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Positive interactions between lactic acid bacteria mediated by peptides containing branched-chain amino acids

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CONTEXT AND AIM

In a chemically defined medium composed of casein and lupin proteins as sole nitrogen sources, in which proteolytic and non-proteolytic lactic acid bacteria (LAB) strains grow in association, different interactions occurred, only impacting the receiving strains (Fig 1). They were mediated by the peptides and amino acids provided by the proteolytic strains. The aim of this study was to identify the characteristics of the peptides involved in these interactions.

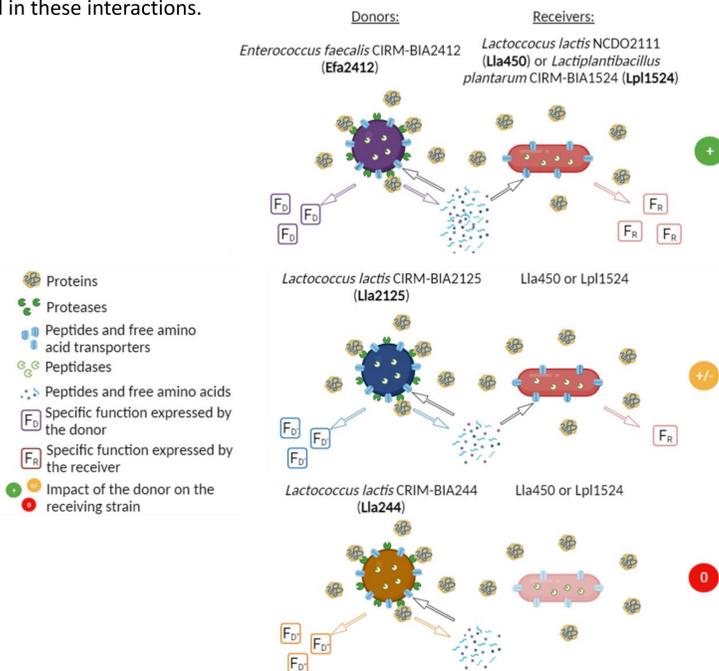


Figure 1 Three types of interactions observed between three proteolytic (=donor) lactic acid bacteria (LAB) strains in co-culture with non-proteolytic (=receiving) strains (Canon et al. 2021)

STRATEGY

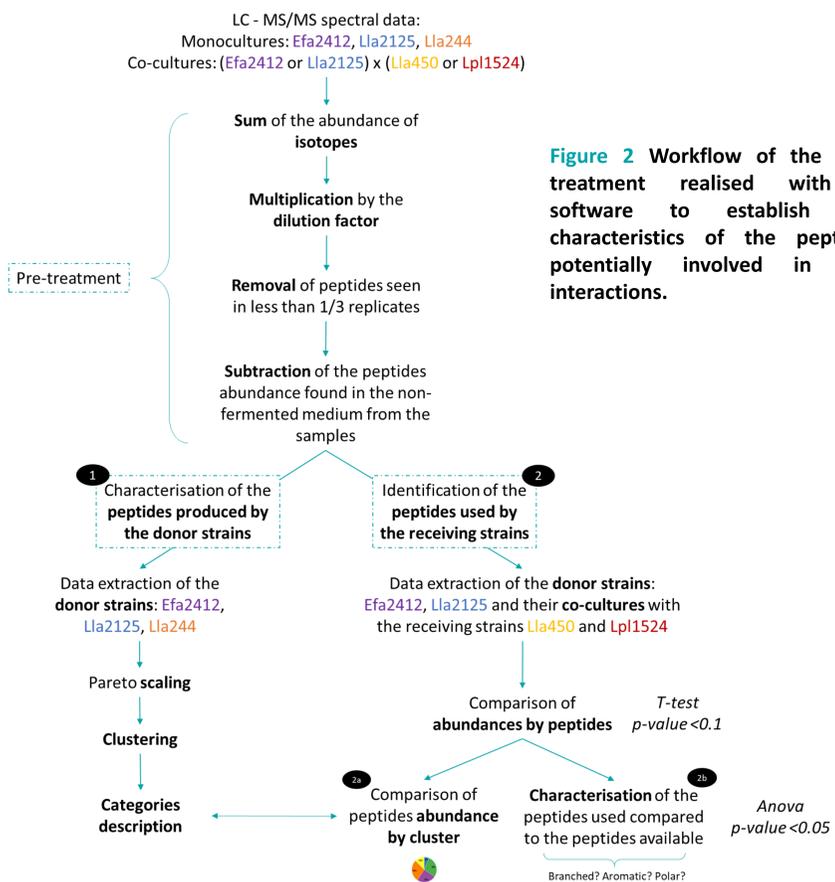


Figure 2 Workflow of the data treatment realised with R software to establish the characteristics of the peptides potentially involved in LAB interactions.

1 Characterisation of the peptides produced by the donor strains

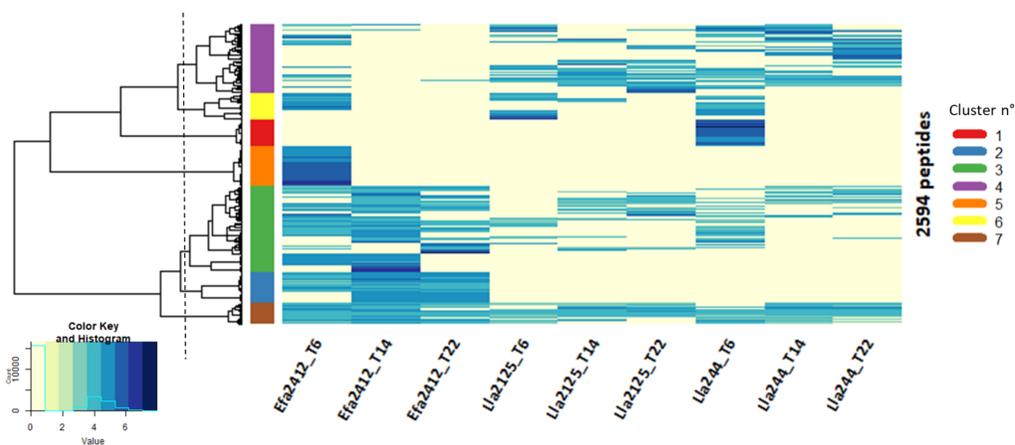


Figure 3 Heatmap of the abundance of the peptides produced by the three donor strains after 6, 14 and 22 h of culture

- ✓ Efa2412 differed from the two other donor strains by a higher concentration and diversity in peptides. The profiles of the two *L. lactis* strains were more similar (Fig 3).
- ✓ Seven clusters identified: clusters 5 and 2 specific to Efa2412; cluster 1 specific to Lla244; no specific cluster for Lla2125 (Fig 3).

Table 1 Description of the clusters according to peptide characteristics and amino acids composition

Category	Cluster n°							Counted amino acids
	1	2	3	4	5	6	7	
Molecular weight	High	Low	Low	Low	Low	Low	Low	
Isoelectric point	Low	Low	Low	Low	Low	Low	Low	
Essential amino acids for LAB (%)	Low	Low	High	Low	Low	Low	Low	R + I + L + V + N + W + Y + T + F + S + M + H + E
Non-polar side chain (%)	Low	Low	Low	Low	High	Low	Low	G + A + V + L + R + I
Polar non-charged side chain (%)	Low	Low	Low	Low	Low	High	Low	S + T + C + P + N + Q
Aromatic side chain (%)	Low	Low	Low	Low	Low	Low	High	F + H + W + Y
Tiny side chain (%)	Low	Low	Low	Low	Low	Low	High	A + C + G + S + T
Small side chain (%)	Low	Low	Low	Low	Low	Low	High	A + C + D + G + N + P + S + T + V
Aliphatic side chain (%)	High	Low	Low	Low	Low	Low	Low	A + I + L + V
Charged side chain (%)	Low	Low	Low	Low	Low	Low	High	N + D + E + H + K + R + Q
Acidic side chain (%)	Low	Low	Low	Low	Low	Low	High	D + E
Basic side chain (%)	Low	Low	Low	Low	Low	Low	High	H + K + R
Branched side chain (%)	Low	Low	Low	Low	High	Low	Low	I + L + V
Hydrophobic side chain (%)	High	Low	Low	Low	Low	Low	Low	I + L + V + F + W + C
Sulphurous side chain (%)	Low	Low	Low	Low	Low	Low	High	M + C

Criteria that are significantly more/less represented in a specific cluster compared to the others. Example: cluster 1 contains peptides that have a significantly lower molecular weight than in clusters 4,5 and 6.

- ✓ Cluster 5 is associated in particular with high amounts in aliphatic, non-polar, and sulphurous side chain amino acids in contrast to cluster 1.
- ✓ Cluster 3 is associated with peptides containing the highest percentage in essential amino acids for LAB and cluster 4 the lowest.

2 Identification of the peptides used by the receiving strains

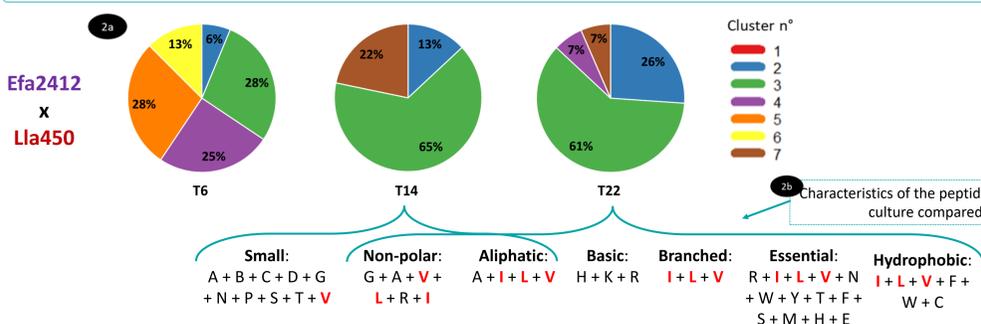


Figure 4 Clusters associated with the peptides used by Lla450 in co-culture with Efa2412 and characteristics of the peptides used compared to the all the peptides available

- ✓ Peptides significantly less abundant in the co-culture Efa2412 x Lla450 compared to Efa2412 monoculture were mostly found in clusters 5 and 3 (Fig 4 & Table 1).
- ✓ Among the peptides produced by Efa2412, Lla450 preferably used peptides containing more non-polar, small side chain, branched-chain, and aliphatic amino acids (Fig 4).
- ✓ Similar results were observed with Llp1524.

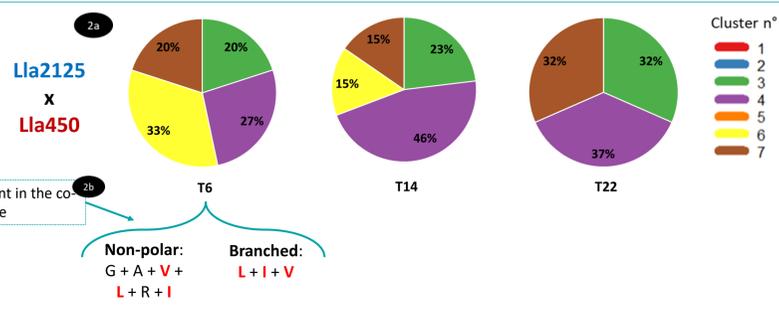


Figure 5 Clusters associated with the peptides used by Lla450 in co-culture with Lla2125 and characteristics of the peptides used compared to the all the peptides available

- ✓ Peptides significantly less abundant in the co-culture Lla2125 x Lla450 compared to Lla2125 monoculture were found in clusters 4, 3, 7 and 6 (Fig 5 & Table 1)
- ✓ Among the peptides produced by Lla2125, Lla450 preferably used peptides containing non-polar amino acids (Fig 5).

CONCLUSION

- ✓ Peptides containing non-polar and aliphatic amino acids, and more specifically branched-chain amino acids were involved in positive interactions.
- ✓ Peptides containing branched-chain amino acids were associated with cluster 5 specific to Efa2412, which showed the strongest positive interactions with receiving strains. In contrast, peptides from cluster 1, specific to Lla244, which did not interact with receiving strains, contained less branched-chain amino acids.
- ✓ The peptide length and charge did not appear as important in the observed interactions.
- ✓ In contrast to Lla244, the low amount of peptides containing branched-chain amino acids produced by Lla2125 is most likely compensated by the production of branched-chain free amino acids, which would explain why weak interactions were observed with receiving strains.
- ✓ The amino acid composition of the proteins and the cleavage sites of the proteases of the donor strain are important factors to consider when in co-culture with proteolytic and non-proteolytic LAB strains.