Evaluation of microencapsulation impact on *Akkermansia muciniphila*' culturability during freeze-drying, storage under different temperatures and atmospheric conditions, and *in vitro* gastrointestinal passage

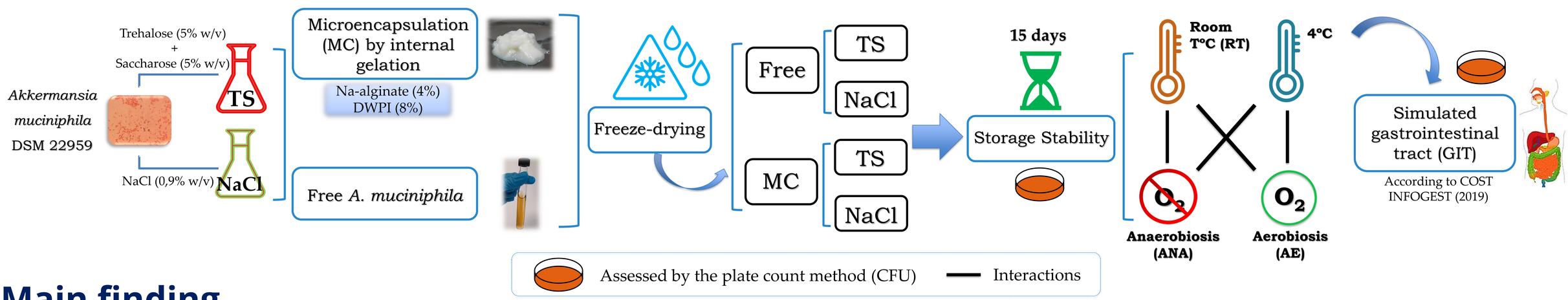
Diana Almeida[a]; Rita Vedor[a]; Joana Cristina Barbosa[a]; Daniela Machado[a]; José Andrade[b]; Ana Maria Gomes[a*]; Ana Cristina Freitas[a]
*Corresponding author: amgomes@ucp.pt

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

Background

With the surge of next-generation technologies the scientific community awareness to gut ecosystem importance on human health has been increasing. In the context of inflammatory and cardio-metabolic disorders, which have major clinical/economic impact [1], the gut resident *Akkermansia muciniphila* emerges as a next-generation probiotic, due to its potential in their prevention/treatment [2][3]. However, the high sensitivity to acidic conditions and its aerotolerant metabolism hampers functional foods/nutraceuticals development [4]. To overcome such challenges, a combination of two cryoprotective agents was evaluated on the protection of microencapsulated *A. muciniphila* when exposed to detrimental conditions.

Methodology



Main finding

During and after freeze-drying cryoprotective solutions offer considerable protection to *A. muciniphila* viability, particularly during gastrointestinal passage while microencapsulation does not.

Results & Discussion

Table 1 - Viability of pre-freeze-dried microencapsulated (CFU/g) and free (CFU/mL) *A. muciniphila* DSM 22959 and post freeze-drying (CFU/g). Data is shown as the mean ± standard deviation (n = 6).

	MC (TS)	MC (NaCl)	Free (TS)	Free (NaCl)
Pre-Freeze-Drying	$(3.02 \pm 2.52) \times 10^9$	$(2.28 \pm 1.72) \times 10^9$	$(2.10 \pm 1.63) \times 10^{10}$	$(3.23 \pm 2.34) \times 10^{10}$
Post-Freeze-Drying (CFU/g)	$(1.32 \pm 0.97) \times 10^8$	$(2.94 \pm 2.79) \times 10^7$	$(1.78 \pm 2.10) \times 10^{11}$	$(5.52 \pm 5.38) \times 10^8$

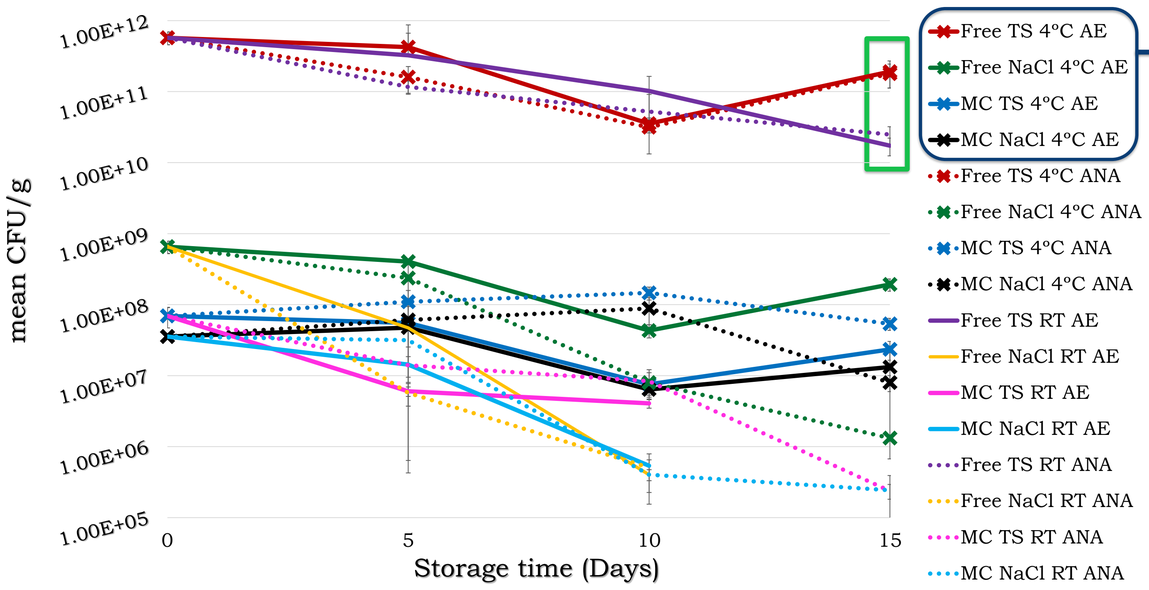


Fig. 1 - Survival of freeze-dried free and microencapsulated (MC) *A. muciniphila* cells throughout 15 days when stored aerobically (AE) and anaerobically (ANA) at 4 ºC and room temperature (RT). TS, trehalose (5% w/v) + saccharose (5% w/v); NaCl, NaCl (0,9% w/v). Straight line (aerobiosis); Dotted line (anaerobiosis); Marker (4ºC); No marker (room temperature).

For microencapsulated *A. muciniphila* cells, TS solution revealed a similar protective effect to that of NaCl during freeze-drying (1.3×10^8 vs 2.9×10^7 CFU/g, respectively), and after 15 days under refrigerated aerobiosis.

Interestingly, free *A. muciniphila* in TS solution exhibited better stability during freeze-drying and, after 15 days under all tested storage conditions (>10^{10} CFU/g of lyophilizate).

Microencapsulation does not seem to offer additional protection *to A. muciniphila* viability when the cells are submitted to freeze-drying stress. However, incorporation of a 10%(w/v) cryoprotectant solution, composed of equal parts of trehalose and saccharose, considerably minimizes the impact of the drying method.

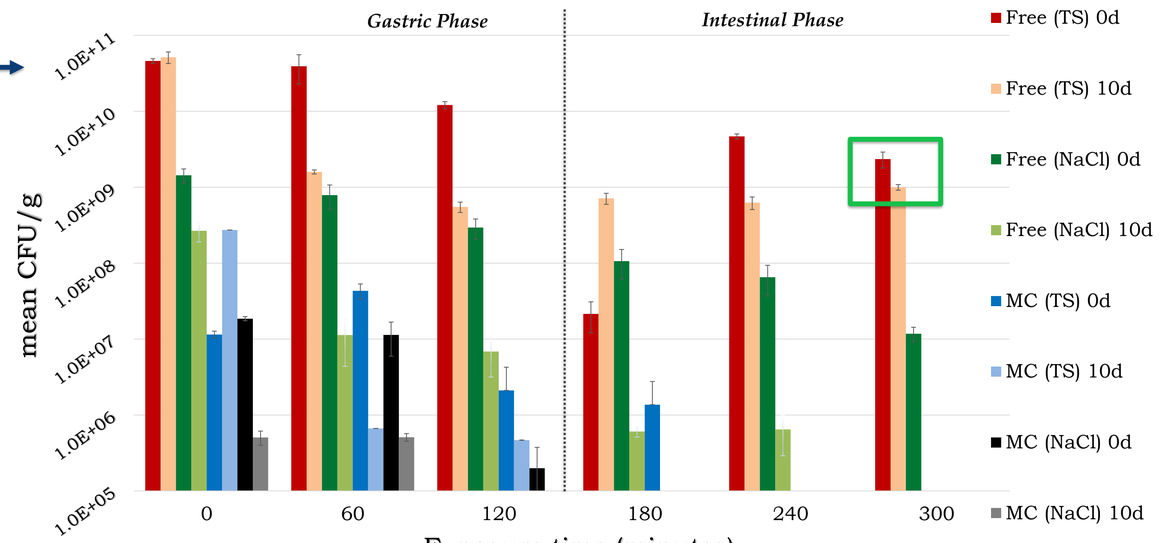


Fig. 2 - Comparison of the evolution of freeze-dried free and microencapsulated (MC) *A. muciniphila* cells throughout simulated gastrointestinal conditions, before and after 10 days of storage at 4ºC in aerobiosis. TS, trehalose (5% w/v) + saccharose (5% w/v); NaCl, NaCl (0,9% w/v).

During the simulated *in vitro* GIT passage TS solution improved free *A. muciniphila* survival, as its viability remained at desired levels (>10^9 CFU/g), even after 10 days of storage under refrigerated aerobiosis.

References

[1] "Health Effects of Overweight and Obesity in 195 Countries over 25 Years,", GBD 2015 Obesity Collaborators et al., N. Engl. J. Med., 377(1):13–27, (2017).
[2] "Gut microbiota — at the intersection of everything?," P. D. Cani, Nat. Rev. Gastroenterol. Hepatol., 14(6):321–322, (2017).
[3] "Evolving trends in next-generation probiotics: a 5W1H perspective," D. Almeida, D. Machado, J. C. Andrade, S. Mendo, A. M. Gomes, and A. C. Freitas, Crit. Rev. Food Sci. Nutr., 60(11):1783-1796, (2019).
[4] "Uncovering Akkermansia muciniphila resilience or susceptibility to different temperatures, atmospheres and gastrointestinal conditions," D. Machado, D. Almeida, C. L. Seabra, J. C. Andrade, A. M. Gomes, and A. C. Freitas, Anaerobe, 61:1–14, (2019).

Acknowledgements

This work was supported by national funds through FCT/MEC (PIDDAC), project references IF/00588/2015, by the Scientific Employment Stimulus - Individual Call (CEEC Individual) - CEECIND/00520/2017/CP1404/CT0001, and by the Operational Program Competitiveness and Internationalization in its FEDER component and by the budget of the Foundation for Science and Technology, I.P. (FCT, IP) in its OE component, project reference POCI-01-0145-FEDER-031400 – PTDC/BAA-AGR/31400/2017. We would also like to thank the scientific collaboration under the FCT project UIDB/50016/2020.