

## Supplementary Material

# Chromatographic characterization and *in vitro* bioactivity evaluation of *Lactobacillus helveticus* hydrolysates upon fermentation of different substrates

Federica Ianni <sup>1\*</sup>, Alessandra Anna Altomare <sup>2</sup>, Beniamino T. Cenci-Goga <sup>3\*</sup>, Francesca Blasi <sup>1</sup>, Luca Grispoldi <sup>3</sup>, Luca Regazzoni <sup>2</sup>, Lina Cossignani <sup>1,4</sup>

<sup>1</sup> Food Science and Nutrition Section, Department of Pharmaceutical Sciences, University of Perugia, 06126 Perugia, Italy; francesca.blasi@unipg.it (F.B.); lina.cossignani@unipg.it (L.C.)

<sup>2</sup> Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli 25, 20133 Milano, Italy; alessandra.altomare@unimi.it (A.A.A.); luca.regazzoni@unimi.it (L.R.)

<sup>3</sup> Department of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06126 Perugia, Italy; grisluca@outlook.it

<sup>4</sup> Center for Perinatal and Reproductive Medicine, University of Perugia, Santa Maria della Misericordia University Hospital, Sant'Andrea delle Fratte, 06132 Perugia, Italy

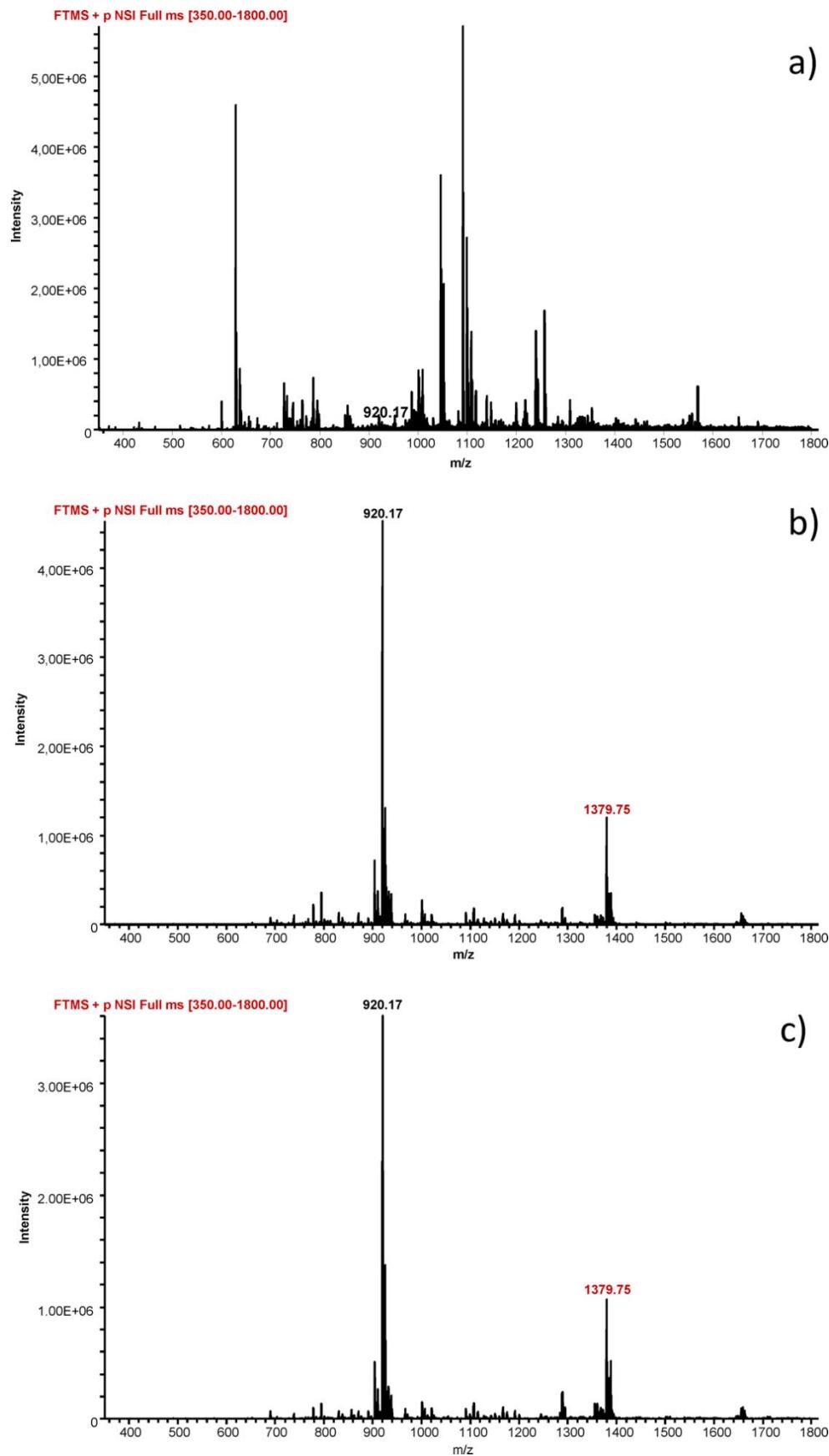
\* Correspondence: federica.ianni@unipg.it (F.I.); beniamino.cencigoga@unipg.it (B.T.C.-G.); Tel.: +39-075-585-7919 (F.I.); +39-075-585-7929 (B.T.C.-G.)

**Table S1.** Time sampling scheme for strain fermentation activity evaluation.

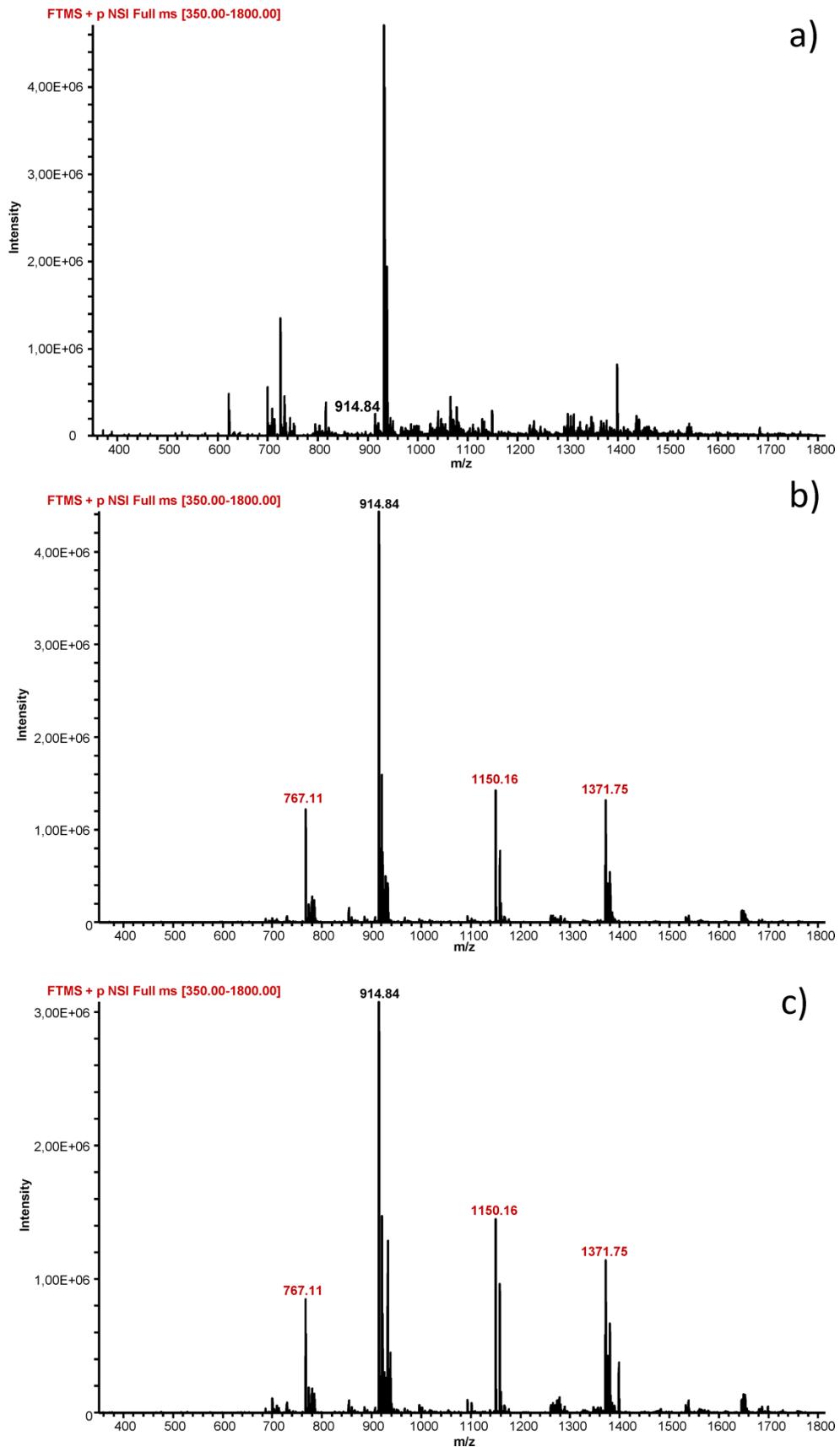
Strain	Time sampling (h)						
	12	36	60	72	120	280	400
<i>Lactococcus lactis</i> ssp. <i>lactis</i> (ref. 79, field strain)	x	x	x	x	x		
<i>Lactococcus lactis</i> ssp. <i>lactis</i> (ref. 81, field strain)	x	x	x	x	x		
<i>Lactobacillus casei</i> ssp. <i>casei</i> (ref. 80, filed strain)	x	x	x	x	x		
<i>Lactobacillus acidophilus</i> ATCC 4356 (ref. 618)	x	x	x	x	x		
<i>Lactobacillus acidophilus</i> LA 14 (ref. 1004)	x	x	x	x	x	x	x
<i>Lactobacillus acidophilus</i> (ref. 80/2, field strain)	x	x	x	x	x		
<i>Lactobacillus acidophilus</i> (ref. 80/3, field strain)	x	x	x	x	x		
<i>Lactobacillus helveticus</i> (ref. LH, field strain)	x	x	x	x	x	x	x
<i>Enterococcus faecium</i> UBEF-41 (ref. 1003)	x	x	x	x	x		
<i>Saccharomyces cerevisiae</i> var <i>boulardii</i> MTCC-5375 (ref. 1005)	x	x	x	x	x		

**Table S2.** Comparison between *L. acidophilus* (LA 14) and *L. helveticus* (LH) activity upon 72 h fermentation, reported as mean area values of reference peaks (measured in mV·s)  $\pm$  standard deviation (SD),  $n=3$  for each selected peak.

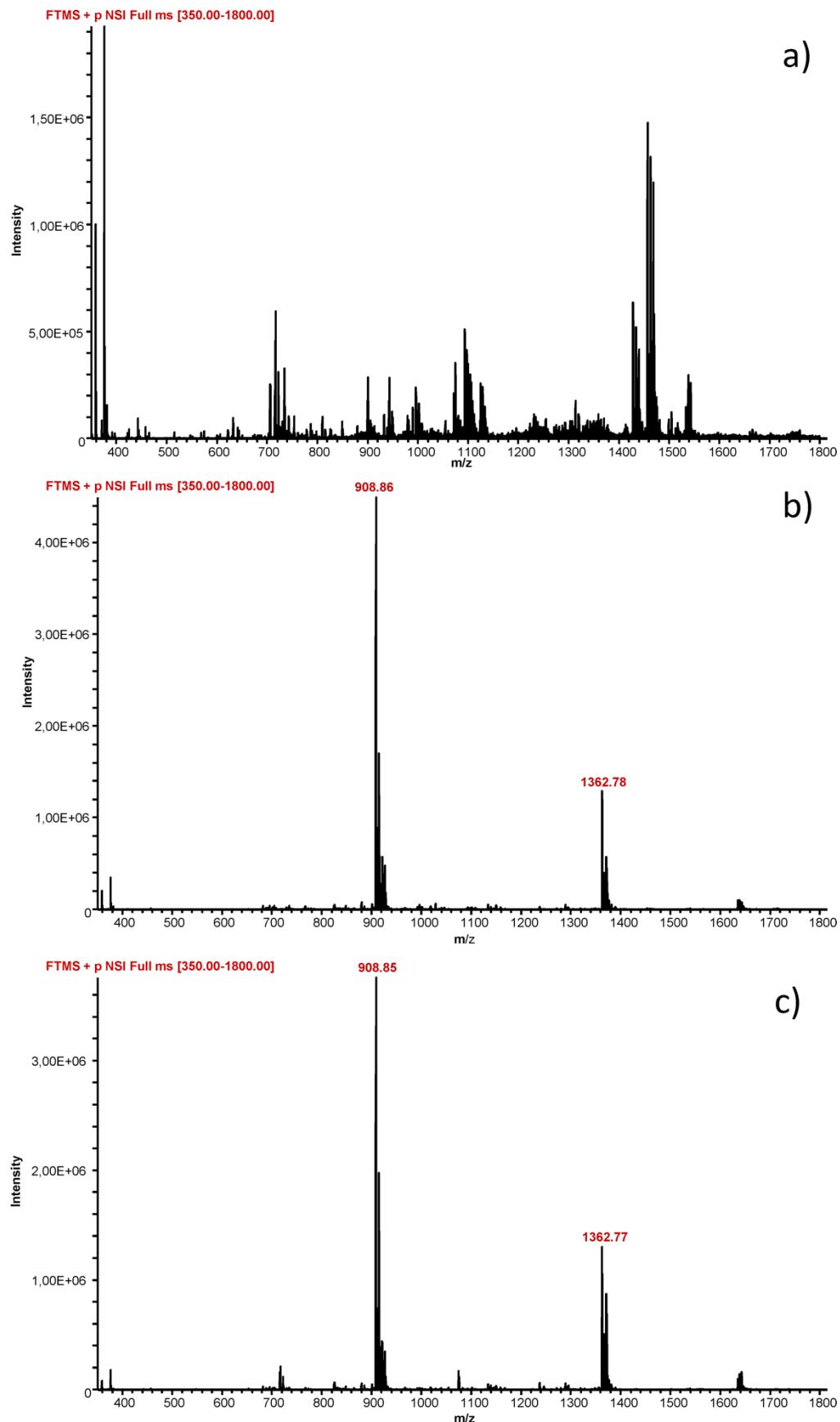
Peak $t_r$ (min)	Peak Area	Peak Area
	LA-72h	LH-72h
4.80	0.00	1726.80 $\pm$ 49.12
5.50	3911.83 $\pm$ 47.25	11294.77 $\pm$ 90.69
9.48	86.41 $\pm$ 0.20	172.29 $\pm$ 16.80
13.15	622.45 $\pm$ 9.34	669.44 $\pm$ 25.53
13.58	570.08 $\pm$ 43.60	826.23 $\pm$ 33.72
16.20	47.49 $\pm$ 1.52	1055.96 $\pm$ 24.01
18.60	51.09 $\pm$ 3.25	334.28 $\pm$ 5.83
23.50	689.81 $\pm$ 49.88	1150.70 $\pm$ 28.40
30.86	153.43 $\pm$ 4.77	1318.87 $\pm$ 53.50



**Figure S1.** Full MS spectrum of the three samples **a)** SM-Substrate, **b)** SM-TCA, **c)** SM-Beads, related to the RT including the peak at  $42.57 \pm 0.16$  min. The m/z of the newly formed peptides are highlighted in red.



**Figure S2.** Full MS spectrum of the three samples **a)** SM-Substrate, **b)** SM-TCA, **c)** SM-Beads, related to the RT including the peak at at  $45.75 \pm 0.19$  min. The m/z of the newly formed peptides are highlighted in red.



**Figure S3.** Full MS spectrum of the three samples **a)** SM-Substrate, **b)** SM-TCA, **c)** SM-Beads, related to the RT including the peak at at  $47.12 \pm 0.15$  min. The  $m/z$  of the newly formed peptides are highlighted in red.



© 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).