TITLE: Probiotic or pathobiont: *Akkermansia muciniphila* induces a-synuclein aggregation in enteroendocrine cells *in vitro* and *in vivo*.

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ABSTRACT:

Since its discovery nearly two decades ago, *Akkermansia muciniphila* (Akk) has been used as a next generation probiotic. However, in patients with Idiopathic Parkinson's Disease (iPD) it has been consistently reported Akk increased population in their dysbiotic gut microbiota. However, whether this bacterium might contribute to the etiology of iPD remains unknown. Enteroendocrine cells (EEC) are intestinal cells featuring neuron-like properties that synaptically connects with sympathetic enteric neurons and constitutively expressing the protein α-synuclein (αSyn) that once aggregated become a hallmark of neurotoxicity in a great proportion of iPD cases. In addition, it has been reported intestinal aSyn aggregation even before its misfolding in the brain. Since they face the intestinal lumen, these cells are directly subject to stimuli from the gut microbiota. Therefore, here we aimed to investigate the effects of Akk in aSyn homeostasis in an EEC line, STC-1, and subsequently *in vivo* using a murine SPF model. Akk secretome was obtained by culturing Akk in BHI media (BHICM) with and without mucin in anaerobiosis at 37°C. Unconditioned media with and without mucin were used as negative controls (BHI). Supernatants were concentrated using 3kDa Centricons. Then, BHICM and BHI were used to stimulate STC-1 cells. Cellular and molecular changes were analyzed by IMF assays, western blotting and confocal microscopy. Our results show that BHI did not induce significant cells responses. BHICM promoted increased cytosolic calcium (Ca^{2+}) signaling by directly activation of RyR receptors. In addition, such increased Ca^{2+}signaling led to mitochondrial (mt) injury due to excessive Ca^{2+mt} uptake causing reduction of mt membrane potential and ROS production. These events were followed by increased expression, phosphorylation and aggregation of αSyn in STC-1 cells. However, Akk secretome-induced cellular injury was efficiently reverted by buffering Ca^{2+mt} using parvalbumin. Finally, after orally administering viable Akk during a 28-consecutive-day treatment in aged mice, we found that cholecystokinin-positive EECs in the mouse ileum exhibited high levels of aSyn-aggregates compared to control. However, no motor changes between the two groups after treatment were observed. In summary, we demonstrated that the Akk secretome and the bacterium itself were able to induce the emergence of aSyn aggregation in EECs *in vitro* and *in vivo*, connecting the gut-brain axis in iPD.

Keywords: Parkinson's disease; alpha-synuclein; gut microbiome; gut-brain; Akkermansia

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