EVALUATION OF MICROENCAPSULATION IMPACT ON AKKERMANSIA MUCINIPHILAS' CULTURABILITY DURING FREEZE-DRYING, STORAGE UNDER DIFFERENT TEMPERATURES AND ATMOSPHERIC CONDITIONS, AND IN VITRO GASTROINTESTINAL PASSAGE

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Almeida, Diana (Portugal)[1]; Vedor, Rita (Portugal)[1]; Barbosa, Joana (Portugal)[1]; Machado, Daniela (Portugal)[1]; Andrade, José (Portugal)[2]; Gomes, Ana Maria (Portugal)[1]; Freitas, Ana Cristina (Portugal)[1]

1 - Escola Superior de Biotecnologia (ESB) - Universidade Católica Portuguesa (UCP); 2 - University Institute of Health Sciences (CESPU)

Body

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 intestinal 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. *Akkermansia muciniphila* (DSM 22959) was microencapsulated – by emulsification/internal gelation method – in Na-alginate (4%), CaCO3 (5%) and denatured whey protein isolate (DWPI; 8%) matrix, in which a trehalose (5%) plus saccharose (5%) (T+S) solution was tested for its cryoprotective impact, versus NaCl (0.9%). Upon freezing at -80ºC, microencapsulated cells were freeze-dried for 48h. Viability of freeze-dried encapsulated *A. muciniphila* during storage at 4ºC and 22ºC, in aerobic and anaerobic conditions was compared for 15 days with that of freeze-dried free cells and assessed using total cultivable cell numbers. Also, stored formulations in refrigerated aerobiosis were evaluated in simulated gastrointestinal (GIT) passage. *Akkermansia muciniphila* was efficiently encapsulated in an alginate:DWPI matrix (93.3 ± 2.8%). As for microencapsulated *A. muciniphila* cells, T+S solution revealed a similar protective effect to that of NaCl during freeze-drying ($1.3x10^8$ vs $2.9x10^7$ CFU/g, respectively), and after 15 days in refrigerated aerobiosis. Interestingly, free *A. muciniphila* in T+S solution exhibited better stability during freeze-drying and, after 15 days under all tested storage conditions (>10^{10} CFU/g of lyophilizate). During the simulated *in vitro* GIT passage T+S solution improved free *A. muciniphila* survival, as its viability remained at desired levels (>10^9 CFU/g), even after 10 days of storage in refrigerated aerobiosis. Overall, microencapsulation appears to evoke susceptibility in *A. muciniphila* but using cryoprotectant solutions such as trehalose and saccharose significantly protects probiotic cells viability from freeze-drying and subsequent exposure to aerobic, non-refrigerated environments, as to *in vitro* simulated GIT transit.

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