

ASSOCIATION OF TARGETED METAGENOMIC ANALYSIS AND CLASSICAL MICROBIOLOGY FOR CLOSTRIDIUM DIFFICILE DETECTION AND MICROBIAL ECOSYSTEM MAPPING OF SURFACES HANDS AND FOODSTUFFS IN A MEAT PROCESSING PLANT

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INTRODUCTION

Proper hygiene practices in meat processing plants are essential for prevention of foodborne disease outbreaks. **Metagenetics** is a **culture independent based strategy** allowing for the **identification of bacterial populations and their proportions** present in a large panel of samples, including foods and surfaces. Such an approach permits detection of **foodborne pathogens and hygiene indicators** without the time-consuming step of culturing individual bacterial species for identification, with a **high resolution taxonomic information**.

PURPOSE

The aim of this study was to **evaluate the hygienic level of a meat processing plant** and to identify possible **sources of cross contamination**. A **microbiological detection scheme** was performed along with an **overall microbial biodiversity study** of the samples by **16S metagenetic analysis**. Detection of the pathogenic bacterium *C. difficile* was also performed.

METHODS

- ✓ The **production line** of two Belgian meat products (pork tomato sauce and white pudding) was **monitored**.
- ✓ Samples from **operator hands** (n=8), **surfaces** (n=9) and **meat products** (n=11) were collected at different production points.
- ✓ All samples were analysed by **classical microbiology** to determine the levels of **total aerobic viable counts, Enterobacteriaceae, Escherichia coli, Staphylococcus aureus** and to detect the presence of *C. difficile*.
- ✓ **Metagenetic analysis** was targeted on the **V1-V3 hyper-variable region of 16S rDNA**.
- ✓ The taxonomical assignment of the populations was performed with **Mothur and Blast algorithms**.

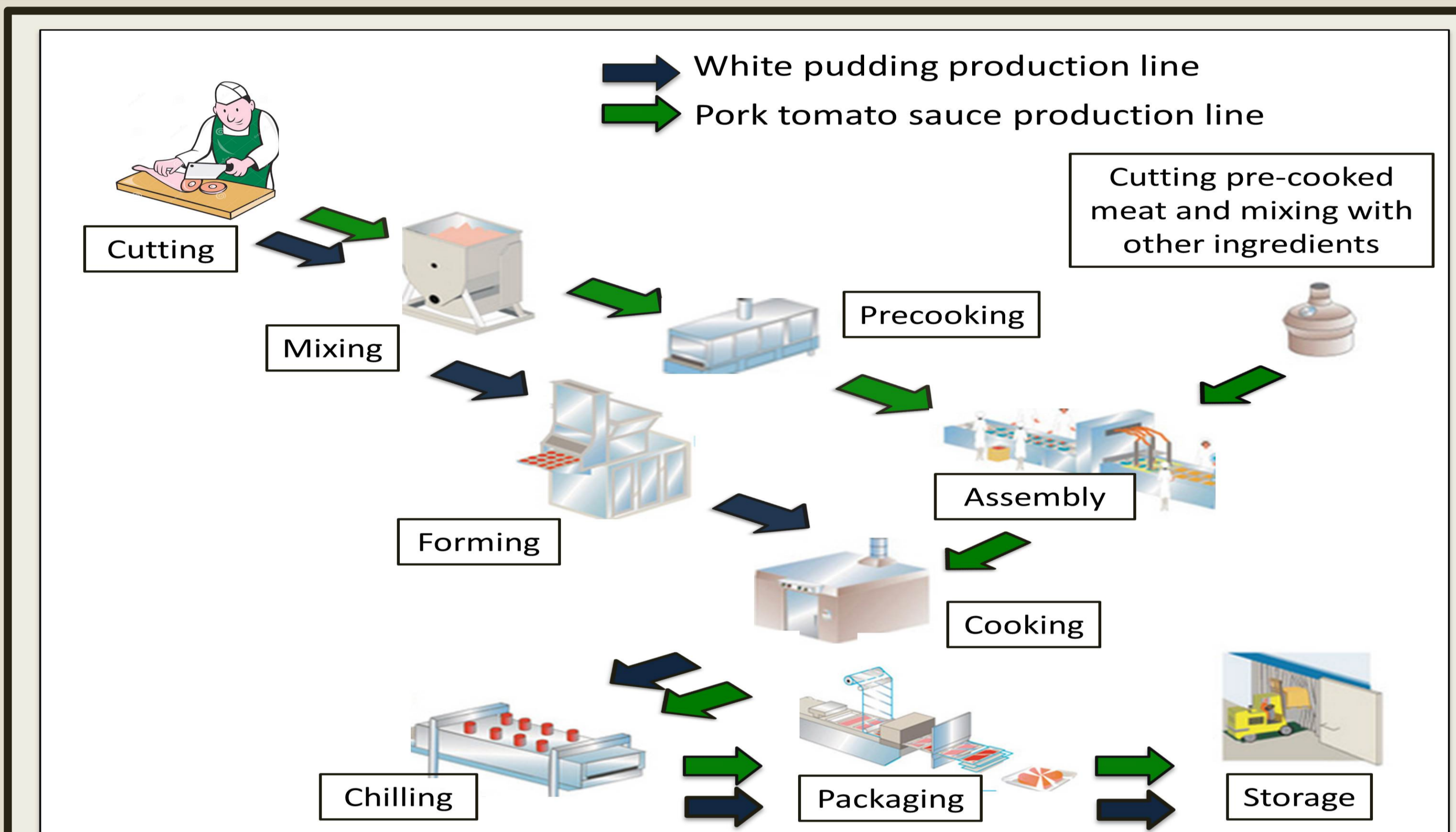


Figure 1. Production lines of pork tomato sauce and white pudding

RESULTS

Classical microbiological analysis

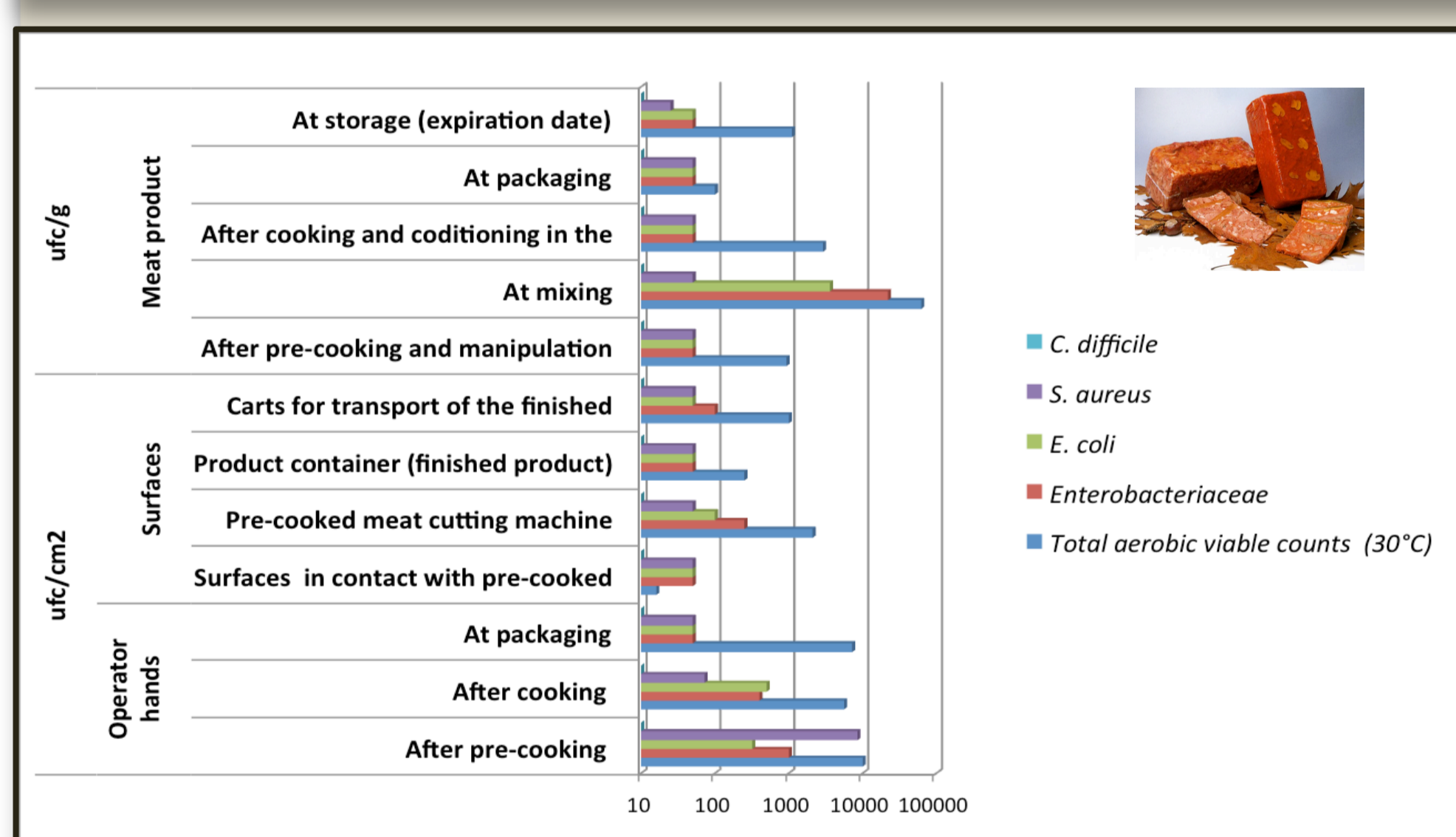


Figure 2. Results of classical microbiological testing of operator hands, surfaces and meat products in the production line of **pork tomato sauce**.

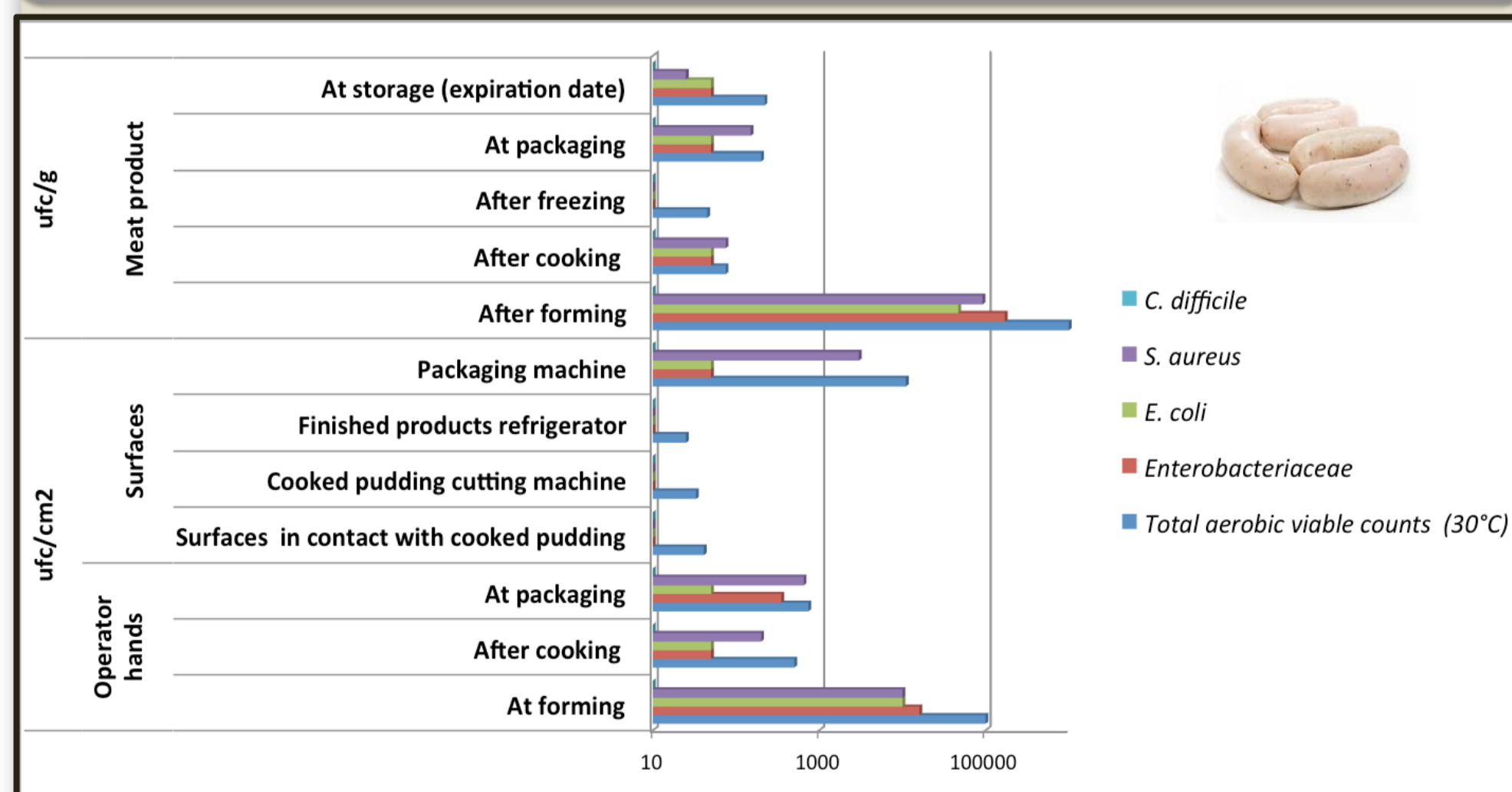


Figure 3. Results of classical microbiological testing of operator hands, surfaces and meat products in the production line of **white pudding**.

Metagenetic analysis

PORK TOMATO SAUCE

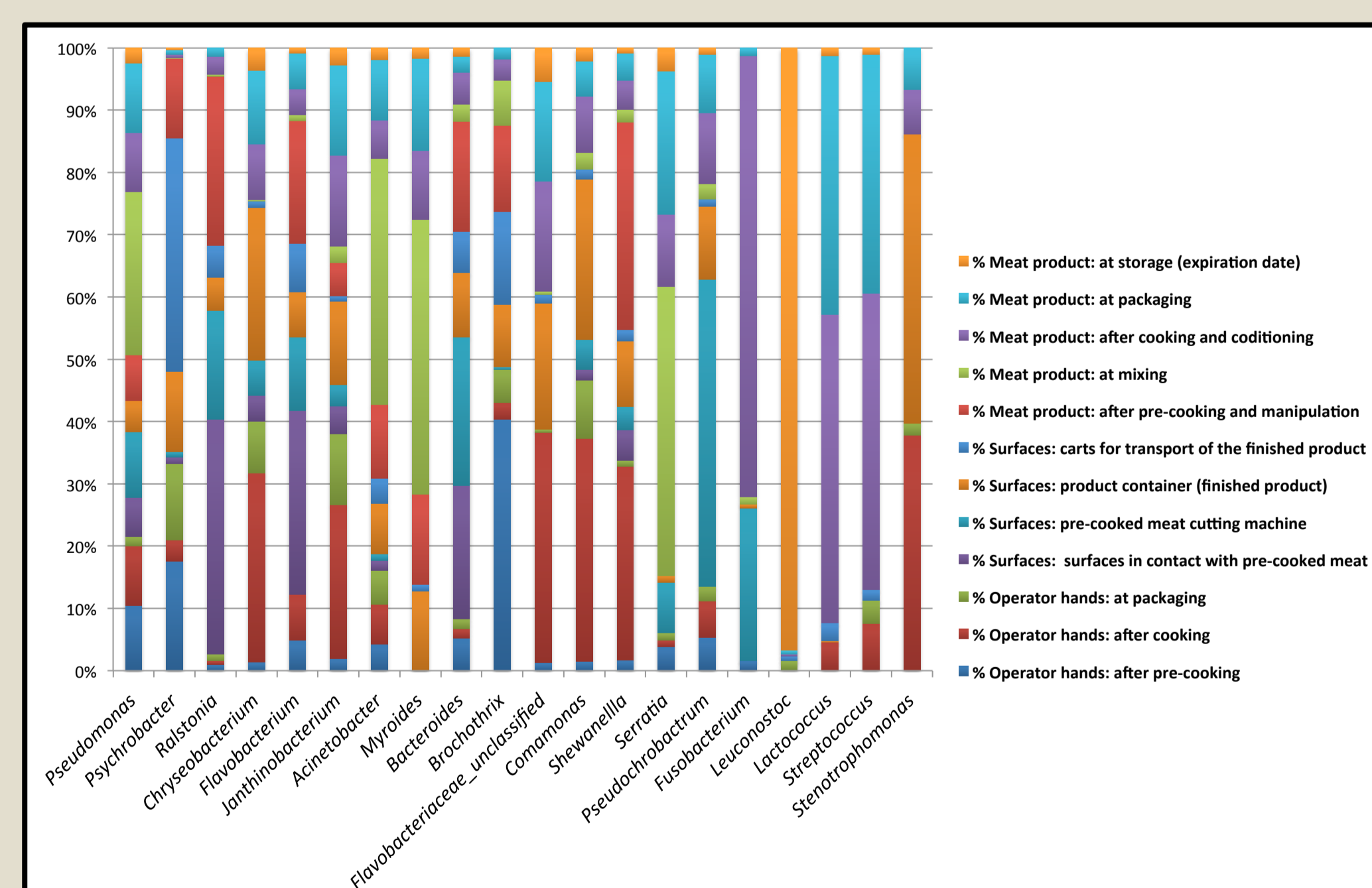


Figure 4. Microbiota composition at genus level (cumulated mean relative abundance >4 % in at least one type of sample). The unclassified populations correspond to defined groups of the genus level for which a taxonomical classification assignment to the genus cannot be attributed.

WHITE PUDDING

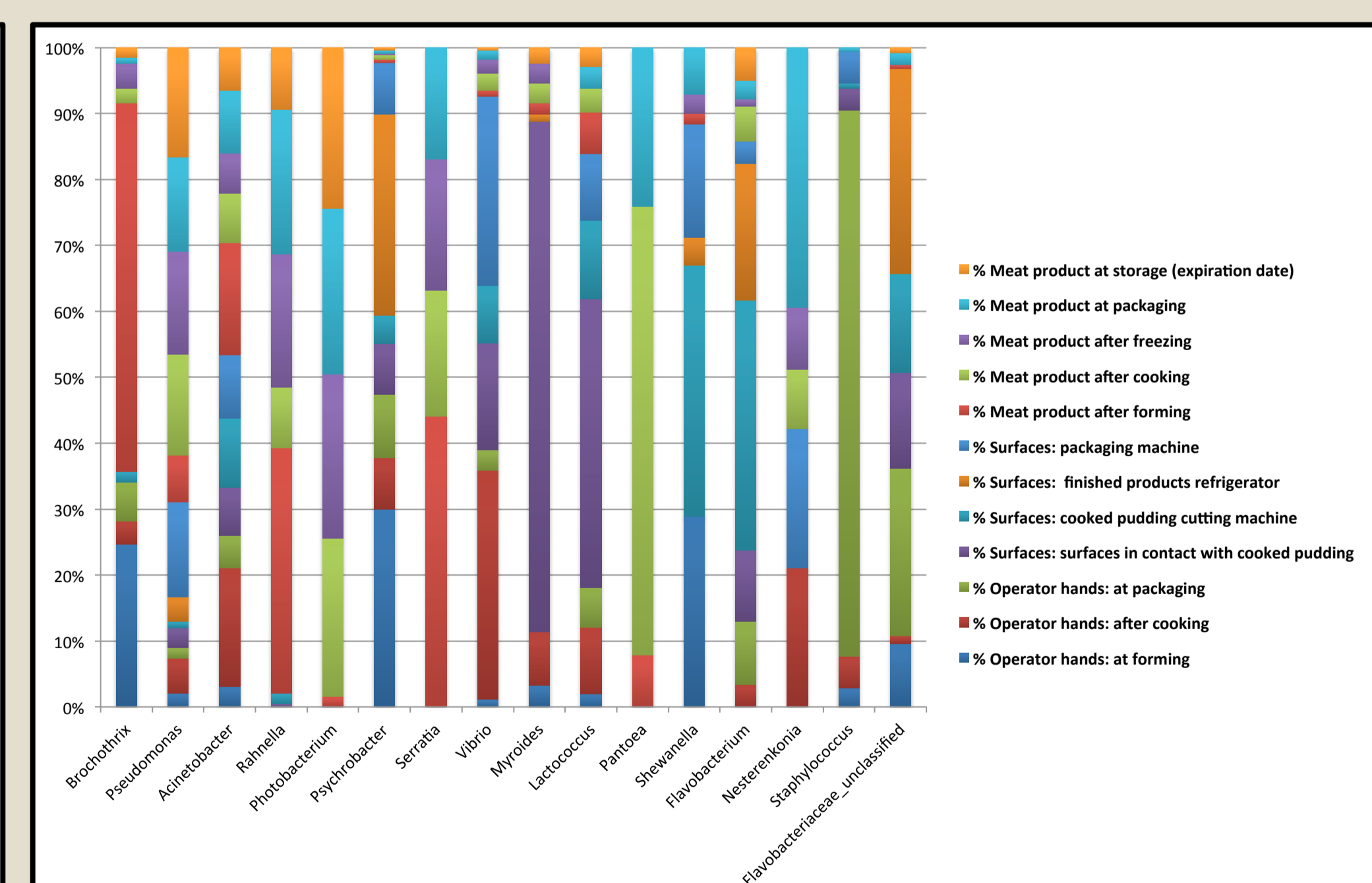


Figure 5. Microbiota composition at genus level (cumulated mean relative abundance > 0,5% in at least one type of sample). The unclassified populations correspond to defined groups of the genus level for which a taxonomical classification assignment to the genus cannot be attributed.

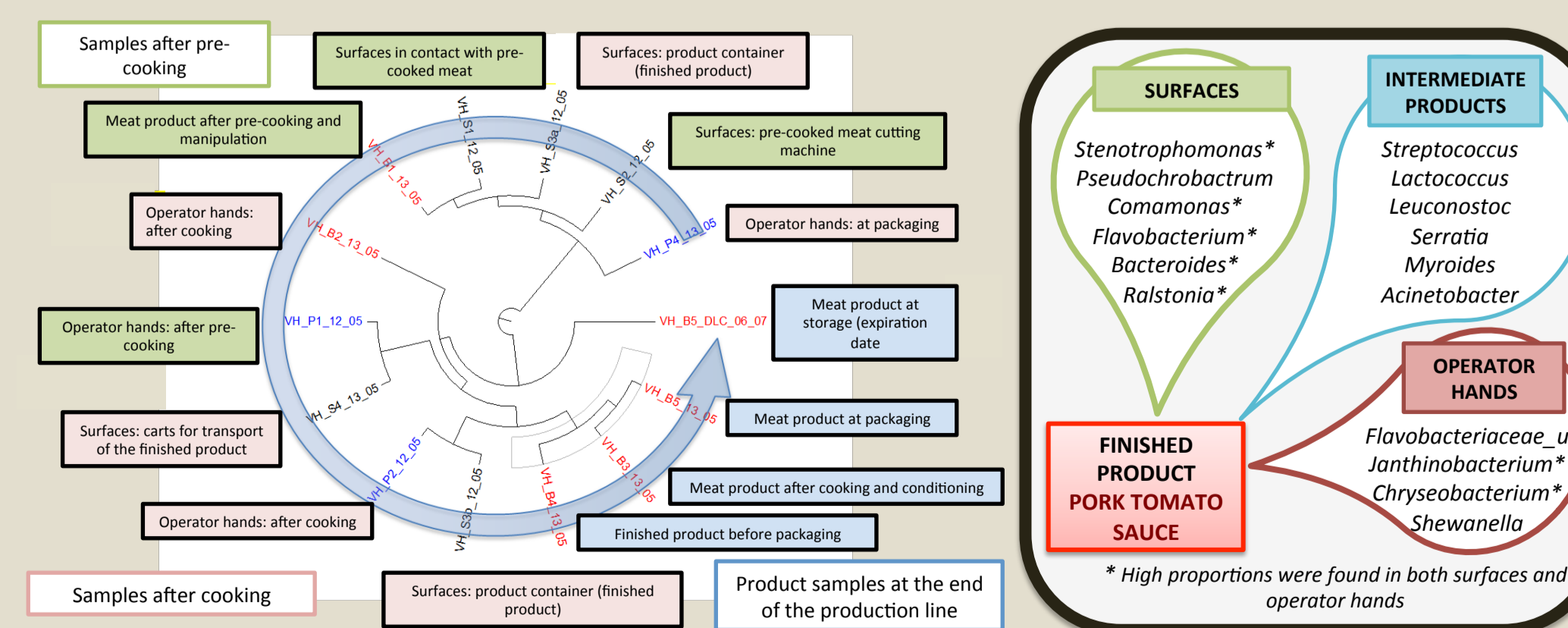


Figure 6. Samples classification based on the presence/absence of different taxa at genus level

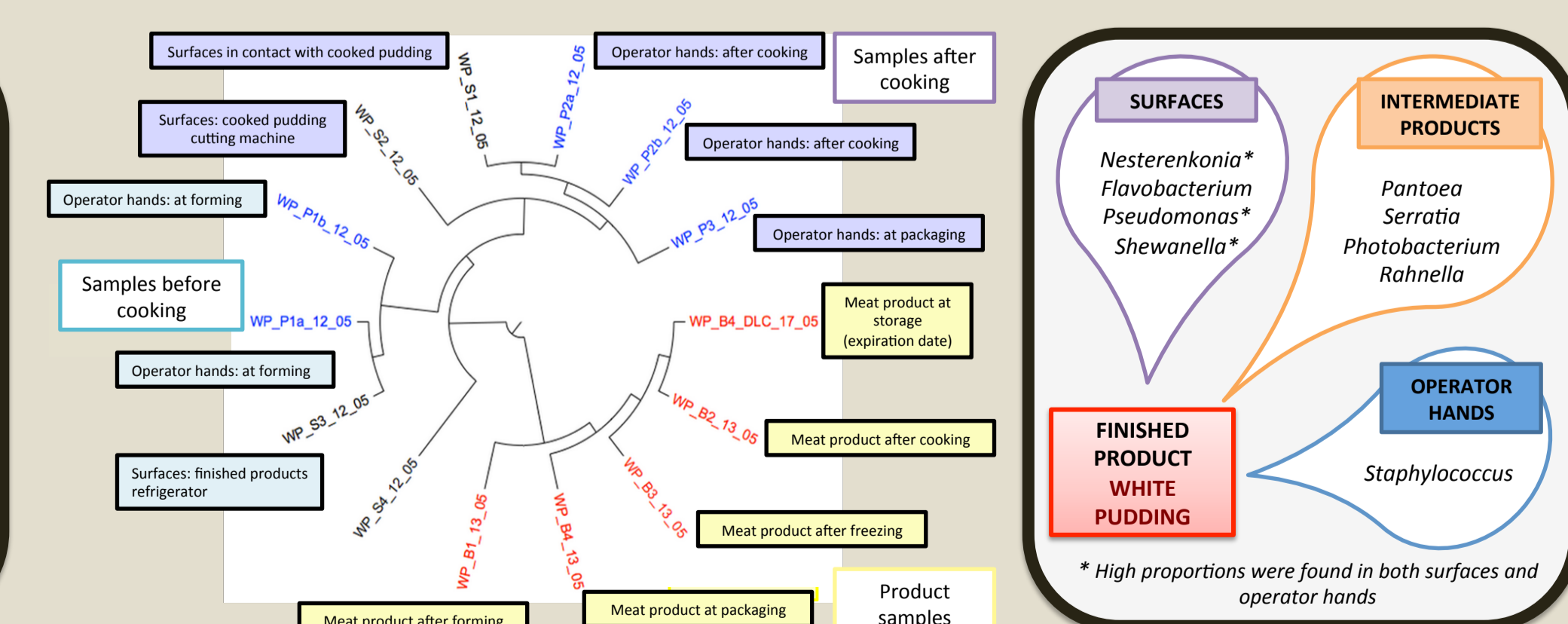


Figure 8. Samples classification based on the presence/absence of different taxa at genus level

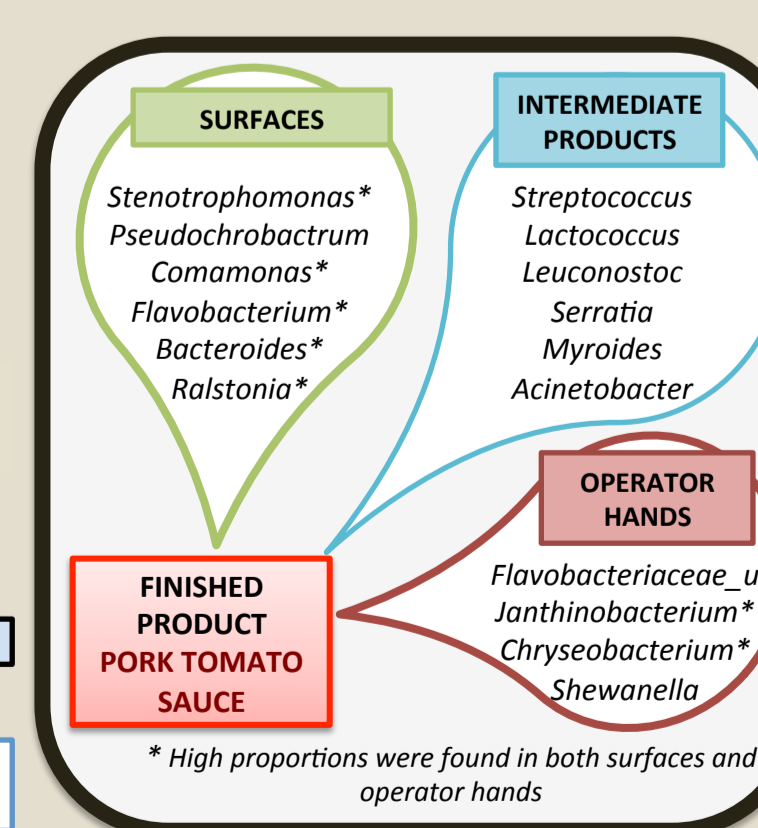


Figure 7. Possible sources of cross-contamination

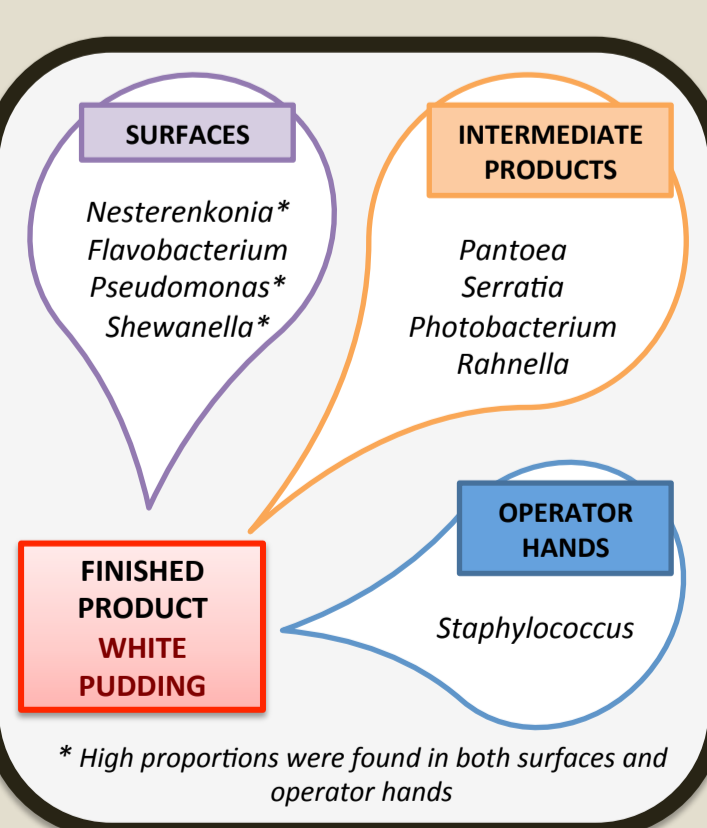


Figure 9. Possible sources of cross-contamination

None of the samples were positive for *C. difficile*, which indicates that surfaces, operators and meat products are unlikely to serve as **vectors of bacterium transmission**. Furthermore, using international standards, **all of the samples contained acceptable levels of the other bacteria studied**.

CONCLUSIONS

Metagenetic analysis revealed the presence of some taxa on surfaces and operator hands that were found in **high abundance in the finished product**, indicating that **cross contaminations may occur**. Results also revealed that **operator hands are a probably vehicle for bacterial dissemination** in the meat processing plant studied.