

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

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Abstract

The objective of this study is to gain a better understanding of the genetic diversity of *Listeria monocytogenes* (*L. monocytogenes*) strains circulating in the pig manure management chain, and to assess transmission routes in the continuum from living pigs, manure processing and application of processed manure on agricultural soils.

Therefore, a collection of 371 *L. monocytogenes* strains isolated along the value chain was characterized using whole-genome sequencing. High genomic diversity (28 CC) was observed in the dataset and, interestingly, the prevalence of CC known to be associated with clinical listeriosis cases (hypervirulent CC) was high: seven hypervirulent CC represented 38.5% of the dataset, CC1 being the most represented with 17% prevalence. These findings highlight the ability for *L. monocytogenes* to persist throughout the chain up to the amended soils. Soil fitness assessment of the isolates will provide further information on the potential of *L. monocytogenes* to circulate within the food chain.

Introduction

Pig manure is widely used for crop production because of its excellent source of nutrients (nitrates, phosphorus). However, intensification of livestock production over the past decades in Europe raised environmental issues such as eutrophication. Consequently, manure treatment processes were developed in order to reduce these environmental impacts and to fully use the potential of manure for the production of renewable energy and biofertilisers. Nitrification-denitrification is an aerobic/anoxic manure treatment designed to mitigate nitrate pollution of soil and water. Anaerobic digestion allows energy recovery by producing methane, promoting a circular bioeconomy. The endpoint of these processes is land spreading of the processed manure as fertiliser and soil improver. This can potentially lead to the reintroduction of zoonotic pathogens in the environment and the food system. Pathogen flows between pig manure management and food sectors are currently poorly understood, in particular for *L. monocytogenes*.

L. monocytogenes is a ubiquitous foodborne pathogen and the causative agent of listeriosis, a rare but severe disease. Most strains of this genetically heterogeneous species can be grouped into major MLST-clonal complexes (CC). Hypervirulent, medium virulent and

hypovirulent CC have been identified by combining epidemiological, clinical and experimental approaches (Maury *et al.* 2016), or risk assessment approaches (Fritsch, Guillier, and Augustin 2018).

In the “One Health” context, this study aims to acquire a systemic and innovative picture of *L. monocytogenes* flows from manure management to the food system. The main objective is to analyse the genomic diversity of *L. monocytogenes* occurring throughout the pig manure management chain and to compare it to the overall diversity found in food and agricultural environments.

Materials and methods

A collection of 371 strains previously isolated from the pig manure management chain between 2008 and 2020 in the Brittany region, France, was used in this study (Boscher, Houard, and Denis 2012, Le Maréchal *et al.* 2019, Denis *et al.* 2022). One hundred twenty-one strains were isolated from pig faeces at farm, 110 before and after denitrification and after anaerobic digestion. DNA extraction and whole-genome sequencing were performed. treatment and 140 before Genomes were assembled and CC were predicted using the Artwork workflow (<https://github.com/afelten-Anses/ARTWORK>). Variant calling was performed with Snippy 4.6.0, using *L. monocytogenes* EGD-e (NC_003210.1) as the reference genome (Seemann 2015). The core-genome SNP phylogenetic tree was created using IQ-TREE 2.1.2 and *L.innocua* Clip11262 (NC_003212.1) was used to root the tree (Nguyen *et al.* 2015).

Results

Figure 1 presents the core-genome SNP phylogenetic diversity of the strain collection. Twenty-eight CC were identified along the pig manure treatment chain. No correlation between CC and the origin of the strain could be found. Hypervirulent clones were over-represented in all compartments of the chain, in the continuum from animals to processed manure, representing 38.5% of the dataset (CC1: 17%; CC6: 8%; CC224: 5.1%; CC4: 3.8%; CC207: 2.2%; CC7: 1.6%; CC220: 0.8%). They are consistent with the CC found in the pork industry, because most of these hypervirulent CC have also been found in living pigs (Félix *et al.* 2018), slaughtered pigs (Oswaldi *et al.* 2022) and pork products (Luo *et al.* 2017).

The prevalence of hypovirulent clones (CC9 and CC121) was low (0.8% and 0% prevalence, respectively) unlike what is observed in the pork processing industry (Félix *et al.*, 2018). CC (CC37, CC59 and CC77) known to be specifically associated with pigs at farm (Félix *et al.* 2018) were prevalent in this dataset, in all compartments of the value chain.

Discussion

L. monocytogenes was found throughout the entire manure management chain. These results show that hypervirulent strains are prevalent in

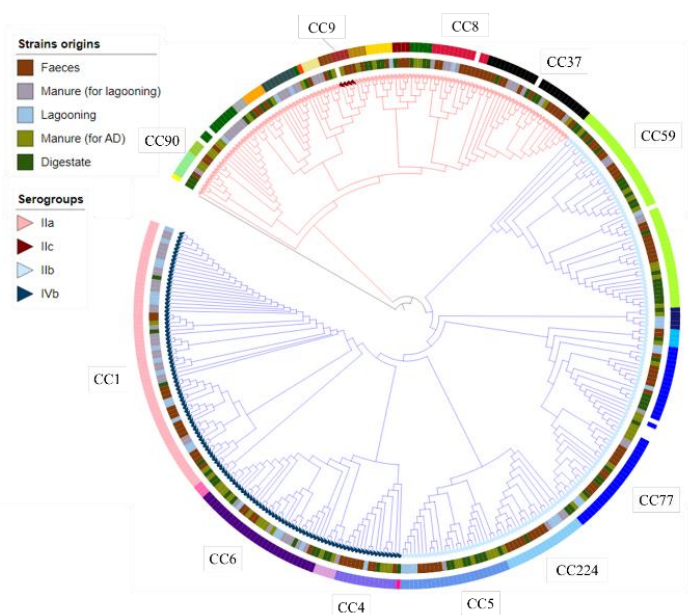


Figure 1. Core-genome SNP based phylogeny of *L. monocytogenes* strains. Blue branches represent lineage I strains, red branches represent lineage II strains.

the processed manure eventually spread on agricultural land. CC1, the most prevalent CC in this study, has been previously associated with pork-related outbreaks (Cantinelli *et al.* 2013). This stresses out the need to assess the fate of *L. monocytogenes* after land application and its potential reintroduction in the food chain.

In-depth genetic characterization of virulence and resistance genes as well as plasmid and prophage content of the strains will allow us to better understand the adaptive mechanisms allowing *L. monocytogenes* to survive throughout the chain. Further experiments will be performed to investigate intrinsic factors underlying survival throughout the value chain from farm effluent to agronomic land spreading. The fitness of these strains in soil and their potential to exploit soil specific resources will be assessed. Pan-Genome-Wide Association Study (GWAS) will be used to identify genetic markers linked to soil survival, which could be further used to develop diagnostic tests for public health surveillance.

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