heads has not been found.

Deliverables (give a short description per project deliverable)

This study aimed to collect experimental evidence for the phage entry and release from the hosts in the Ur culture.

- Strains containing (pro)phages labelled with antibiotic resistance marker or green fluorescence protein gene cassette were successfully constructed.
- No evidence was found to demonstrate the phage entry to the hosts and DNA transfer with the conditions tested so far, but phage adsorption to the hosts was shown to happen.
- Evidence for non-lytic phage release was provided by demonstrating that 80% of the cell population was positively producing phages, and still no detectable lysis was observed.
- In this study, the constructed plasmids all contained a thermosensitive replication origin *repA*^{Ts} and a gene encoding a lactococcal orotate transporter (*oroP)*. To the best of our

knowledge, the combination of these two elements has not been used in plasmid construction in any other study before, and is therefore an innovation point of the methodology in this study.

Number of delivered products in 2018 (give titles and/or descriptions of products, or a link to the products on the project website, or other public websites).			
Scientific articles	Reports	Articles in professional journals	Lectures/workshops
4	1		
Titles/descriptions of prominent products in 2018 (max. 5) and their target groups			
Liu, Y. S. Alexeeva, K.A.Y. Defourny, E. J. Smid and T. Abee. 2018. Tiny but mighty: bacterial membrane vesicles in food biotechnological applications. Curr. Opinion in Biotechnol. 49:179-184			
Liu, Y., K.A.Y. Defourny, E. J. Smid and T. Abee. 2018. Gram-positive bacterial extracellular vesicles and their impact on health and disease. Frontiers in Microbiol. 9:1502.https://doi.org/10.3389/fmicb.2018.01502			
Liu, Y., E.J. Smid, T. Abee and R. A. Notebaart. 2019. Delivery of genome editing tools by bacterial extracellular vesicles. Microbial Biotechnol. 12(1): 71–73			
Alexeeva, S, J. A. Guerra Martínez, M. Spus and E. J. Smid. 2018. Spontaneously induced prophages are abundant in a naturally evolved bacterial starter culture and deliver competitive advantage to the host. BMC Microbiology 18:120. https://doi.org/10.1186/s12866-018-1229-1			