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# Genetic characterization of *Listeria monocytogenes* strains circulating in the pig manure management chain



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\* Shared contribution



## Introduction

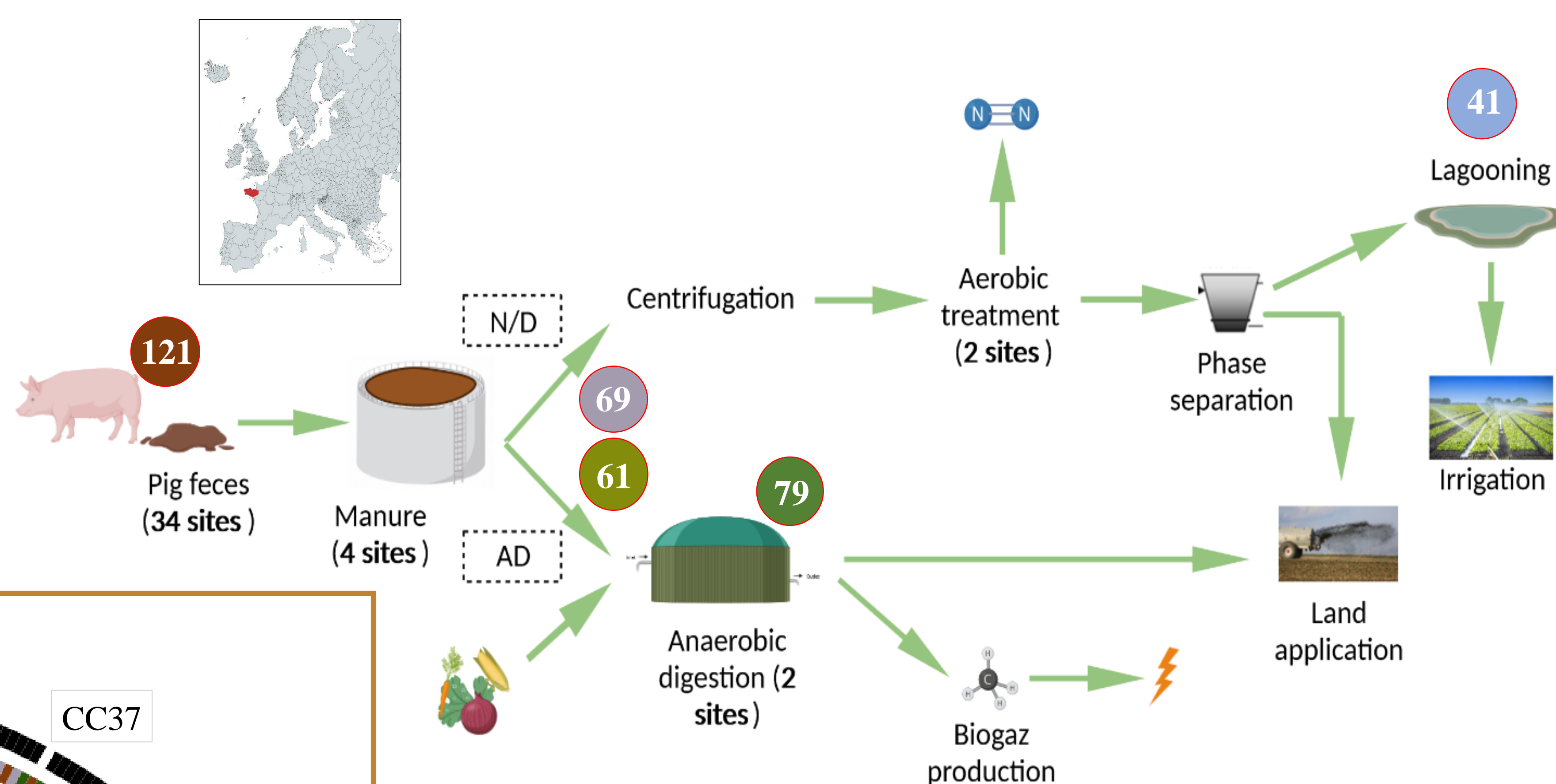
**Pig manure** is a valuable resource for renewable energy production and crop fertilisation, but inappropriate manure management can raise environmental issues. Consequently, technology blocks such as **anaerobic digestion (AD)** and **nitrification/denitrification (N/D)** can be added to the value chain to produce energy and organic fertiliser, and mitigate eutrophication issues.

Occurrence of pathogens along the value chain raises further concerns. *Listeria monocytogenes* is a zoonotic agent responsible for listeriosis and a genetically heterogeneous species. Strains are divided in **Clonal Complexes (CC)**. Some of these CC are more prevalent in specific ecological niches.

This project aims to analyse the genomic diversity of *L. monocytogenes* occurring throughout the pig manure management chain. In the “**One Health**” context, CC found along the manure treatment value chain were described, to draw a systemic and innovative picture of **pathogen flows from manure to land application, thus the food system**.

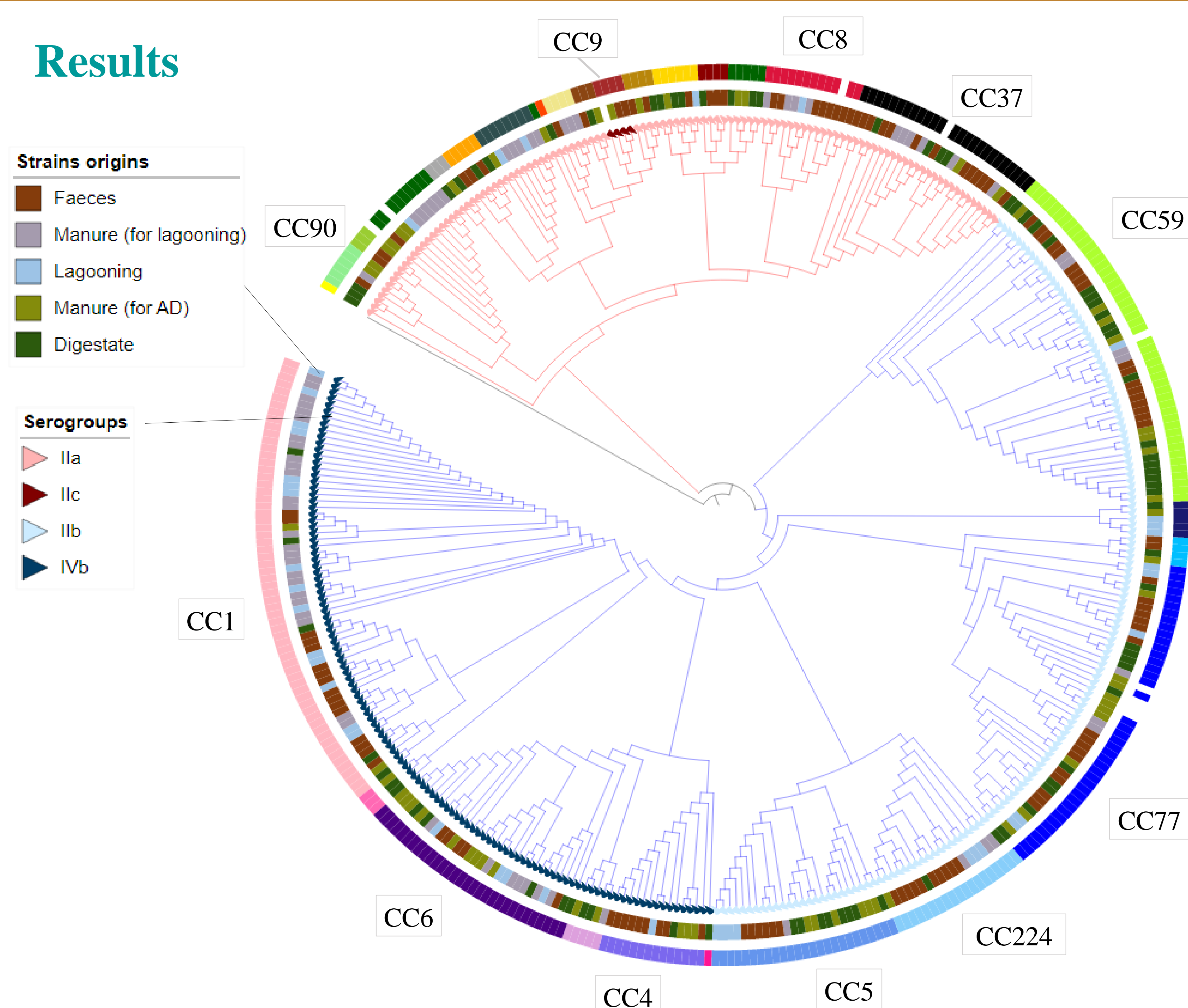
## Materials & Methods

- 371 strains collected from previous projects<sup>(1-2)</sup> (2008-2019)
- Genome sequencing using Illumina technology
- Reads assembly and genome annotation using ARTwork<sup>(3)</sup>
- Core-genome SNP identified with Snippy<sup>(4)</sup>
- Phylogenetic inference using the maximum likelihood (IQ-TREE)<sup>(5)</sup>



Overview of the two manure management strategies studied. Colored dots indicate the number of strains isolated and the sampling zones.

## Results



Core-genome SNP based phylogeny of *Listeria monocytogenes* strains. SNPs were identified using EGD-e (NC\_003210) as the reference genome, and a *Listeria innocua* strain was used to root the tree. Blue branches represent lineage I strains, red branches represent lineage II strains

- High diversity** (28 CC out of 371 strains)
- Over-representation of hypervirulent CC**: mainly CC1, and to a lesser extent, CC4 and CC6
- Low occurrence of the hypovirulent, food-associated CC**: CC9 and CC121 (0,8% and 0% respectively)
- Predominance of CC59, CC1, CC37, CC77, CC224 in **pig feces**
- Predominant CCs are conserved through N/D and AD, and only marginal CC shifts after treatment

## Discussion

- Manure treatments have **no significant impact** on *L. monocytogenes* strains diversity
- Hypervirulent clones are found all along** the value chain, up to treated manure applied on agricultural soils
- The clonal diversity differs from the diversity found in food processing environments and the food industry

In-depth **pangenome characterization** (virulence factors, plasmidic content) and **soil survival** assessment of the strains are on-going analysis. Genome-Wide Association Studies (**GWAS**) will then be conducted to identify **new genetic markers** linked to soil survival. These data can help future development of diagnostic tests for public health surveillance.

## References

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- Denis M *et al.*, Occurrence and diversity of *Listeria monocytogenes* isolated from two pig manure treatment plants (2022) Microbes and Environment
- <https://github.com/afelten-Anses/ARtWORK>
- <https://github.com/tseemann/snippy>
- <https://github.com/iqtree/iqtree2>