

# Study of a new putative species of *Fusobacterium* which could inhibit *Listeria monocytogenes* in Herve cheese

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## Introduction

A new putative species of *Fusobacterium* was found as a major component of the rind of a farmhouse Herve cheese and its genome was sequenced before isolation of the species. Herve cheese is a smear-ripened soft cheese made from raw cow's milk and the only PDO cheese in Belgium. A challenge-test study has also shown that *Listeria monocytogenes* cannot multiply on this type of cheese during shelf-life at  $8 \pm 1$  °C

## Materials and methods

Aging of six Herve cheeses which are a **soft cheese**, which can be **refined or not**. In this study, only the « Herve Blanc » is unripened. The characteristics of the different samples are transcribed in the table below :  
Samples were studied by **qPCR** and **metagenetics**. At the same time, **attempts to isolate this new species** on Skim Milk agar have been made with different culture conditions.

## Objective

Validate the presence of this new species of *Fusobacterium* in different types of cheeses from the same operator in order to make assumptions about the sources of contamination and the conditions of multiplication

	Kind of milk	Organic	Protected Designation of Origin	Sample collection day after purchase		
Herve blanc	Cow	No	No	J-5	J-12	J-21
Herve doux	Cow	No	Yes	J-5	J-12	J-21
Herve doux	Cow	Yes	Yes	J-5	J-12	J-21
Herve piquant	Cow	No	Yes	J-5	J-12	J-21
Palet du vieux Moulin	Cow	No	No	J-5	J-12	J-21
Chèvre du vieux Moulin	Goat	No	No	J-5	J-12	J-21

Table 1. Table summarizing the different characteristics of the cheese samples

## Results

The genus *Fusobacterium* currently includes **19 species**, according to NCBI Taxonomy, among which some are known pathogenic bacteria. In a previous study, **full sequence of 16S rRNA gene** of this unknown *Fusobacterium sp.*, was aligned with **known sequences of *Fusobacterium spp.*** using Basic Local Alignment Search Tool (BLAST)(Table 1).  
Most sequences **corresponded to bacteria identified in marine environments or organisms**. The most closely related known species was *Fusobacterium perfoetens*.

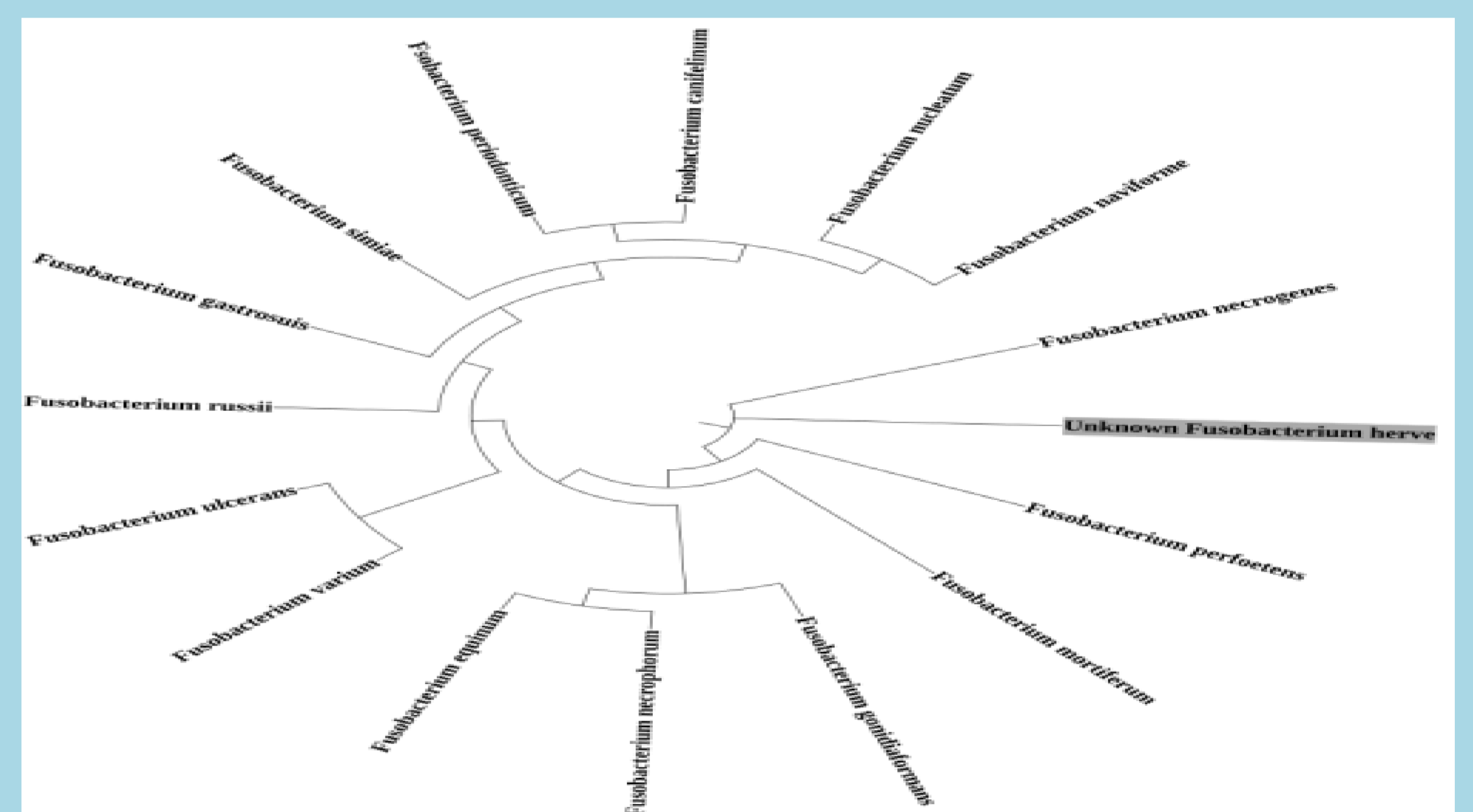


Figure 1. Phylogenetic tree of *Fusobacterium* genus, including the new putative *Fusobacterium spp.*

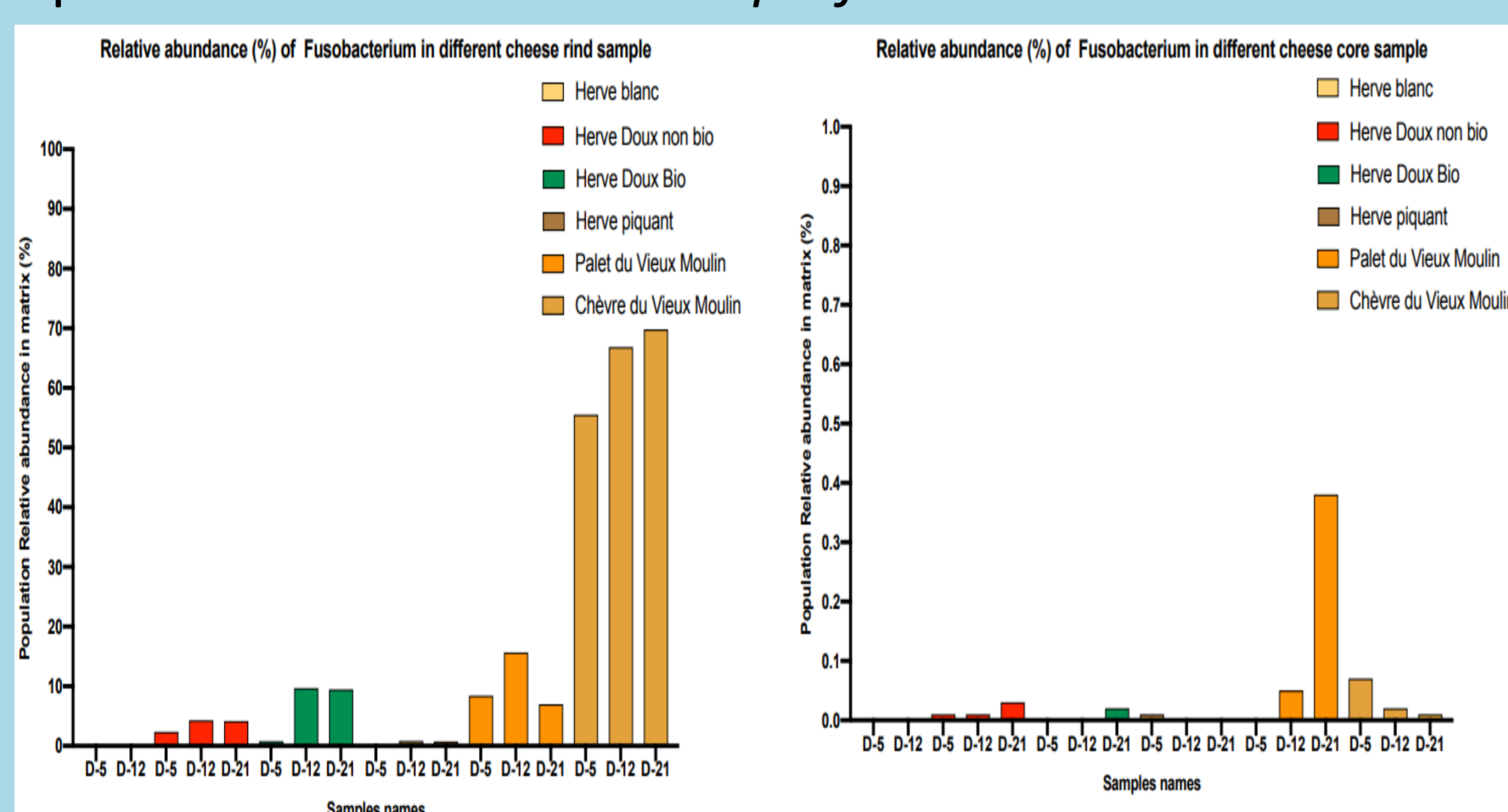


Figure 2. Relative abundance of the new putative species of *Fusobacterium* based on the metagenetic results

***Fusobacterium* DNA is present in every ripened cheese (Table 2).** The new species DNA is present in cheeses from all cow and goat milk origins, so it **does not come probably from the raw material**. The relative abundance of *Fusobacterium* is **higher on the rind of the cheeses** and the level is **very high** on the studied goat cheese rind, "**Chèvre du Vieux Moulin**".  
It has been impossible to date to isolate the new species from cheeses despite numerous trials. A new hypothesis to take into account is that this new species would come from the smear allowing to wash the cheeses.

## Conclusion

The new putative species DNA was found to be **present in all the washed ripened cheeses** with much **higher proportions in the rind samples** and not from unripened cheeses refuting probably the hypothesis that the bacterium has raw milk as a reservoir.  
New isolation tests will be conducted to isolate and characterize this bacterium from cheese and in the environment of the cheese dairy.