FORMULATION STRATEGIES FOR ENHANCING GROWTH OF *AKKERMANSIA MUCINIPHILA* AND ITS SURVIVAL THROUGH LYOPHILISATION AND STORAGE AT AIR AMBIENT

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Introduction:
Gut bacterium *Akkermansia muciniphila* has been recognized as a new potential probiotic due to promising outcomes in the prevention and treatment of several human diseases. However, despite its multiple beneficial effects, its viability is compromised by the presence of oxygen. This has so far precluded its application namely in the development of nutraceutical/therapeutic formulations. In order to enhance growth of *A. muciniphila* and its survival through lyophilisation and storage at air ambient, formulation strategies are presented based on use of prebiotic compounds, antioxidants and cryoprotectants. According to our best knowledge, there is no information regarding lyophilisation procedures involving *A. muciniphila* as well as its subsequent storage at ambient air.
Methods:
Akkermansia muciniphila (DSM 22959) was initially grown in PYGM at 37ºC/24h, under anaerobic conditions (80% N2/10% H2/10 %CO2). Growth in the presence of prebiotics: Bacterial suspension (300 µL) was inoculated in 30 mL of PYGM medium with or without inulin or FOS (2.5-5.0%, w/v) and incubated at 37ºC/42h under anaerobic conditions. After incubation, optical density at 600 nm, number of viable cells (CFU/mL) and biomass (g/mL) were determined. Lyophilisation in antioxidants/cryoprotectants based formulations: Bacterial pellets from growth in presence or absence of inulin or FOS were re-suspended in 200 µL of riboflavin (16.5 mM) and 400 µL of inulin (10%) with or without 0.2% (w/v) cysteine or glutathione. Upon freezing at -80 ºC, all formulations were lyophilised for 24h. Viable cells through storage at ambient air: After lyophilisation, freeze-dried granules were exposed to atmospheric air at room temperature (22ºC) for 0, 10 and 24h as well as to anaerobic conditions.
Results:
The incorporation of inulin or FOS in growth media did not increment the number of viable cells of *A. muciniphila* (10^6 CFU/mL). After lyophilisation, 50-60 mg of freeze-dried granules was obtained from biomass grown in PYGM and PYGM with 2.5% of inulin, respectively. The exposure of freeze-dried granules to air ambient at room temperature for 24h maintained the number of viable cells of *A. muciniphila* practically constant and similar to those after 24h exposure to anaerobic atmosphere.
Discussion:
These results are very promising from a technological point of view because although *A. muciniphila* is being considered tolerant to oxygen, capable to profit from nanomolar concentrations of oxygen [1], atmospheric oxygen levels are detrimental provoking for example 20% reduction of *A. muciniphila* viability after only 1 hr of exposure [2]. Thus, these findings indicate that cells of *A. muciniphila* can be kept alive at ambient air at least for 24h in the form of freeze-dried granules containing inulin at 10%, riboflavin at 16.5mM and glutathione at 0.2%. While riboflavin and glutathione were used because they can act as antioxidants and redox mediators to shuttle electrons oxygen, favouring the survival of anaerobic bacteria under oxygenized conditions, inulin was included in the formulation due to its cryoprotectant and prebiotic properties.
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References
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We have included the Acknowledgments at the end of Discussion because is obligatory to us to include the funded projects behind the presented research.

Keywords: Akkermansia muciniphila, Formulations, Lyophilisation, Storage, Survival