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"Chorizo de León" is a high-value Spanish dry fermented sausage traditionally manufactured without the use of starter cultures, owing to the activity of a house-specific autochthonous microbiota that naturally contaminates the meat from the environment, the equipment and the raw materials. Lactic acid bacteria (LAB), particularly *Lactobacillus* and coagulase-negative cocci (CNC, mainly *Staphylococcus*) have been reported as the most important bacterial groups regarding the organoleptic and safety properties of the dry fermented sausages. In this study, samples at different producing steps from raw minced meat to final products were taken from five different producers and the microbial diversity was investigated by high-throughput sequencing of 16S rRNA gene amplicons.

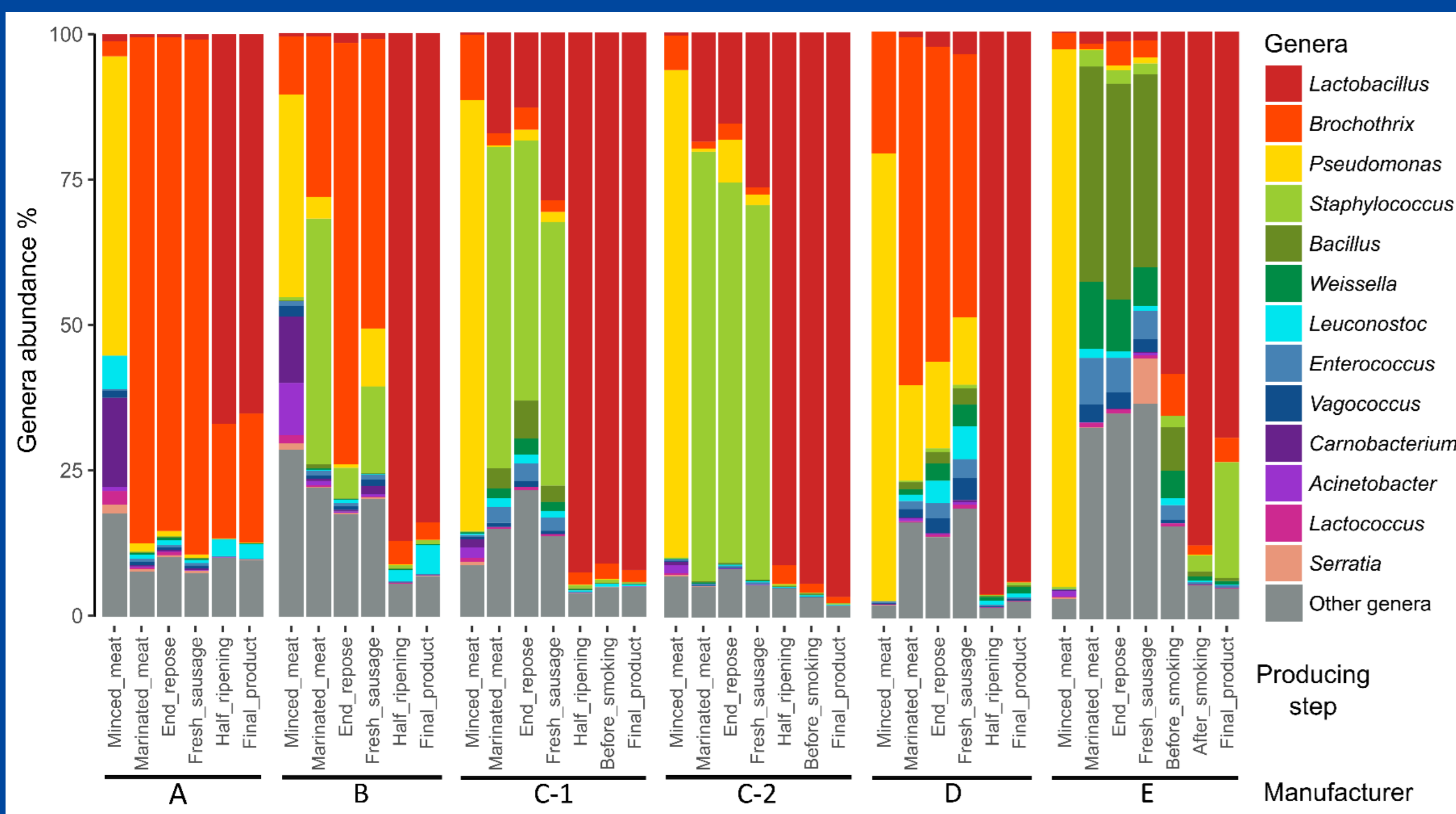


Figure 1A. Relative abundance of the 13 most abundant genera in the different manufacturing steps from the different making companies

Evolution of microbial population involved in "Chorizo de León" ripening

Microbial communities varied along "Chorizo de León" manufacture (Fig. 1A, 1B and 2). *Pseudomonas* and *Brochothrix* were found as dominant in the first stages of "Chorizo de León" manufacture (from mince meat to the fresh sausage). These microorganism are associated to meat spoilage during refrigerated storage and may produce undesirable off-flavors. *Staphylococcus* also dominated during the marinating stage in some manufacturers.

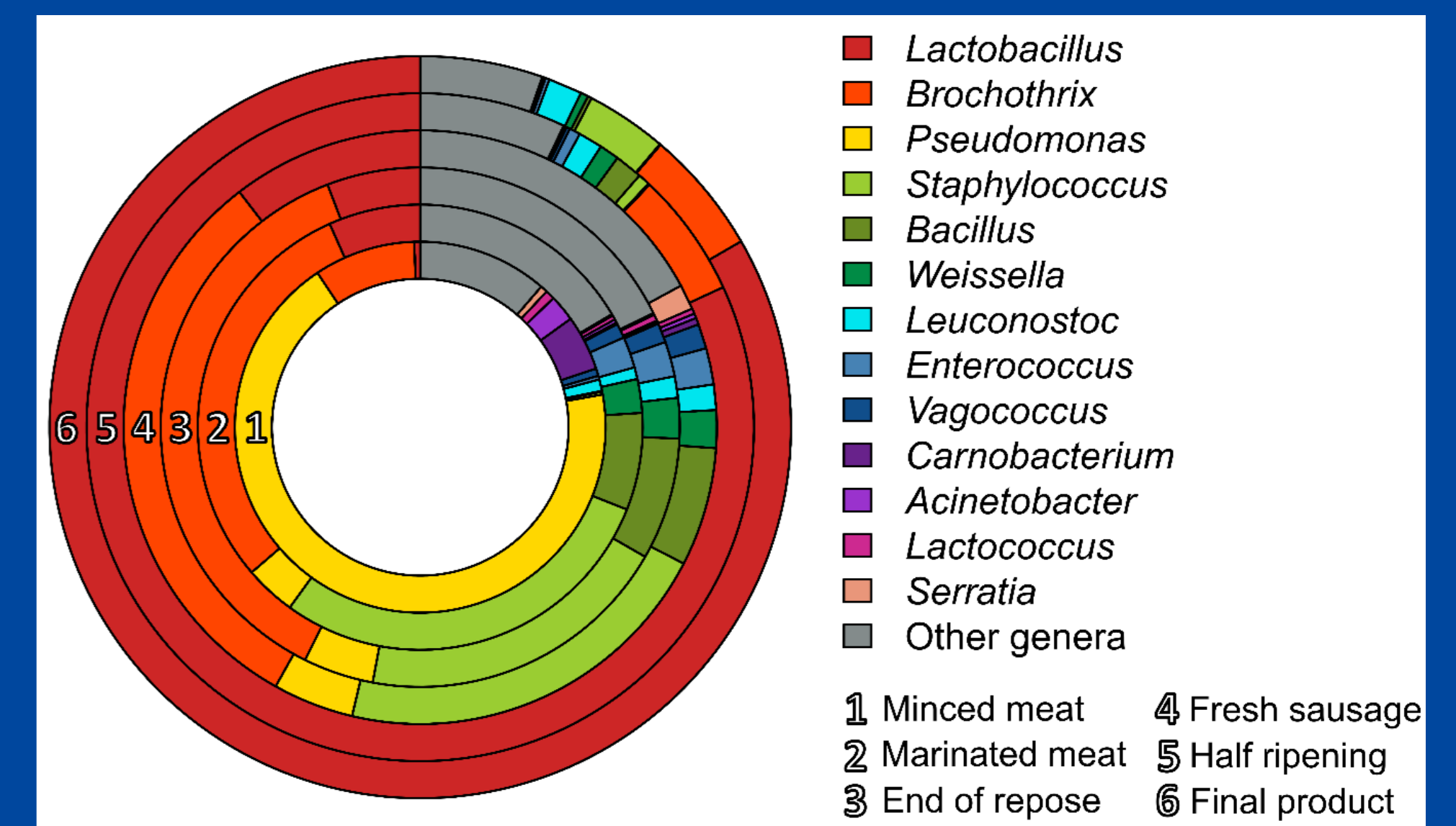


Figure 1B. Relative abundance of the 13 most abundant genera in the different manufacturing steps from the overall process

As "Chorizo de León" ripening proceeded (after the fresh sausage performance), *Lactobacillus* became dominant in samples from all the manufacturers and dominated in the final products. LAB presence during ripening (enhanced by the addition of sugar during the marinating process) is crucial, as they are responsible of some organoleptic properties and safety of the products.

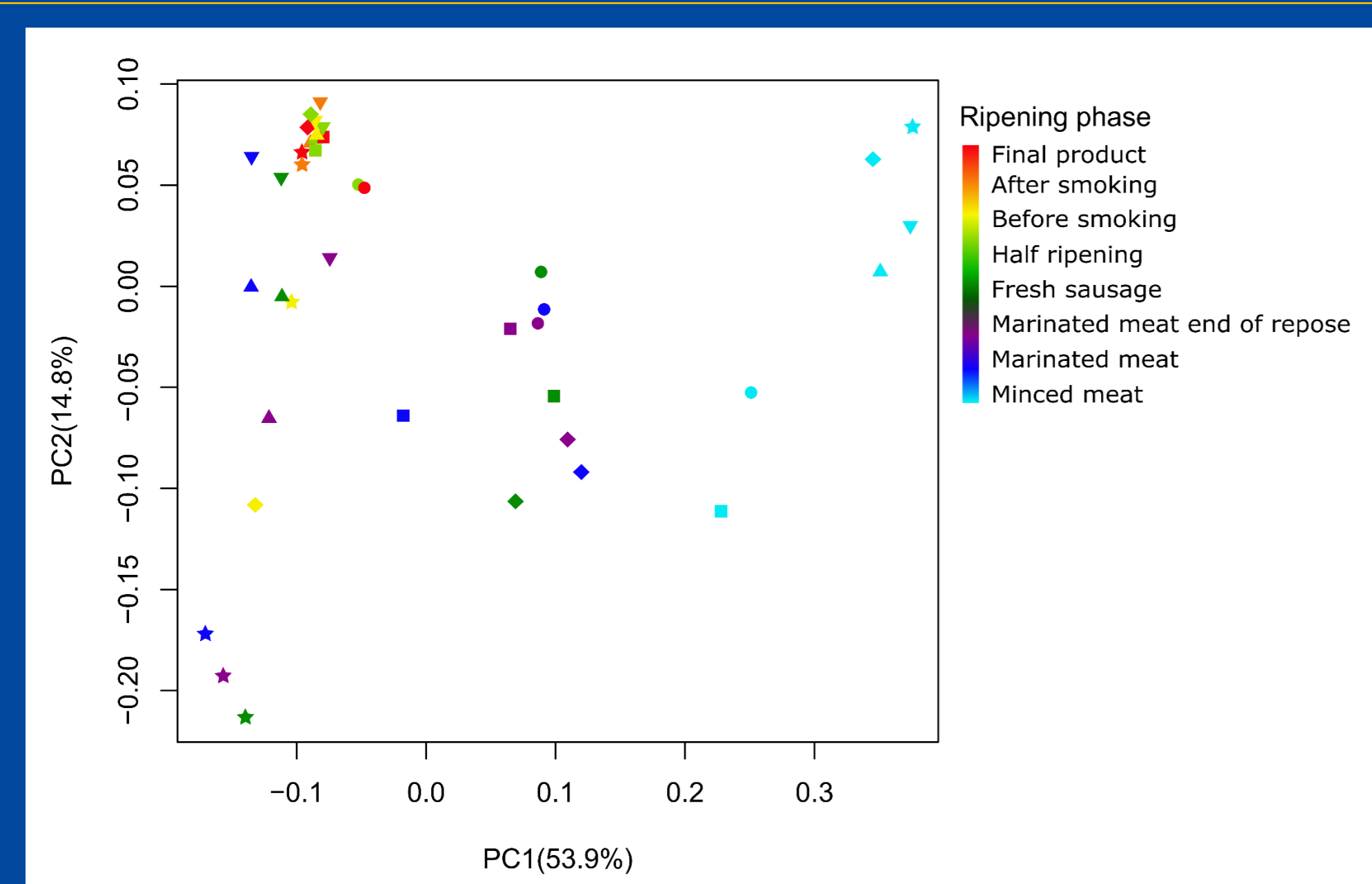


Figure 2. Differences between samples evaluated by Weighted UniFrac phylogenetic metric. The microbial composition of the samples evolved from the minced meat through the marinating and repose phases, where the microbiota from the different samples showed differences according to the making company. Nevertheless, samples from the late-ripening stages clustered together, showing a similar microbial evolution in "Chorizo de León" ripening (where *Lactobacillus* almost dominate) independently from the initial meat contamination.

Sub-genus level analysis of *Staphylococcus* populations revealed 23 different oligotypes and *S. xylosus* was as the most abundant specie. Surprisingly, the most abundant *Staphylococcus* oligotype found in E, the only manufacturer where *Staphylococcus* was found at higher levels in the final product, was identified as *S. equorum* (Figure 4).

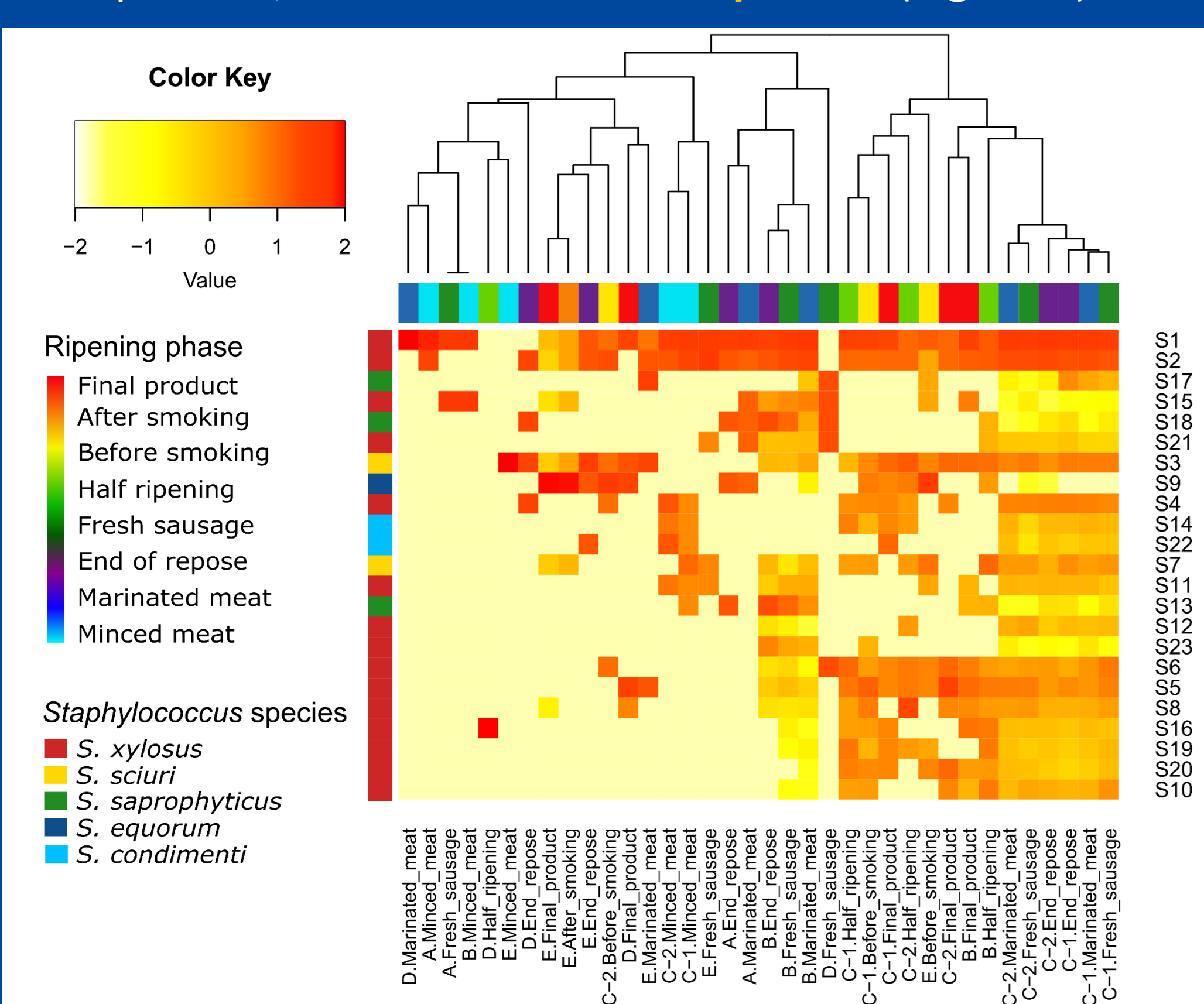


Figure 4. Distribution of *Staphylococcus* oligotypes (rows) through the different samples (columns).

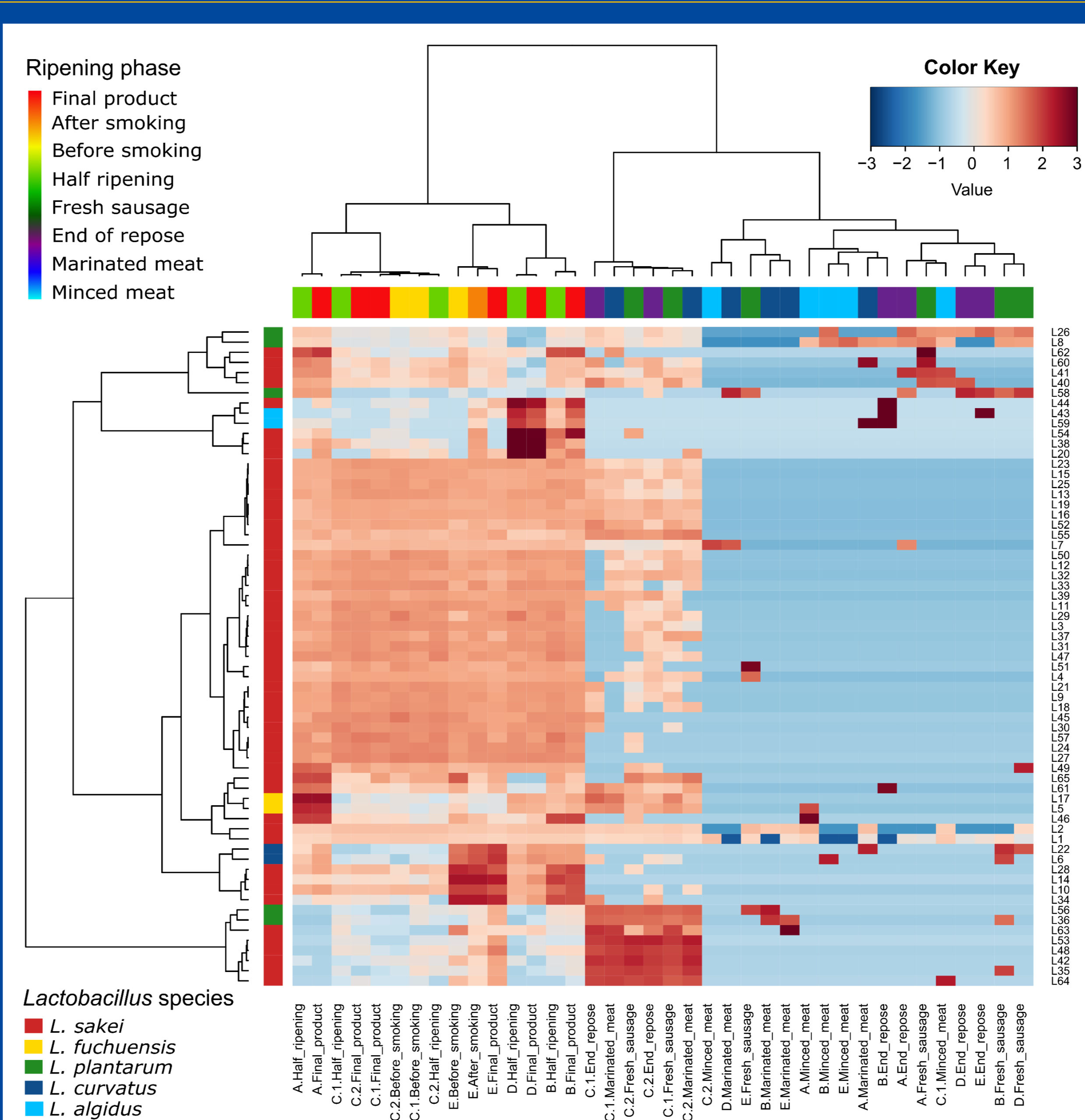


Figure 3A. Distribution of *Lactobacillus* oligotypes (each row represent the 100%) through the different samples (in columns). Manufacturing stage of each sample is represented in the horizontal bar over the figure. Species level of each *Lactobacillus* oligotype can be seen in the vertical bar in the left side of the figure.

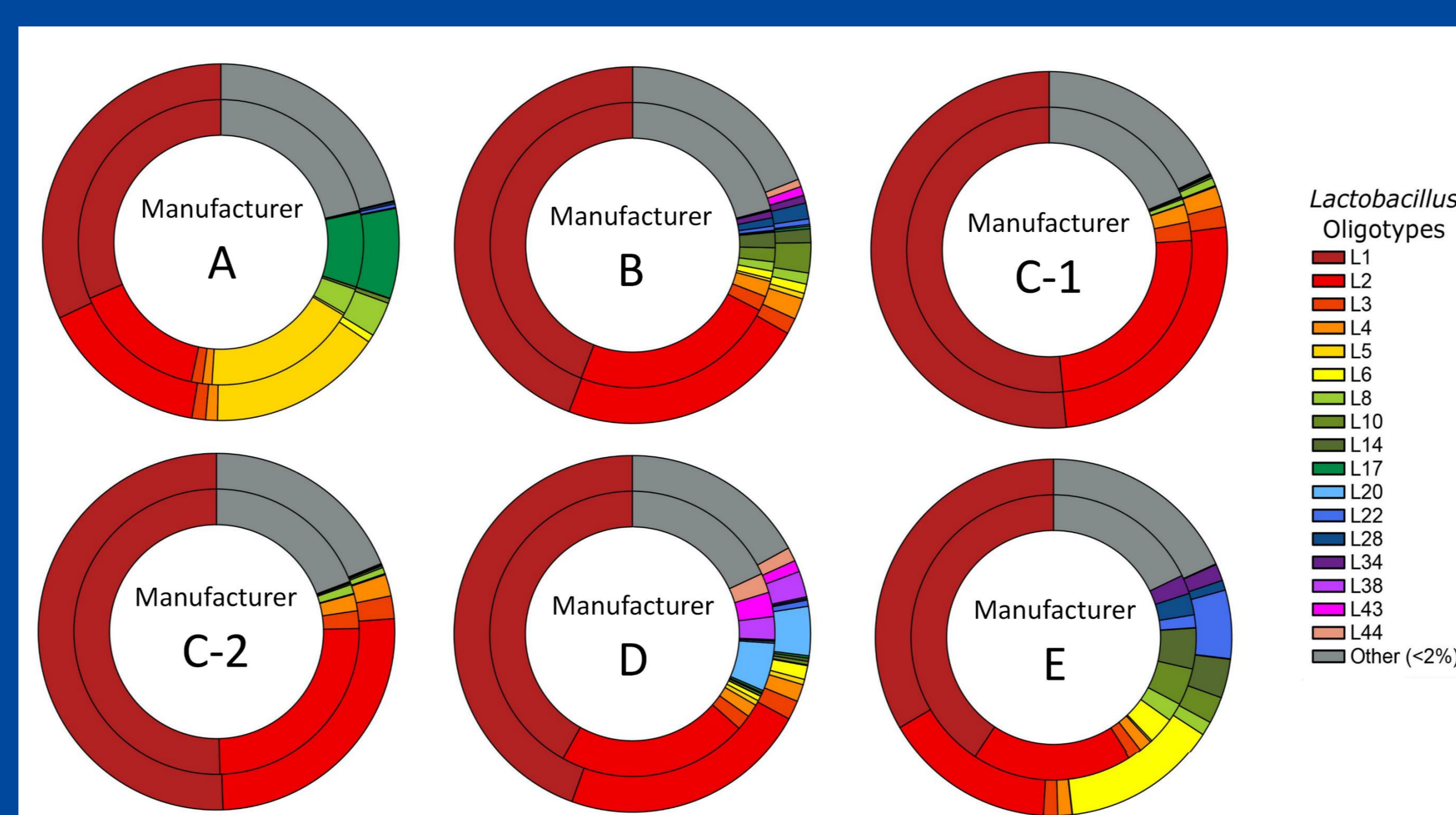


Figure 3B. Relative abundance of *Lactobacillus* oligotypes in the half of the ripening process (inner circle) and in the final products (outer circle) in the different making companies.

Sub-genus analysis revealed different *Lactobacillus* populations that dominated during "Chorizo de León" making

Oligotyping of 16S rRNA gene was performed on *Lactobacillus* populations in order to explore differences at sub-genus level, as closely related species may harbor different genetic pools and therefore may be responsible of the unique properties of "Chorizo de León". *Lactobacillus* was present in all samples and its relative abundance increased widely as the ripening process begun, dominating in the final products. 65 oligotypes were found and BLASTn taxonomic assignment revealed five different species occurring in "Chorizo de León" manufacture. *L. sakei* was found as the most abundant specie (93% of all *Lactobacillus* oligotypes), followed by *L. fuchuensis*, *L. plantarum*, *L. curvatus* and *L. algidus*. *L. sakei* has been reported as the most abundant LAB in dry fermented sausages, due to its superior competitiveness. Figure 3A shows the distribution of *Lactobacillus* oligotypes among the different samples. Minced meat and intermediate samples clearly clustered apart from samples at the half and the end of ripening, that showed a higher number of different *Lactobacillus* oligotypes. Figure 3B shows the relative abundance of *Lactobacillus* oligotypes in the half of the ripening process and in the final products in the different making companies. L1 and L2 (both *L. sakei*) were the most abundant overall and present in all manufacturers. Nevertheless, different *Lactobacillus* oligotypes profile can be observed for each manufacturer.

CONCLUSIONS

- Microbial diversity evolved during ripening to at least 65% of *Lactobacillus*.
- Oligotyping of 16S rRNA gene was used for sub-genus level analysis
- Sub-genus diversity of *Lactobacillus* and *Staphylococcus* allowed to differentiate between manufacturers.