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Strict anaerobic bacteria involvement in pain and well-being: Novel probiotics strategies for the modulation of the gut-brain axis

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Strict anaerobic bacteria involvement in pain and well-being: Novel probiotics strategies for the modulation of the gut-brain axis

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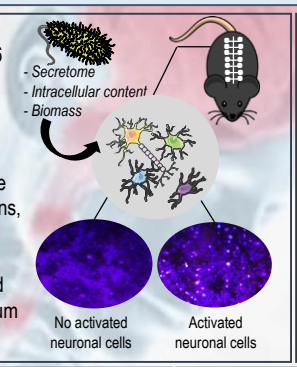
Introduction : The potential role of gut microbiota in visceral pain modulation arouses an emerging interest during recent years. The composition of the gut microbiota could have an influence on the neurological component, both on behavior and pain processing. This could be due to an indirect communication *per* immune response or direct host/microbiota interaction involving activation of nociceptive neurons by metabolites. Although probiotics are recommended for the treatment of visceral pain, mechanisms involved in bacteria/host interaction are not well understood. Commensal anaerobic bacteria constitute a novel target of investigation in the field of probiotics. Several bacterial species or strains of gut microbiota may have beneficial effects. This is the case of *Faecalibacterium prausnitzii* which exhibits anti-nociceptive properties. The aim of this study was to highlight and characterize potential interactions between other commensal anaerobic bacteria and neuronal cells.

Material and Methods (1) : Seven intestinal anaerobic bacterial strains (6 reference strains and 1 strain isolated from healthy donor) were cultivated before being lysed to separate soluble intracellular bacterial fractions from insoluble membrane parts. To study the neuromodulator potential of these strains, bacterial fractions were applied on primary culture of neuronal cells from mouse Dorsal Root Ganglia (DRG) and neuronal activity was followed by calcium imaging.



Results (1)* :

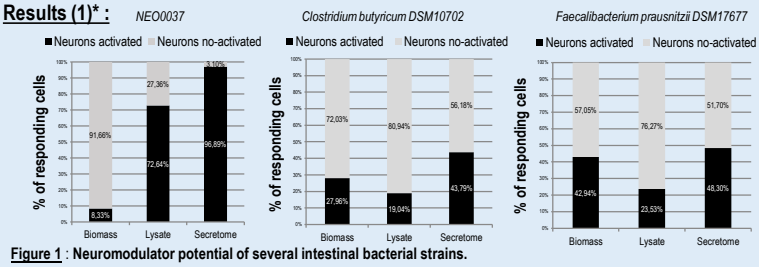


Figure 1 : **Neuromodulator potential of several intestinal bacterial strains.**
In addition to revealing a direct interaction between bacteria and neuronal cells, different levels of activation are observed between strains but also within the same strain according to the tested fraction.

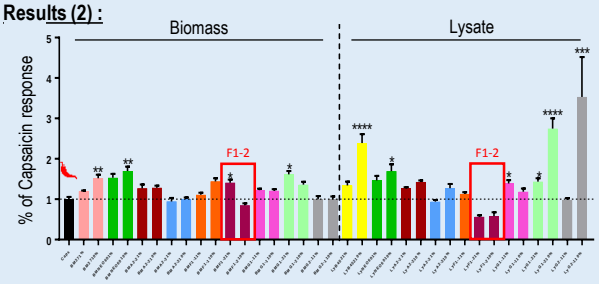
Other tested strains : *Roseburia hominis* (DSM16839), *Bacteroides thetaiotaomicron* (DSM2255), *Roseburia intestinalis* (DSM14610), *Bacteroides fragilis* (DSM1396)

**All strains tested are not presented here.*

Material and Methods (2) : After isolating strict anaerobic bacterial strains from healthy donors, these strains have been cultivated. Intracellular content (lysate) and membrane fraction (biomass) have been tested for their potential to inhibit neuronal activation under Capsaicin stimulation thanks to high-throughput screening method. Neuronal cells from DRG rat have been incubated with bacterial fractions before stimulated by Capsaicin (4µM). Intensity of neuronal activation have been compared.

Statistical analysis : non parametric test Krustal-Wallis to evaluate differences between groups, and Post-hoc Dunn's test for multiple comparisons.

Results (2) :



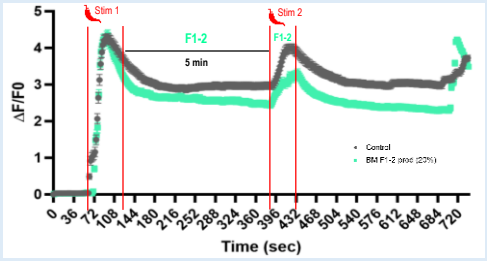
Diverse responses are observed. While some strains seem to increase neuronal activation under Capsaicin stimulation, the strain F1-2 seems to have positive effect with potential neuroprotective effect.

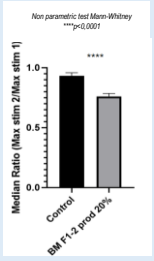
Post-hoc Dunn's test
p<0,05;p<0,01; ***p<0,001; ****p<0,0001*

Figure 2 : **Neuroprotective effects of strict anaerobic bacterial fractions isolated form healthy donor, under Capsaicin stimulation**.

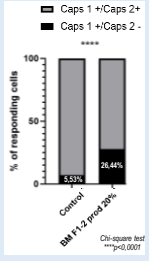
Material and Methods (3) : The biomass of strain F1-2 was tested for its neuroprotective effect on neuronal cells from DRG mice by calcium imaging. This technique allows single cell and sub populations analysis. After a first stimulation with Capsaicin (100nM), neuronal cells were incubated with F1-2 biomass during 5 min before a second stimulation with Capsaicin + F1-2 biomass.

Results (3) :





1) Decrease of intensity response to the second stimulation

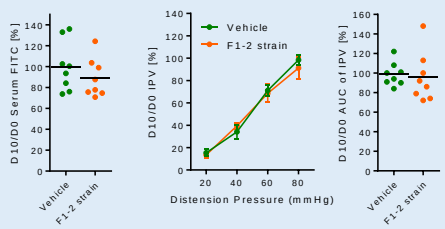


2) Increase of % of cells no responding to the second stimulation

Figure 3 : **Neuroprotective effect of F1-2 strain under Capsaicin stimulation.**

Material and Methods (4) *(in vivo)* : C57BL/6 pups were separated from their mother between day 2 and day 14, 3 hours per day in order to induce chronic stress. At the age of 8-10 weeks, colonic sensitivity of neonatal maternal separated (NMS) mice were assessed by colorectal distension (CRD). The NMS mice with colonic hypersensitivity were then gavaged for 10 days (with vehicle or F1-2 strain) and their colonic sensitivity were assessed. The CRD were performed using a barostat to apply constant pressure coupled with intracolonic pressure sensors to evaluate the response to CRD. In addition, intestinal permeability were also assessed before and after gavage treatment using the Dextran-FITC (4kDa) gavage protocol.

Results (4) :



The intestinal permeability and intracolonic pressure variations (IPV) were measured in NMS mice before and after treatment with vehicle or F1-2 strain by gavage. The intestinal permeability were established to 100% for each mice before treatment. Similarly, IPV for each mouse were established to 100% for the response to the 100 mmHg CRD before treatment.

Figure 4 : **The F1-2 strain exhibited no *in vivo* effect on intestinal permeability or colonic hypersensitivity in a NMS paradigm model.**

Conclusion : These preliminary data have shown that anaerobic bacterial content can modulate the activity of primary culture neurons *in vitro*. Further studies are necessary to characterize these interactions, and to evaluate the ability of some bacterial fractions cross the intestinal barrier to rich peripheral and central nervous system. Other *in vivo* models in which intestinal permeability is more affected could be used to evaluate bacterial effects on pain processing, well-being and behavior component.