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Genomic diversity of *Listeria monocytogenes* in the pig manure recovery sector

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* Shared contribution

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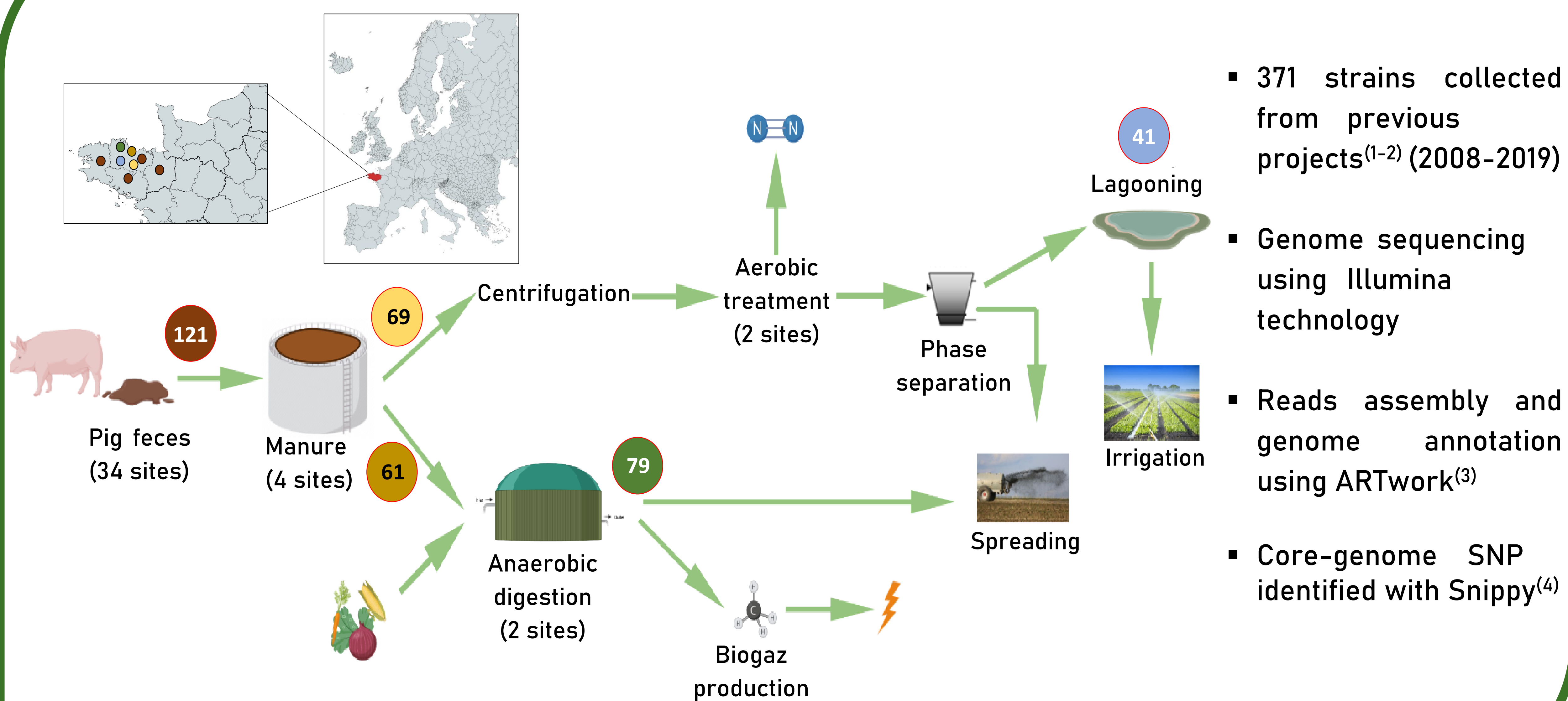
Introduction

Listeria monocytogenes is a zoonotic agent responsible for listeriosis and a genetically heterogeneous species. Strains are divided in Clonal Complexes (CCs). Some of these CCs are more prevalent in specific ecological niches.

Pig manure is widely used as a source of nutrients for crop production, but intensification of livestock production raises eutrophication issues. Consequently, manure treatment techniques, such as nitrification/denitrification (N/D) or anaerobic digestion (AD), were developed in order to reduce environmental impacts and to produce energy and fertiliser.

This project aims to analyse the genomic diversity of *L. monocytogenes* occurring throughout the pig manure management chain. In the "One Health" context, CCs found along the manure treatment chain were described, to acquire a systemic and innovative picture of pathogen fluxes from farm effluent management to the soils, thus the food system.

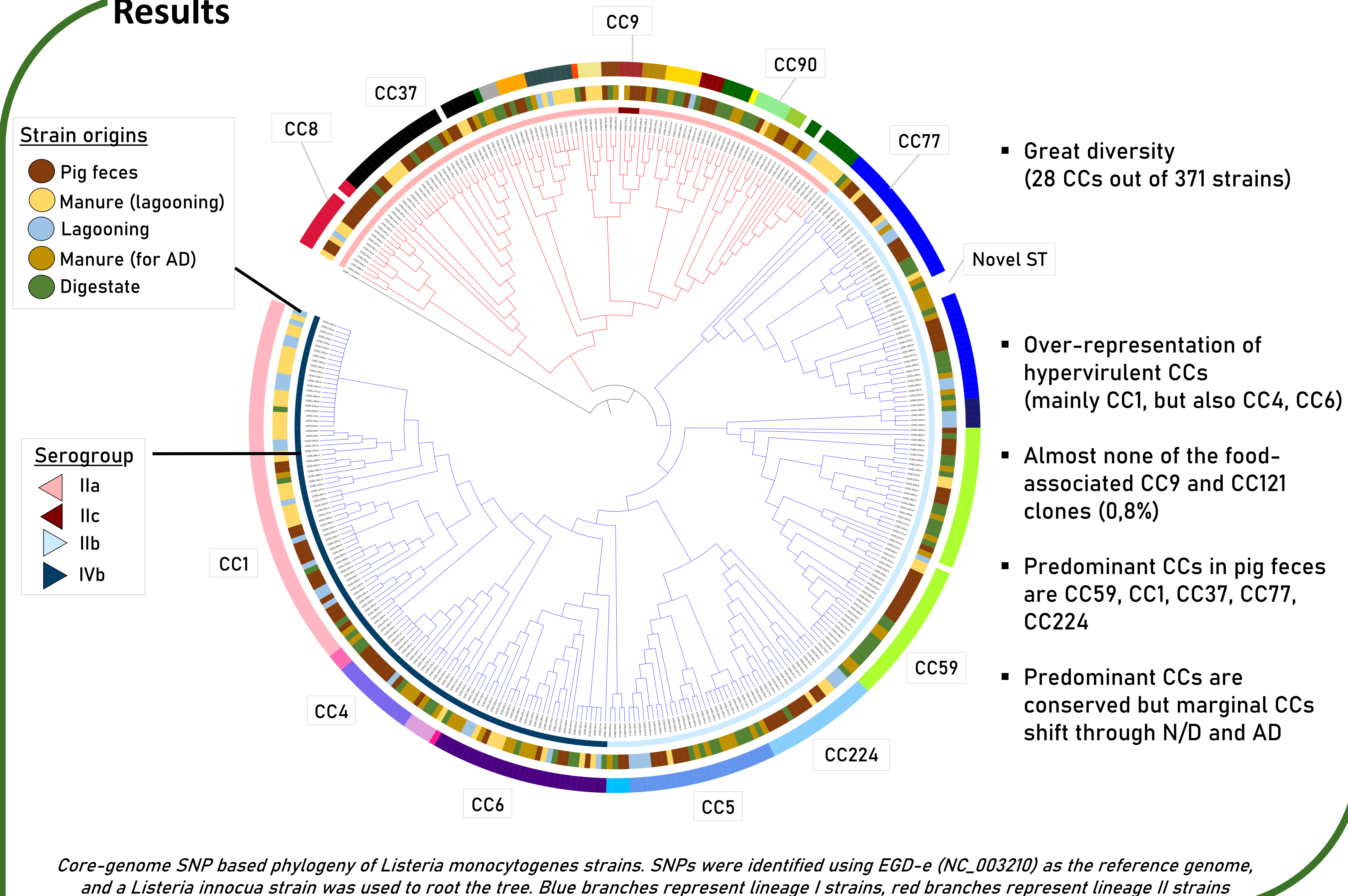
Materials and Methods



Overview of the manure treatments studied. Colored dots are placed at the sampling steps, and display the number of isolated strains

- 371 strains collected from previous projects⁽¹⁻²⁾ (2008-2019)
- Genome sequencing using Illumina technology
- Reads assembly and genome annotation using ARTwork⁽³⁾
- Core-genome SNP identified with Snippy⁽⁴⁾

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

- Great diversity (28 CCs out of 371 strains)
- Over-representation of hypervirulent CCs (mainly CC1, but also CC4, CC6)
- Almost none of the food-associated CC9 and CC121 clones (0,8%)
- Predominant CCs in pig feces are CC59, CC1, CC37, CC77, CC224
- Predominant CCs are conserved but marginal CCs shift through N/D and AD

Ongoing analysis:

- Pan-genome analysis → Identification of known genetic markers linked to virulence and soil survival
- Analysis of the genomic impact of the manure treatment techniques
- Phenotypic soil survival assessment of the strains
- Genome-Wide Association Studies (GWAS) → Identification of new genetic markers linked to soil survival

➔ Development of diagnostic tests for public health surveillance

1. Boscher E, Houard E, Denis M. Prevalence and distribution of *Listeria monocytogenes* serotypes and pulsotypes in sows and fattening pigs in farrow-to-finish farms (2012). J Food Prot. 2012.
 2. Denis M, Ziebal C, Boscher E, Picard S, Perrot M, Vila Nova M, Roussel S, Diara A, Pourcher A, Occurrence and diversity of *Listeria monocytogenes* isolated from two pig manure treatment plants (2022) Microbes and Environment
 3. <https://github.com/afelten-Anses/ARTWORK>
 4. <https://github.com/tseemann/snippy>