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Title	Detecting Lactococcus lactis prophages by Mitomycin C-mediated induction coupled to flow cytometry analysis
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Publication date	2017
Original Citation	Oliveira, J., Mahony, J., Hanemaaijer, L., Kouwen, T. R. H. M., Neve, H., MacSharry, J. and van Sinderen, D. (2017) 'Detecting Lactococcus lactis prophages by Mitomycin C-mediated induction coupled to flow cytometry analysis', Frontiers in Microbiology, 8, 1343 (11pp). doi: 10.3389/fmicb.2017.01343
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://journal.frontiersin.org/article/10.3389/fmicb.2017.01343/full - 10.3389/fmicb.2017.01343
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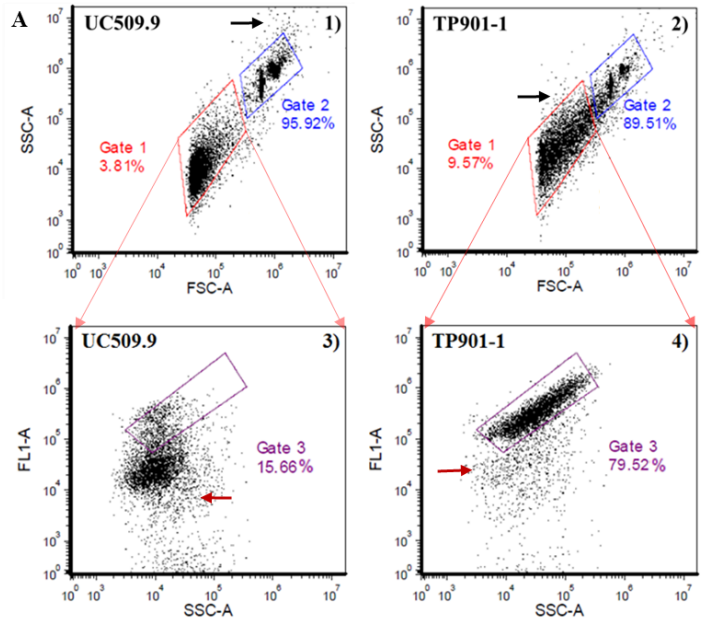

Parameters
Run limits: 5000 events.ml^{-1}
Medium flow rate: 33 µl.min^{-1}
Phage particles detection: SSC (Side Scatter) *versus* FSC (Forward Scatter)
Purpose: Discriminate different particles based on size and complexity in the induced lysate

Analysis
Gate 1- Phage particles population (virions produced by cell lysis)
Gate 2- Microspheres (used as a fluorescence & size reference control)
Gate Outliers- Cell debris and/or background noise (dots outside of each gate; black arrow)

Parameters
Fluorescence detection: FL1 (BP Filter 530/30) *versus* SSC
Purpose: Detect phage DNA particles stained with SYTO-9

Analysis
Gate 3- Phage DNA stained with SYTO-9 in our induced lysate sample (phage population detected in Gate 1)
Gate Outliers- Background noise (dots outside of each gate; red arrow)

Flow cytometry analysis

		% of events (SSC *versus* FSC)			% of fluorescence (FL1 *versus* SSC)	
		Sample population	**Beads**	**Cell debris/ noise**	**Phage particles**	**Background noise**
***L. lactis* strains**	**Features**	Gate 1	Gate 2	Outliers of Gates 1 & 2	Gate 3	Outlier of Gate 3
UC509.9	No prophage released (negative control)	6.73 ± 2.07	92.93 ± 2.12	0.34 ± 0.05	16.33 ± 3.76	83.67 ± 3.76
NZ9000 (TP901-1 *erm*)	TP901-1*erm* prophage (positive control)	10.98 ± 1.13	87.97 ± 1.26	1.05 ± 0.15	80.60 ± 2.39	19.39 ± 2.39

Figure S1. Schematic representation of the flow cytometry parameters, analysis and general results for the two *L. lactis* control strains. BD Accuri™ C6 flow cytometer was used for the implementation of the correct parameters to detect and enumerate phage DNA particles stained with SYTO-9 dye. **(A1** and **A3)**: Cytograms of 3 µg.ml^{-1} MmC-treated *L. lactis* UC509.9 (prophage-free lactococcal strain used as negative control); **(A2** and **A4)**: Cytogram of 3 µg.ml^{-1} MmC-treated *L. lactis* NZ9000 TP901-1*erm* (lactococcal strain harbouring the TP901-1 prophage used as a positive control).