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Positive interactions between lactic acid bacteria promoted by nitrogen-based nutritional dependencies

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CONTEXT & AIM

- ✓ Lactic acid bacteria (LAB) are associated and interact in fermented food products but the mechanisms underlying their interactions have rarely been investigated in depth.
- ✓ **Nutritional dependencies**, especially those regarding **nitrogen sources**, govern many microbial **positive interactions** (Canon *et al.*, 2020).
- ✓ This study aims to investigate the exploitation of the proteolytic activity and amino acid auxotrophies of LAB strains **to promote positive interactions between proteolytic ("donors") and non-proteolytic "receivers" strains.**

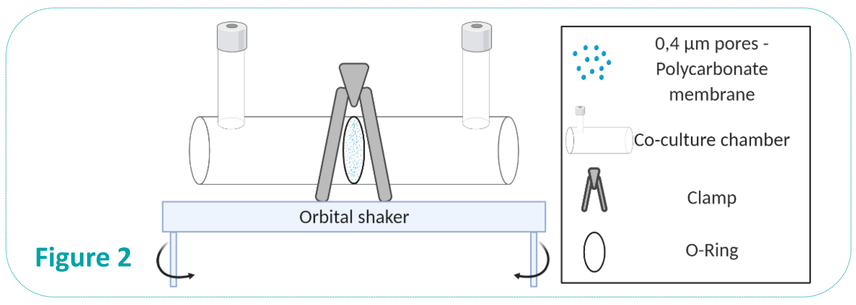


Figure 2

STRATEGY

- ✓ Selection of six LAB strains:
  3 **donors** : proteolytic activity + volatile compounds production + lactose consumption
  3 **receivers** : no proteolytic activity + hydrolysis of raffinose family oligosaccharides
- ✓ Development of a chemically defined medium containing caseins and lupine proteins as sole nitrogen sources (growth of proteolytic strains only)
- ✓ **Association** of pairs of donor/receiver strains to **favour positive interactions** (Figure 1)

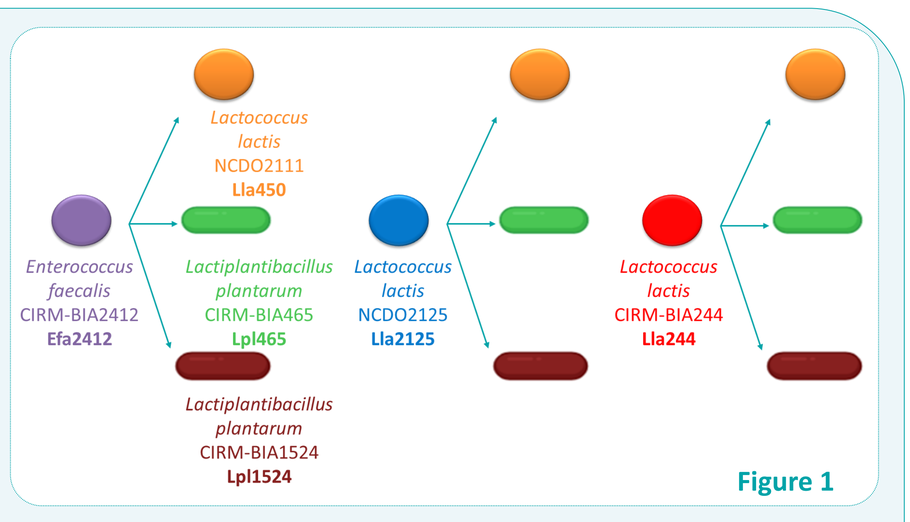
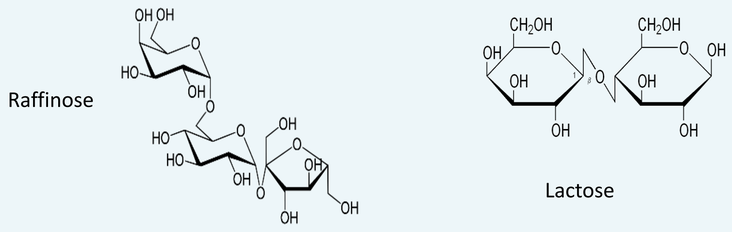


Figure 1

- ✓ **Growth of each pair strains in compartmented chambers** (Figure 2) to facilitate bacterial growth monitoring at 30 °C for 24 h, orbital shaking 65 rpm
- ✓ Characterization of the **resulting functional outputs**:
  - **Carbohydrate** consumption, quantified by anion exchange chromatography
  - **Volatile compound** production, analysed by heas space GC-MS



RESULTS

Bacterial growth

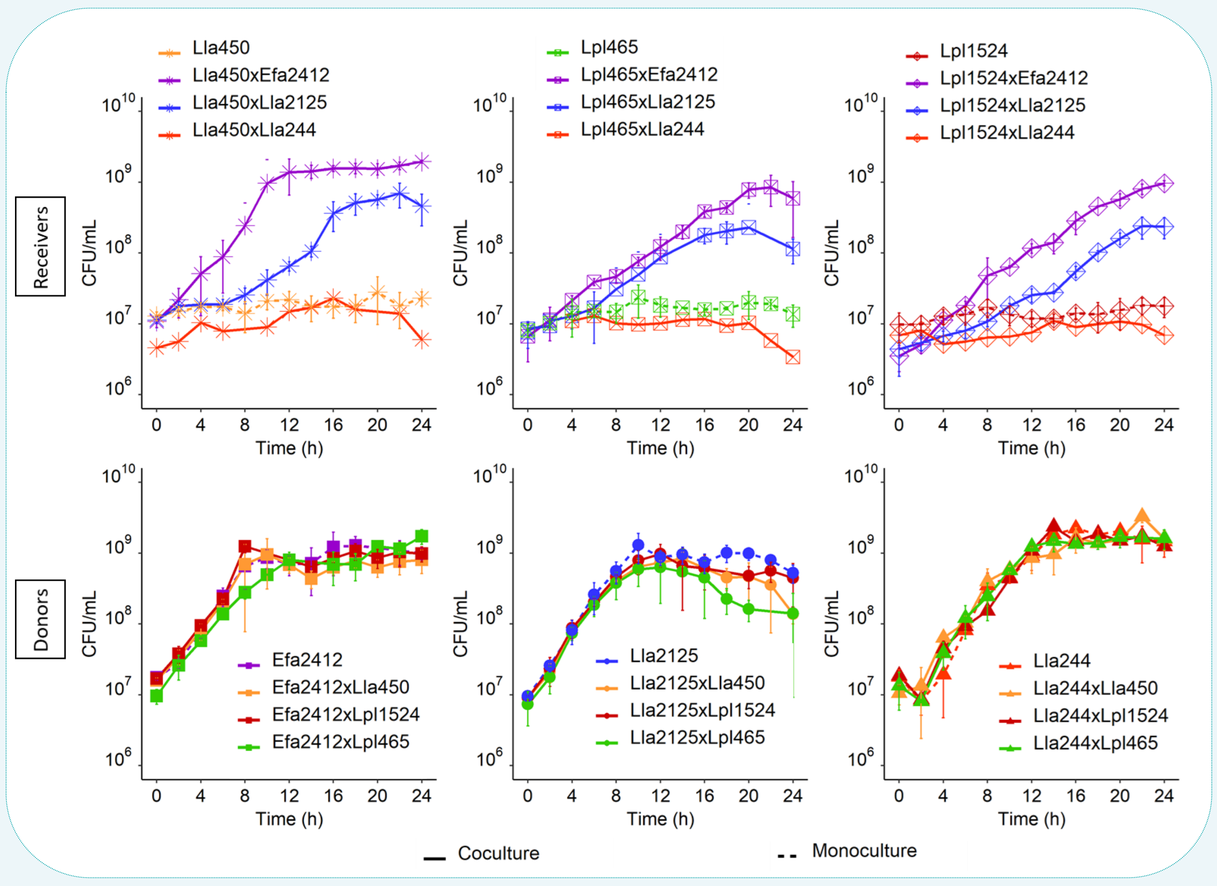


Figure 3 **Three different donor stains resulted in three different types of interactions**

- ✓ **No growth** of the three **receiver** strains in monoculture, as expected
- ✓ The three **receiver** strains grew in co-cultures with Efa2412 and Lla2125, but not with Lla244
- ✓ Three types of interactions can be distinguished: strong interactions with Efa2412, weak interactions with Lla2125, and no interaction with Lla244.

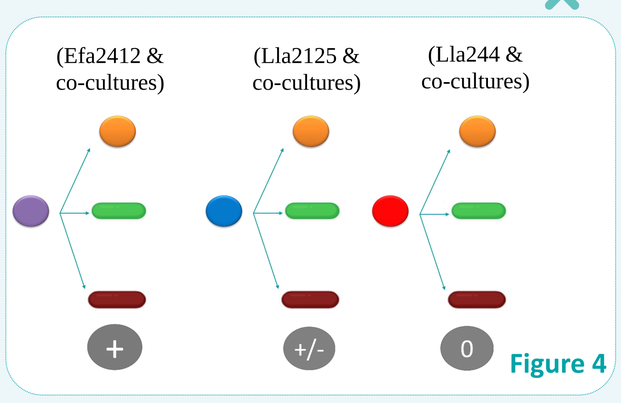


Figure 4

Carbohydrate consumption

Lactose consumption,%

	Efa2412 (+)	Lla2125 (+/-)	Lla244 (0)
∅	-72.4^{c}	-91.9^{a}	-63.9^{cd}
Lla450	-30.0^{e}	-81.5^{b}	-62.6^{d}
Lpl465	-62.9^{d}	-90.2^{ab}	-63.5^{d}
Lpl1524	-86.2^{ab}	-94.6^{a}	-66.6^{cd}

Raffinose consumption, %

	Efa2412 (+)	Lla2125 (+/-)	Lla244 (0)
∅	-6.2^{b}	-7.1^{b}	-6.8^{b}
Lla450	-13.4^{ab}	-10.1^{ab}	-6.4^{b}
Lpl465	-14.5^{ab}	-8.6^{b}	-6.5^{b}
Lpl1524	-20.1^{a}	-10.6^{ab}	-12.0^{ab}

Increase/Decrease compared to the monoculture of the donor

- ✓ Co-cultures influenced lactose and raffinose consumption, especially when the strains exhibited strong interactions

Volatile compounds

- Ratio culture:control calculated for each volatile compound identified

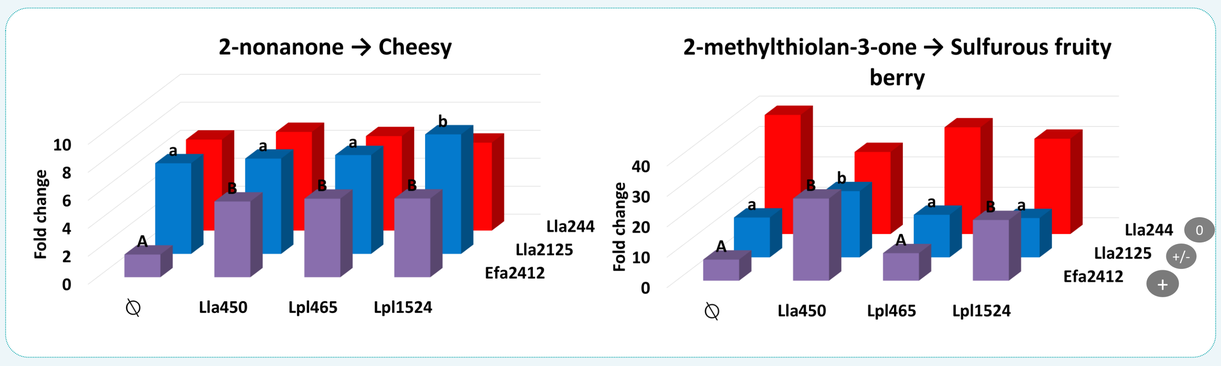


Figure 5 **Co-cultures increased the concentrations of volatile compounds associated with desirable flavours**

- ✓ Co-cultures influenced volatile compounds production
- More differences were observed with stronger interactions
- Diacetyl (buttery), acetoin (milky), 2,3-pentanedione (buttery), benzaldehyde (nutty) concentrations also increased

Global overview

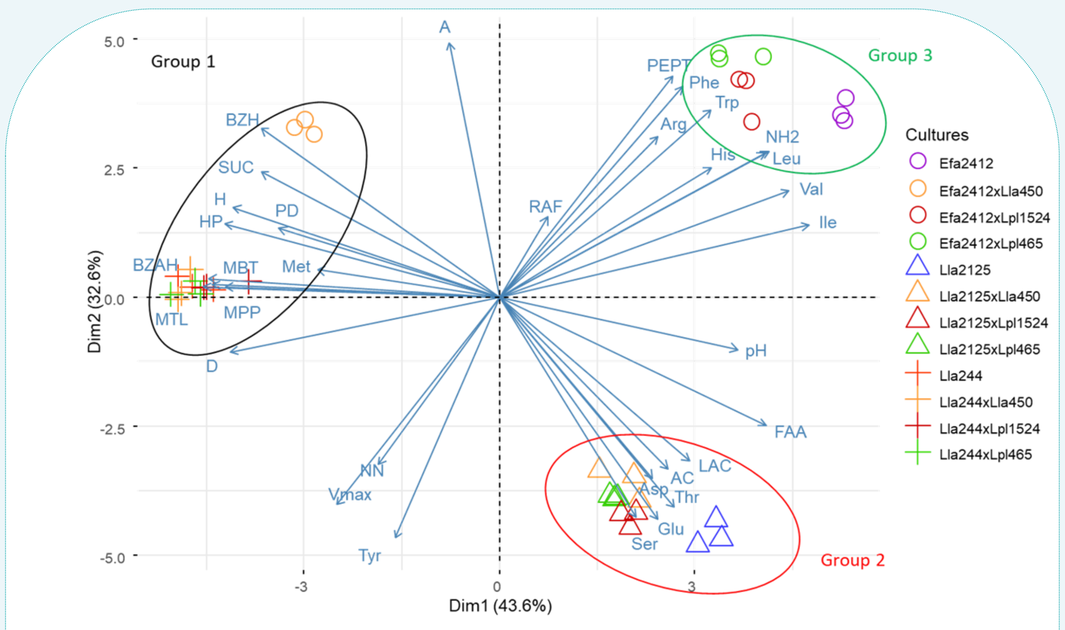


Figure 6 **PCA on the whole dataset**

- Vmax = maximal acidification rate, pH
- LAC/SUC/RAF = percentage of lactose, sucrose and raffinose consumed
- NH2 = free NH_2 groups concentration, FAA= free amino acids concentration, PEPT = peptides concentration
- H = hexanal, HP= heptanal, MBT= 3-methylbutanal, BZH = benzaldehyde, BZAH = benzene acetaldehyde, MTL = 2-methylthiolan-3-one, D= diacetyl, PD = 2,3-pentanedione, AC = acetoin, NN = 2-nonanone, A = acetic acid, MPP = 2-methyl-1-propanol.

- ✓ The proteolytic activity of Efa2412 (group 3), which led to strong positive interactions was characterised with higher concentrations in NH_2 compounds: more specifically in peptides, branched amino acids, Trp, Phe and Arg

CONCLUSION & PERSPECTIVES

- The proteolytic activity of LAB can **favour the growth** of non-proteolytic LAB
- All proteolytic activity are **not equally stimulating** : moderate activities such as for Lla2125 and Lla244 lead to weak or no interactions
- Positive interactions changed carbohydrate consumption and production of volatile compounds
- The study of the nitrogen compounds used by the receiver strains will be further investigated to understand how the proteolytic and non-proteolytic strains positively interact

Centre
Bretagne - Normandie

Canon, F., Nidelet, T., Guédon, E., Thierry, A., & Gagnaire, V. (2020). Understanding the mechanisms of positive microbial interactions that benefit lactic acid bacteria co-cultures. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.02088

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