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# Spontaneously European fermented sausages as source of new starter or bioprotective cultures

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## INTRODUCTION

Fermented meat products represent an important cultural heritage. In the traditional production processes the presence of indigenous microorganisms confers peculiar technological and organoleptic features to these products, also linked to the geographical origin (Van Reckem et al., 2019). On the other hand, the introduction of starter cultures to enhance product safety in the meat industry led to an impoverishment of their typical characteristics (Cocconcelli and Fontana, 2010).

## AIM OF THE RESEARCH

The study of traditional spontaneously fermented sausages microbiota can represent an important tool to find new technological and functional strains able to preserve product microbial biodiversity and traditional features. With this aim, 15 spontaneously fermented European sausages, characterized for their features and microbial biodiversity, were used as a source of isolation for new lactic acid bacteria (LAB) strains. The isolates, tested for their biodiversity and genetically identified, were evaluated for their safety properties.

## METHODS

- ✓ Collected sausages sample (Italy, Slovenia, Spain and Croatia) characterization → **Microbial counts:** cultivation-dependent technique on selective media and metagenomic analysis; **pH, $a_w$, biogenic amines and aroma profiles** according to Tabanelli et al. (2020).
- ✓ **LAB strains isolation, genetical identification and characterization as regard their safety properties** according to Dentice Maidana et al. (2020).

## RESULTS

| **Characteristics of the tested sausages** | **Italy** | | | **Slovenia** | | **Spain** | | | | | | | **Croatia** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **IM1** | **IM2** | **IAL** | **SN** | **SWO** | **ESA** | **ESB** | **ESE** | **ESO** | **ECB** | **ECE** | **ECO** | **HNS** | **HS** | **HZK** |
| **Section** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **LAB** | $7.07^{a}$ | $8.52^{b}$ | $8.26^{b}$ | $6.57^{c}$ | $7.28^{a}$ | $7.85^{d}$ | $6.96^{a}$ | $6.32^{c}$ | $7.78^{d}$ | $4.41^{e}$ | $5.88^{c}$ | $7.73^{d}$ | $8.67^{b}$ | $8.43^{b}$ | $8.54^{b}$ |
| **Staphylococci** | $7.12^{a}$ | $7.13^{a}$ | $5.22^{b}$ | $<0.5^{c}$ | $1.44^{d}$ | $3.65^{e}$ | $3.05^{e}$ | $4.54^{f}$ | $5.40^{b}$ | $<0.5^{c}$ | $<0.5^{c}$ | $<0.5^{c}$ | $5.34^{b}$ | $5.09^{b}$ | $7.24^{a}$ |
| ***Enterobacteriaceae*** | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $<0.5^{a}$ | $3.55^{b}$ | $2.71^{b}$ | $5.12^{c}$ |
| **pH** | $6.42^{a}$ | $6.09^{b}$ | $5.88^{b}$ | $5.20^{ce}$ | $5.39^{c}$ | $5.83^{b}$ | $5.63^{d}$ | $5.80^{b}$ | $5.13^{e}$ | $4.77^{f}$ | $5.04^{f}$ | $4.52^{f}$ | $5.81^{b}$ | $5.72^{bd}$ | $6.05^{b}$ |
| **$a_w$** | $0.824^{a}$ | $0.760^{b}$ | $0.879^{c}$ | $0.823^{a}$ | $0.832^{a}$ | $0.917^{d}$ | $0.811^{a}$ | $0.848^{e}$ | $0.911^{d}$ | $0.895^{f}$ | $0.870^{df}$ | $0.908^{df}$ | $0.928^{d}$ | $0.903^{df}$ | $0.890^{f}$ |
| **Ketones** | $23.46^{a}$ | $29.36^{b}$ | $14.35^{c}$ | $20.26^{ac}$ | $18.02^{c}$ | $3.83^{d}$ | $25.70^{a}$ | $25.37^{a}$ | $27.18^{ab}$ | $64.33^{e}$ | $45.09^{f}$ | $45.21^{f}$ | $144.71^{g}$ | $24.84^{a}$ | $33.19^{b}$ |
| **Aldehydes** | $22.52^{a}$ | $60.92^{b}$ | $33.00^{c}$ | $19.59^{ad}$ | $27.17^{c}$ | $14.42^{d}$ | $22.54^{a}$ | $20.67^{a}$ | $68.57^{b}$ | $11.21^{d}$ | $36.27^{c}$ | $44.18^{c}$ | $269.54^{e}$ | $143.19^{f}$ | $36.43^{c}$ |
| **Alcohols** | $24.27^{a}$ | $18.59^{b}$ | $26.46^{a}$ | $32.99^{c}$ | $28.50^{ac}$ | $288.37^{d}$ | $111.00^{e}$ | $35.81^{c}$ | $192.46^{f}$ | $64.17^{g}$ | $53.81^{c}$ | $34.81^{c}$ | $166.79^{i}$ | $182.70^{f}$ | $58.40^{h}$ |
| **Acids** | $43.75^{a}$ | $52.98^{b}$ | $45.61^{a}$ | $142.64^{c}$ | $109.69^{d}$ | $45.12^{a}$ | $131.80^{c}$ | $43.55^{a}$ | $120.82^{c}$ | $302.91^{e}$ | $413.24^{g}$ | $232.80^{g}$ | $125.11^{c}$ | $175.96^{h}$ | $35.27^{a}$ |
| **Esters** | $5.62^{a}$ | $2.82^{b}$ | $7.48^{a}$ | $8.93^{a}$ | $7.00^{a}$ | $52.15^{c}$ | $45.64^{c}$ | $6.65^{a}$ | $69.20^{d}$ | 81.96d | $27.79^{a}$ | $7.11^{a}$ | $9.42^{a}$ | $48.82^{c}$ | $2.07^{b}$ |
| **Biogenic amines** | $73.9^{a}$ | $47.7^{b}$ | $194.3^{c}$ | $268.4^{d}$ | $349.0^{e}$ | $267.2^{d}$ | $612.1^{f}$ | $192.7^{c}$ | $178.5^{c}$ | 245.3d | $423.8^{e}$ | $358.5^{e}$ | $1059.4^{h}$ | $925.0^{h}$ | $105.3^{a}$ |

**Tab 1.** Characterization of different traditional sausages. The analyses were performed in triplicate. Different letters indicate significant differences in the results between samples.

The results showed that LAB were the predominant microbial population in each product, while in several samples staphylococci were under the detection limit because the low pH inhibit their growth. *Enterobacteriaceae* were detected only in Croatian sausages, that were characterized also by the highest content of biogenic amines. The geographical origin and manufacturing processes (*e.g.* smoking) affected chemico-physical parameters and aroma profile.

Metagenomic analysis → differences in the amplicon sequence variants. The dendrogram evidenced 4 cluster groups: *Latilactobacillus sakei* was the principal responsible for the description of the first larger group, while *Staph. xylosus* determined the cluster of ESA. The third cluster was mainly influenced by the presence of *Companilactobacillus*, *Corynebacterium* and *Brochothrix thermosphacta*, while the last group depended on the variable *Staph. equorum*.

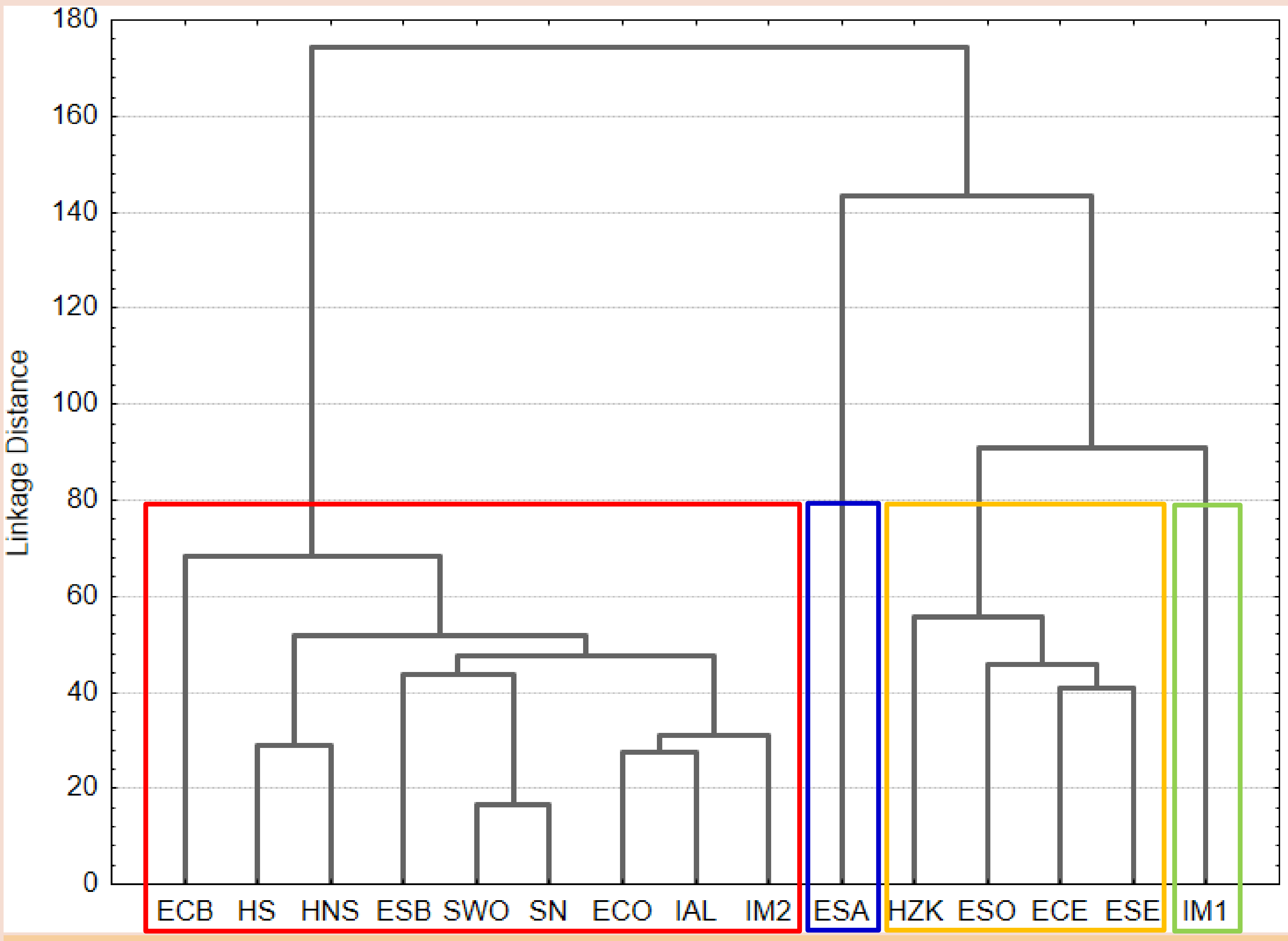

**Fig 1.** Dendrogram derived from the similarity of sausages metagenomic analysis. The squares show the clusterization result.

Total of **915 isolates** of presumptive LAB

**140 identified biotype** belonging to 5 different species

**50 strains** with **safety** characteristics

The most **promising strains** have been evaluated for their **technological properties**

**REFERENCES:** Cocconcelli and Fontana (2010). *Handbook of Meat Processing*; Dentice Maidana et al. (2020). *Int. J. Food Microbiol.*, doi:10.1016/j.ijfoodmicro.2019.108425; Tabanelli et al. (2020). *Food Control*, doi:10.1016/j.foodcont.2020.107304; Van Reckem et al. (2019). *Front. Microbiol.*, doi:10.3389/fmicb.2019.02302.

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