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

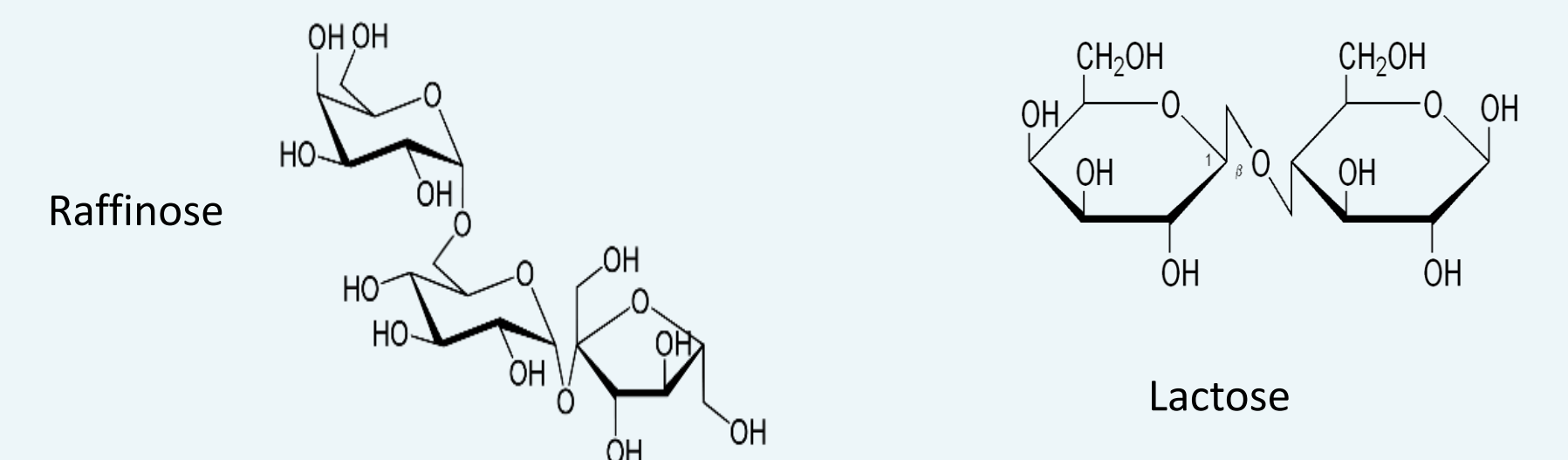
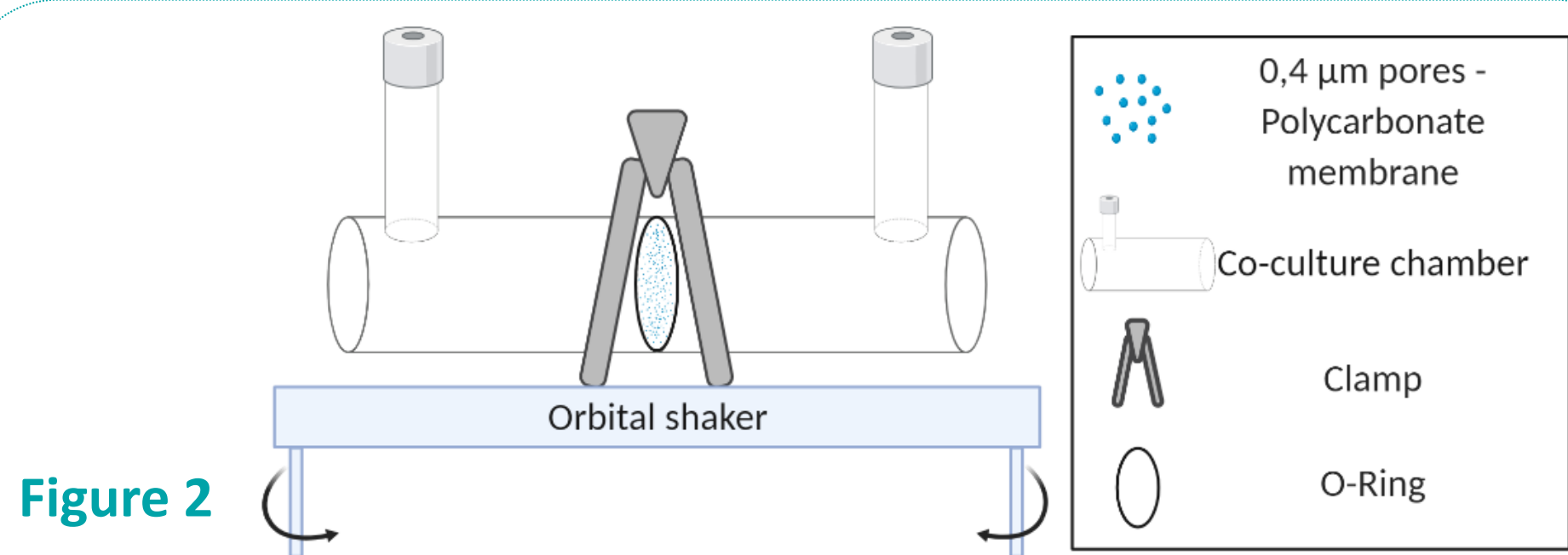
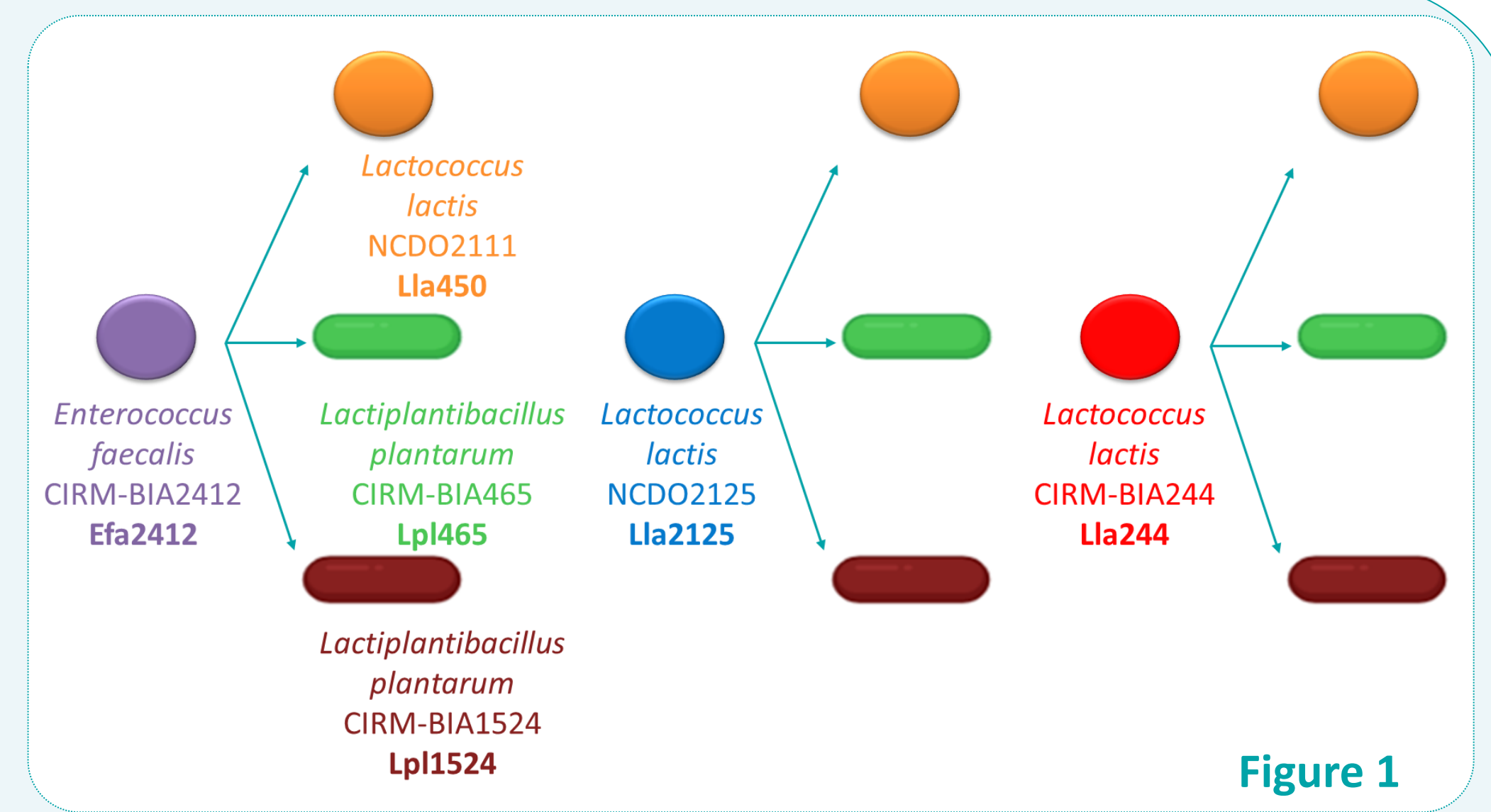
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CONTEXT & AIM

- ✓ 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.**

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)**
- ✓ **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 headspace GC-MS



Bacterial growth

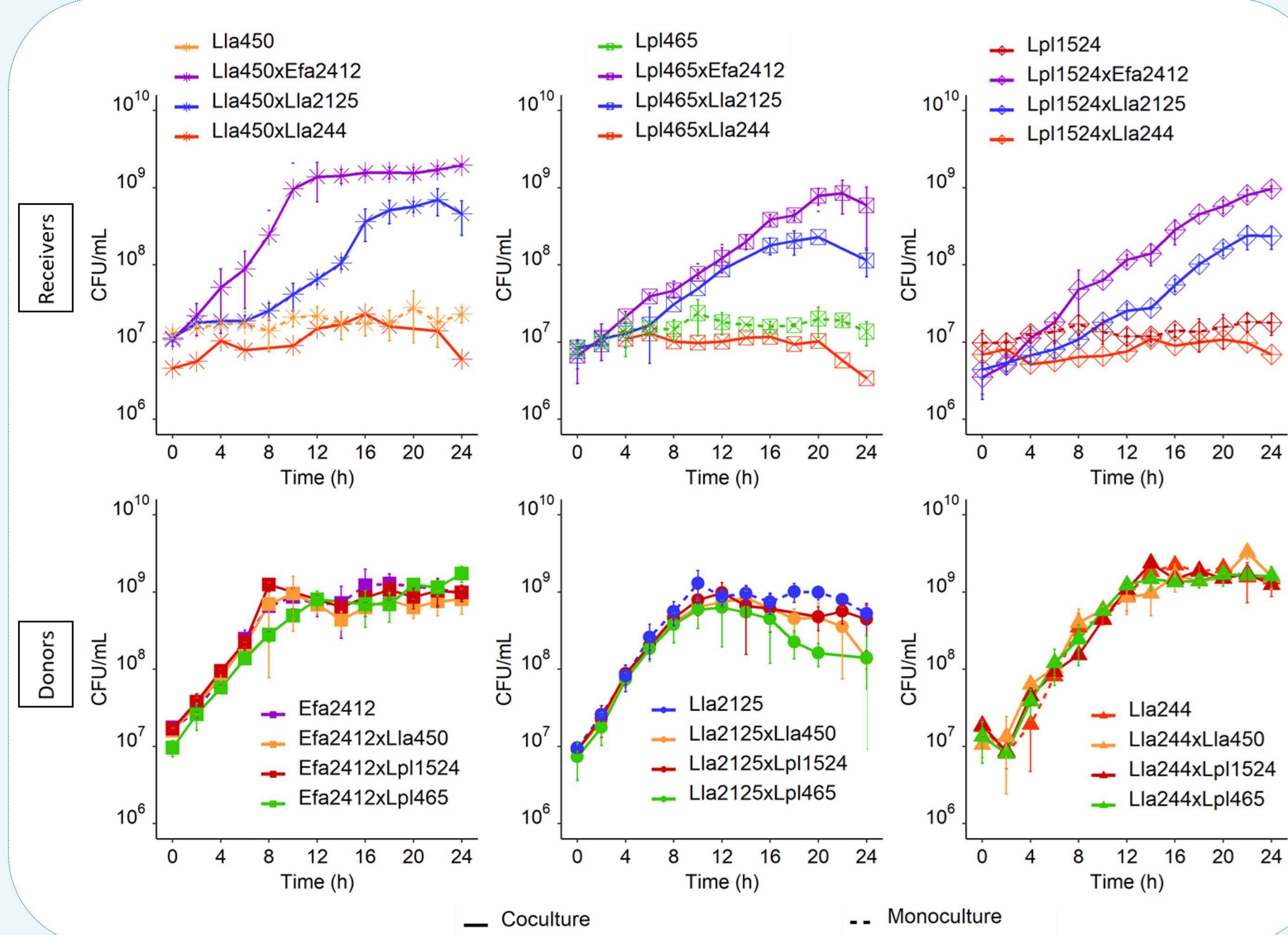
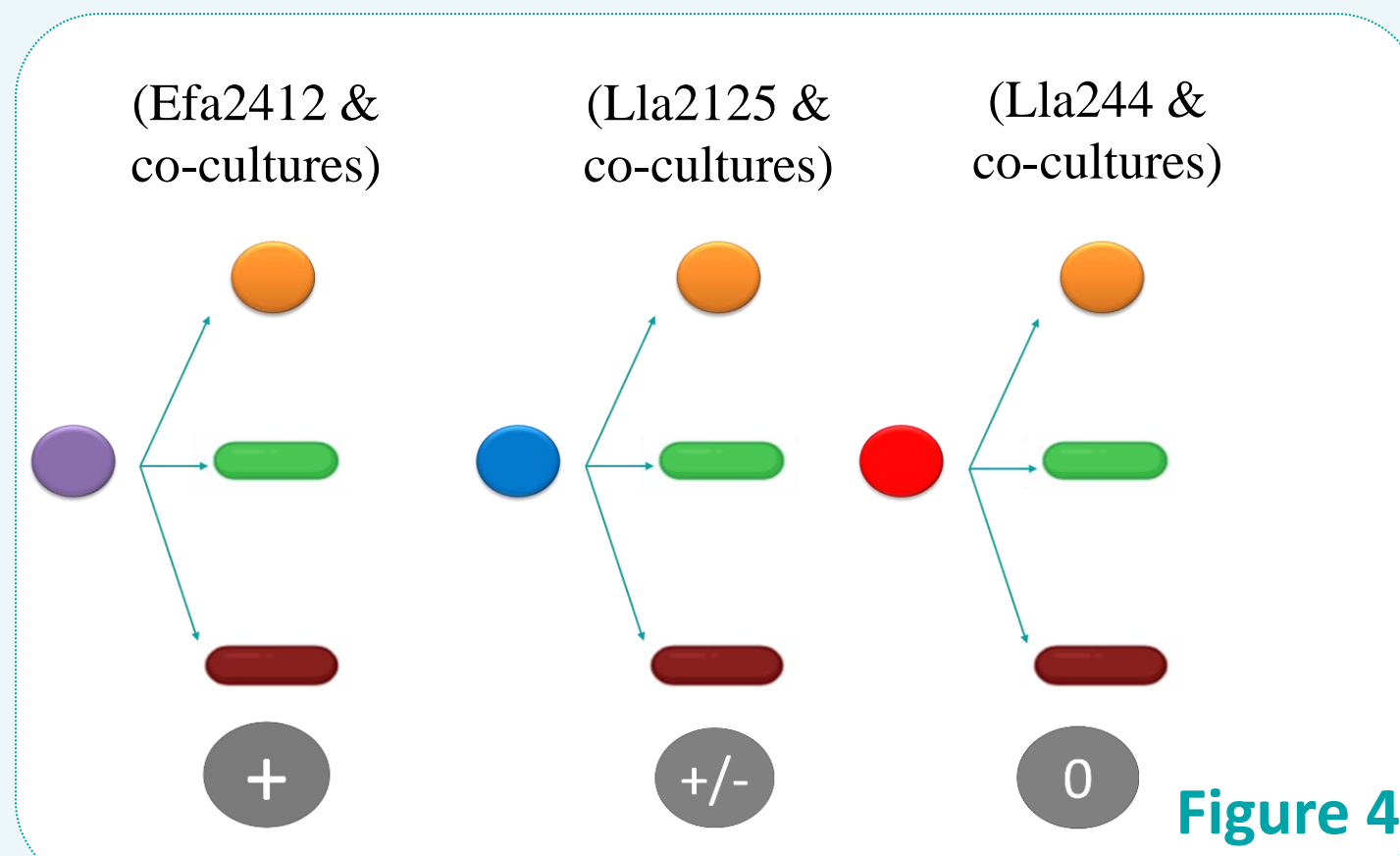


Figure 3 Three different donor stains resulted in three different types of interactions

- ✓ **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.



Carbohydrate consumption

	Lactose consumption, %			Raffinose consumption, %		
	Efa2412 (+)	Lla2125 (+/-)	Lla244 (0)	Efa2412 (+)	Lla2125 (+/-)	Lla244 (0)
☉	-72.4 ^c	-91.9 ^a	-63.9 ^{cd}	-6.2 ^b	-7.1 ^b	-6.8 ^b
Lla450	-30.0 ^e	-81.5 ^b	-62.6 ^d	-13.4 ^{ab}	-10.1 ^{ab}	-6.4 ^b
Lpl465	-62.9 ^d	-90.2 ^{ab}	-63.5 ^d	-14.5 ^{ab}	-8.6 ^b	-6.5 ^b
Lpl1524	-86.2 ^{ab}	-94.6 ^a	-66.6 ^{cd}	-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

RESULTS

Volatile compounds

- Ratio culture:control calculated for each volatile compound identified

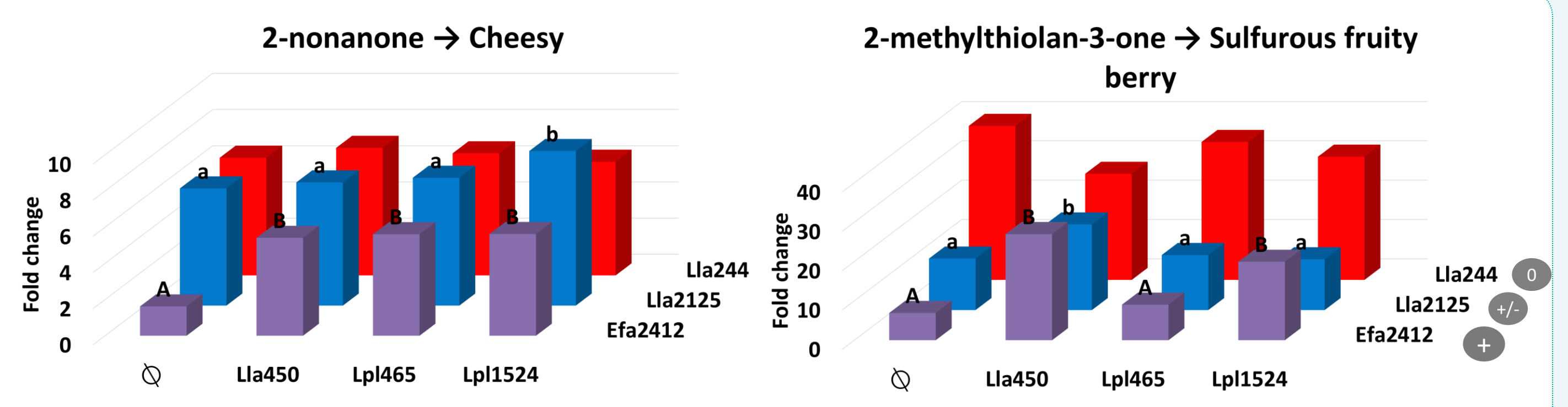


Figure 5 Co-cultures increased the concentrations of volatile compounds associated with desirable flavours

- ✓ 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

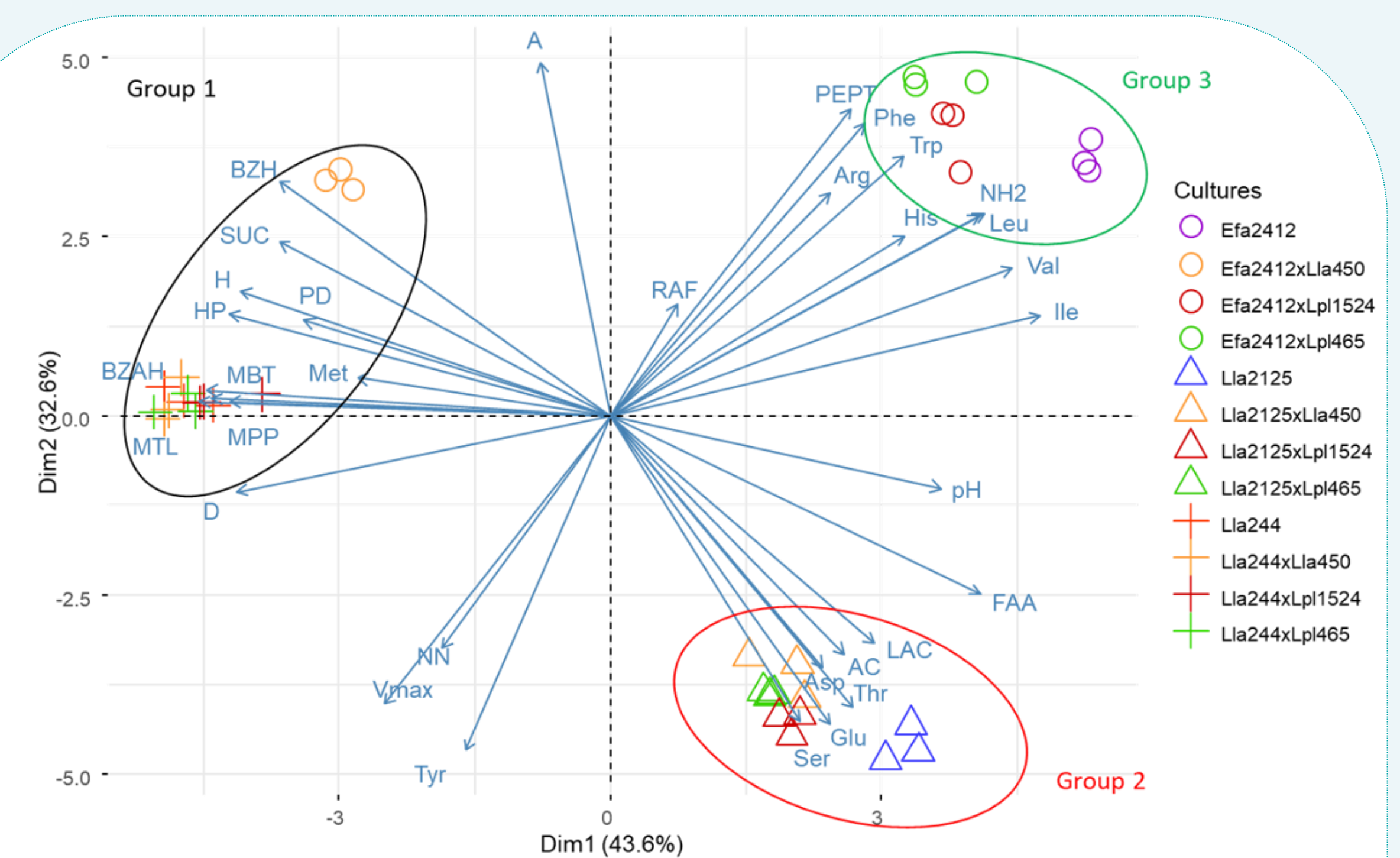


Figure 6 PCA on the whole dataset

- Vmax = maximal acidification rate, pH
- LAC/SUC/RAF = percentage of lactose, sucrose and raffinose consumed
- NH₂ = free NH₂ 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 NH₂ 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