Understanding the parameters leading to sporulation and promoting resistance of *Clostridium perfringens* spores in slaughterhouses

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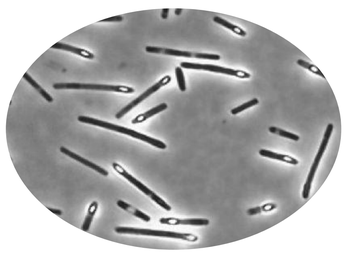
INTRODUCTION

In 2021 in Europe, *Clostridium perfringens* was responsible for 40 outbreaks, 778 human cases, 25 hospitalisations and 4 deaths, making it the 6th most common foodborne pathogen [1]. This spore-forming bacteria is present in the digestive tract of humans and animals [2], which can lead to contamination of meat and carcasses during improper slaughterhouse practices, especially during the evisceration step. However, few studies have focused on the risk assessment of *C. perfringens* in slaughterhouses in France.

This study is carried out in the framework of the **ANR ClostAbat project** entitled "Characterization of the *Clostridium perfringens* and *Clostridioides difficile* hazards in the bovine, pig and poultry sectors in slaughterhouses". **The objective of this study is to investigate the impact of the sporulation medium on the thermoresistance of *C. perfringens* spores.**

METHODS

1 commercial strain :
***C. perfringens* CIP 104880**
Type F, *cpe*+, α toxins,
origin : boiled salted beef



Sporulation media tested :

- Duncan Strong
- Tryptone-Peptone-Glucose (TPG)
- Reinforced Clostridial Medium (RCM)
- Liver Infusion Broth
- Pancreatic Peptone Broth

+ Buffer and sporulation salts

Anaerobic incubation
37°C – 3, 7 and 10 days

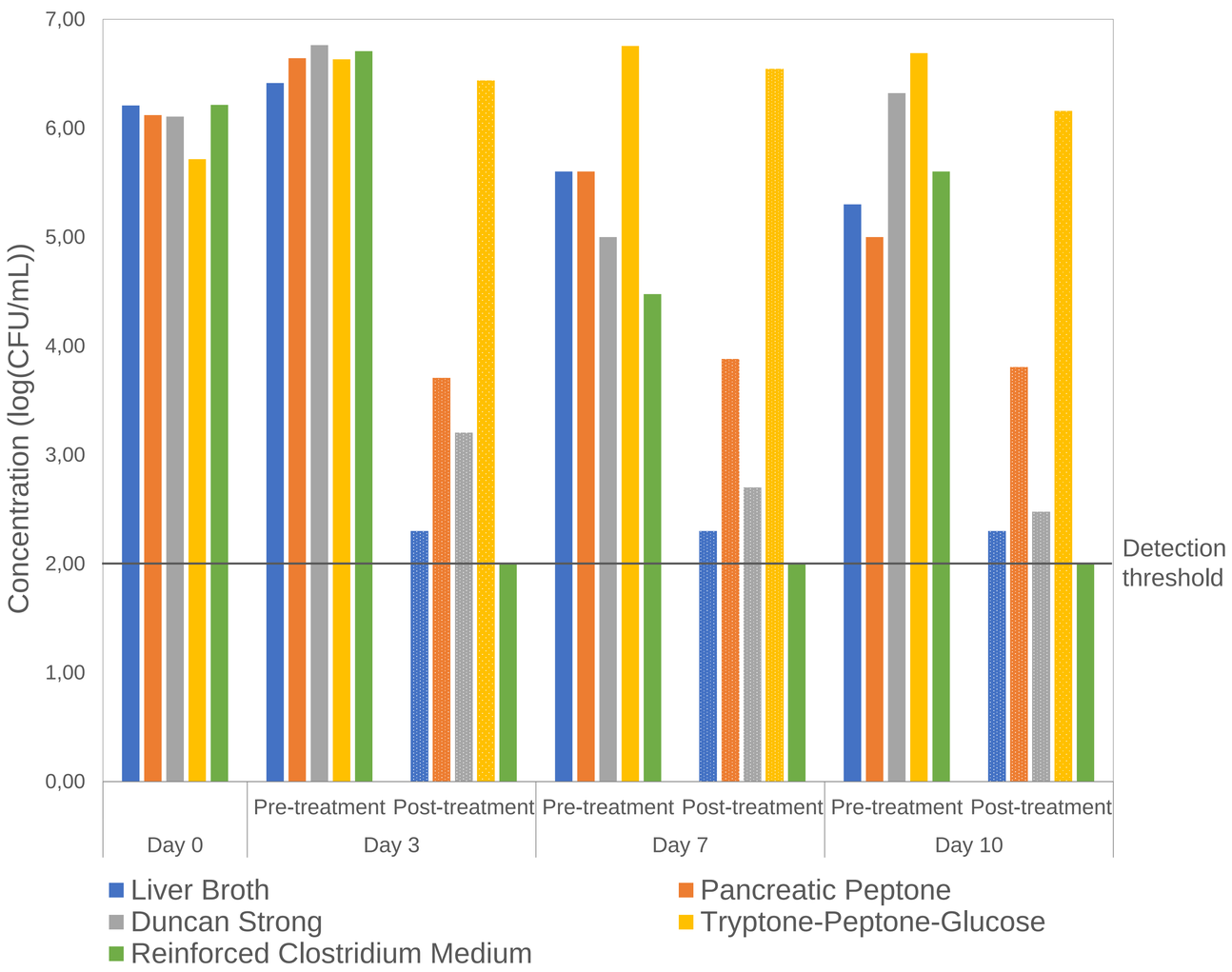
Heat stress
75°C – 15 minutes

Total cells

Spores

Enumeration on Columbia agar (COS)

RESULTS



Sporulation yield between post-treatment and pre-treatment (%)

Incubation time \ Sporulation media	Liver Broth	Pancreatic Peptone	Duncan Strong	TPG	RCM
Day 3	0.01	0.39	0.13	63.72	0.00
Day 7	0.01	0.58	0.04	61.40	0.00
Day 10	0.01	0.48	0.00	29.39	0.00

- The bacteria survival of the *C. perfrincens* CIP 104880 strain is maintained between 10^5 and 10^6 UFC/mL over the 10 days before the treatment for all the media.
- No heat resistant cell forms are produced in RCM.
- For the other media, on the third day of culture, spores are already viable and the rate of heat resistant spores stays constant until 10 days.
- The sporulation yield was the highest for the TPG medium with 10^6 viable spores/mL after heat treatment over the 10 days.
- TPG is considered the best sporulation medium for *C. perfringens* among the tested media.

CONCLUSION & FURTHER WORK

We were able to show that the sporulation medium has an influence on the resistance of spores to heat stress. Other media, similar in composition to meat, will also be tested for this experiment in order to reproduce the conditions encountered in slaughterhouses. In addition, other parameters can have an influence on the spore resistance, for example the **temperature**, the **relative air humidity** or the **oxygen**.

Bacteriological sampling campaigns are currently being carried out in several French cattle, pig and poultry slaughterhouses at the evisceration and cutting steps. The resistance of *C. perfringens* strains isolated from these slaughterhouses will be tested for different parameters (temperature, relative air humidity, oxygen and sporulation medium). **This knowledge could lead to a better understanding of the persistence of these pathogenic strains and the resulting contamination in slaughterhouses.**

REFERENCES

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