

Supplementary Information

Spray-drying encapsulation of the live biotherapeutic candidate *Akkermansia muciniphila* DSM 22959 to survive aerobic storage

Joana Cristina Barbosa¹, Diana Almeida¹, Daniela Machado^{1*}, Sérgio Sousa¹, Ana Cristina Freitas^{1†}, José Carlos Andrade², Ana Maria Gomes¹

¹ *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal;* ² *TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116 Gandra, Portugal*

*corresponding author: Daniela Machado, Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

E-mail address: dmachado@ucp.pt

Table S1. Viability of spray dried *Akkermansia muciniphila* DSM 22959 under aerobic storage conditions. The CFU counts correspond to three different matrices – SM: 10% skim milk; WPC: 10% whey protein concentrate (80 %); WPI: 10% whey protein isolate (90%); processed using 4 different combinations of inlet and outlet temperatures, as described. The resulting powders were aerobically stored, under controlled humidity, at 4 and 22 °C, over a period of 28 days. Results are expressed in CFU/g \pm standard deviation, unless stated otherwise; the CFU concentration initially inoculated is also indicated, as well as the concentration after the spray drying technique (corresponding to Day 0). BDL = Below Detection Limit.

Conditions	Initial (CFU/ml)	4 °C					22 °C			
		Day 0	7	14	21	28	7	14	21	28
SM (10 %)										
#1	1.35 ± 0.25 x 10 ⁸	3.23 ± 0.34 x 10 ⁷	1.58 ± 0.41 x 10 ⁷	1.90 ± 0.40 x 10 ⁷	1.17 ± 0.43 x 10 ⁷	1.87 ± 0.34 x 10 ⁷	1.07 ± 0.11 x 10 ⁷	2.90 ± 0.65 x 10 ⁶	1.08 ± 0.23 x 10 ⁶	7.83 ± 1.86 x 10 ⁵
#2		1.20 ± 0.22 x 10 ⁸	5.30 ± 1.41 x 10 ⁷	5.58 ± 1.60 x 10 ⁷	5.72 ± 1.07 x 10 ⁷	4.82 ± 1.16 x 10 ⁷	1.85 ± 0.28 x 10 ⁷	1.33 ± 0.49 x 10 ⁷	1.40 ± 0.75 x 10 ⁶	1.17 ± 0.25 x 10 ⁶
#3		1.10 ± 0.20 x 10 ⁸	3.40 ± 1.09 x 10 ⁷	4.15 ± 0.47 x 10 ⁷	3.98 ± 0.96 x 10 ⁷	4.53 ± 0.85 x 10 ⁷	1.52 ± 0.27 x 10 ⁷	4.05 ± 1.57 x 10 ⁶	1.90 ± 0.66 x 10 ⁶	1.12 ± 0.46 x 10 ⁶
#4		3.83 ± 0.33 x 10 ⁷	1.17 ± 0.21 x 10 ⁷	1.08 ± 0.28 x 10 ⁷	1.25 ± 0.35 x 10 ⁷	1.08 ± 0.34 x 10 ⁷	2.88 ± 0.59 x 10 ⁶	1.45 ± 0.46 x 10 ⁶	4.85 ± 3.88 x 10 ⁵	4.25 ± 0.82 x 10 ⁵
WPC (10 %)										
#1	1.16 ± 0.11 x 10 ⁹	2.00 ± 0.30 x 10 ⁷	7.67 ± 0.75 x 10 ⁶	1.70 ± 0.62 x 10 ⁶	3.32 ± 1.52 x 10 ⁶	1.38 ± 0.53 x 10 ⁶	6.33 ± 1.25 x 10 ⁵	BDL	BDL	BDL
#2		2.40 ± 1.02 x 10 ⁷	1.88 ± 0.30 x 10 ⁷	1.22 ± 0.66 x 10 ⁷	1.43 ± 0.48 x 10 ⁷	9.33 ± 2.52 x 10 ⁶	3.33 ± 1.89 x 10 ⁶	BDL	BDL	BDL

#3		3.83 ± 1.03 $\times 10^7$	8.00 ± 3.58 $\times 10^6$	BDL	BDL	BDL	5.33 ± 3.40 $\times 10^5$	BDL	BDL	BDL
#4		9.70 ± 0.00 $\times 10^6$	2.67 ± 0.94 $\times 10^6$	BDL	BDL	BDL	7.00 ± 4.24 $\times 10^5$	BDL	BDL	BDL
WPI (10 %)										
#1	$5.57 \pm 0.50 \times 10^8$	4.10 ± 0.71 $\times 10^6$	2.55 ± 0.68 $\times 10^6$	$9.33 \pm 3.40 \times 10^5$	$4.80 \pm 1.60 \times 10^5$	$2.37 \pm 0.85 \times 10^5$	2.67 ± 1.11 $\times 10^5$	BDL	BDL	BDL
#2		1.47 ± 0.33 $\times 10^7$	7.97 ± 4.89 $\times 10^6$	$2.72 \pm 0.32 \times 10^6$	$1.03 \pm 0.64 \times 10^6$	$2.25 \pm 0.77 \times 10^6$	8.60 ± 1.74 $\times 10^5$	BDL	BDL	BDL
#3		1.33 ± 0.47 $\times 10^7$	1.03 ± 0.62 $\times 10^7$	$1.92 \pm 0.56 \times 10^6$	$1.83 \pm 0.51 \times 10^6$	$1.80 \pm 0.34 \times 10^6$	9.80 ± 4.83 $\times 10^5$	$2.55 \pm 0.87 \times 10^5$	$1.83 \pm 0.57 \times 10^5$	$1.03 \pm 0.39 \times 10^5$
#4		3.80 ± 0.59 $\times 10^6$	1.92 ± 0.47 $\times 10^6$	$1.15 \pm 0.27 \times 10^6$	$4.52 \pm 0.69 \times 10^5$	$4.65 \pm 0.73 \times 10^5$	2.04 ± 0.39 $\times 10^5$	$2.13 \pm 0.65 \times 10^4$	$3.44 \pm 1.04 \times 10^4$	BDL

Table S2. Resistance to simulated GIT conditions of skim milk-incorporated, spray dried *Akkermansia muciniphila* DSM 22959 (SM). The samples were aerobically stored, at 4 °C, for 60 days. The survivability to GIT conditions was evaluated every 2 weeks, following a standard protocol, as described [1], and CFU counts were evaluated every hour, with t = 0 corresponding to the *A. muciniphila* concentration at the beginning of the simulated GIT conditions; t = 2 to the end of the gastric phase; and t = 5, to the end of the intestinal phase.

Timepoints (h)	SM (CFU/g)				
	1	14	28	45	60
0	$3.52 \pm 0.00 \times 10^8$	$6.73 \pm 0.20 \times 10^7$	$4.69 \pm 0.97 \times 10^7$	$2.75 \pm 0.45 \times 10^7$	$2.60 \pm 0.24 \times 10^7$
1	$9.11 \pm 3.71 \times 10^7$	$1.62 \pm 0.21 \times 10^8$	$4.55 \pm 0.55 \times 10^7$	$2.07 \pm 1.28 \times 10^7$	$2.62 \pm 1.39 \times 10^7$
2	$1.30 \pm 0.23 \times 10^8$	$1.58 \pm 0.51 \times 10^8$	$3.63 \pm 0.77 \times 10^7$	$2.43 \pm 0.37 \times 10^7$	$2.42 \pm 0.69 \times 10^7$
3	$7.16 \pm 1.90 \times 10^7$	$4.17 \pm 0.43 \times 10^7$	$4.08 \pm 0.95 \times 10^7$	$3.27 \pm 1.04 \times 10^7$	$2.71 \pm 0.74 \times 10^7$
4	$5.50 \pm 0.74 \times 10^7$	$1.43 \pm 1.59 \times 10^7$	$4.61 \pm 1.75 \times 10^7$	$2.49 \pm 0.34 \times 10^7$	$3.06 \pm 1.61 \times 10^7$
5	$1.00 \pm 0.31 \times 10^8$	$4.54 \pm 0.16 \times 10^7$	$5.15 \pm 1.66 \times 10^7$	$2.28 \pm 1.15 \times 10^7$	$3.19 \pm 1.22 \times 10^7$

References

- [1] Minekus, M.; Alminger, M.; Alvito, P.; Ballance, S.; Bohn, T.; Bourlieu, C.; Carrière, F.; Boutrou, R.; Corredig, M.; Dupont, D.; et al. A Standardised Static in Vitro Digestion Method Suitable for Food – an International Consensus. *Food & Function* 2014, 5, 1113–1124, doi:10.1039/C3FO60702J.