Conclusion
Increased resistance against Salmonella colonization in pigs could reduce food safety concerns by lowering Salmonella spread and transmission from farm to fork. These findings indicate that Salmonella shedding in pigs is associated with certain genetic variants and suggests that SNPs in innate immune genes could be used to breed pigs that are more resistant to Salmonella colonization and shedding. Further work, including the impact of these variants on production parameters, is required to evaluate the potential to include the identified genetic markers involved in resistance to Salmonella infection in swine improvement programs in the swine genetic industry. This study examined Salmonella shedding at one point in time, (i.e. at weaning) but further studies are needed to determine SNPs that are associated with carrier stage and intermittent shedding of Salmonella in pigs from birth until marketing.

Acknowledgements
We would like to thank the OMAFRA Food Safety Research Program, and Swine Innovation Porc for funding this research.

References

Abstract
Listeria monocytogenes (L. monocytogenes) is an ubiquitous bacterium that causes a severe foodborne illness. It is established that the contamination of food production facilities over long time period, are potentially one of the major food product contamination sources. L. monocytogenes persistence was observed in almost all food sectors and particularly in pork production facilities. The characterization of such L. monocytogenes contamination is therefore crucial to improve the food safety and prevent outbreaks. These strains are called persistent but this trait remains loosely defined, and no genetic determinants have been firmly associated with it. This study aims at identifying molecular markers associated with the persistence. A panel of 13 presumed persistent (PP) strains, versus 9 strains not exposed to food processing environment (NEP) strains, was constructed from the databases of the French Institute for Pig and Pork Industry (Ifip), the French National Reference Laboratory (NRL) and the European Union Reference Laboratory (EURL), the last two are hosted by Anses.

The genome sequences obtained in the present study were compared to 180 genomes of the Anses strains reference collection and 340 genomes publicly available. Two analysis were performed on the genomes, (i) an allele diversity analysis of 14 loci gathered from a review of significant functions potentially involved in the persistence capacity and (ii) a whole genome variant calling analysis to detect single nucleotide polymorphisms (SNPs) and insertions, deletions specific of persistent strains.

The preliminary results were obtained on 4 strains. The comparison of these whole genome sequencing (WGS) data with those of the whole strain panel is ongoing.

Introduction
Listeria monocytogenes (L. monocytogenes) is an ubiquitous Gram-positive bacterium responsible for a foodborne disease: the listeriosis. Its ability to survive and thrive within the environment and readily colonize a wide range of substrates make this bacterium able to persist in the food factory environment. Food production facilities are thus potentially one of the major food product contamination sources.

In France, L monocytogenes contamination in foods is extensively surveyed to improve the food safety and prevent outbreaks (Tournджman et al., 2014). The pork sector was strongly impacted by several health crisis, in particular in 1993 (Goulet et al., 1998), 1999 and 2000 (de Valk et al., 2001). At European level, from 2006 to 2012, L. monocytogenes prevalence in veal meat products was reported at the second rank for products at risk (EFSA-ECDC, 2010, 2014). In USA food authority reports showed the relatedness between consumption of contaminated ready to eat deli meats and listeriosis cases (Pradhan et al., 2010).

L monocytogenes can persist in food associated environments for months to years in particular in companies that process pork meat (Ferreira et al., 2011, Giovannaci et al., 1999). This study aims to identify molecular markers underlying the phenomenon of persistence. For this, we investigated the whole genome of a panel of strains presumed persistent (PP), mainly isolated from pork processing environments, versus...
strains not exposed to processing environment (NEP). PP strains were selected according to the criteria defined in the literature (PFGE typing and isolation timeline). The genome sequences obtained in the present study were compared to 180 genomes of the Anses strains reference collection and 340 genomes publicly available. Two analysis were performed on the genomes, (i) an allele diversity analysis of 14 loci gathered from a review of significant functions potentially involved in the persistence capacity and (ii) a whole genome variant calling analysis to detect single nucleotide polymorphisms (SNPs) and insertion deletions specific of persistent strains.

Material and Methods

Strain origin

A panel of 13 presumed persistent (PP) strains, versus 9 strains not exposed to food processing environment (NEP), was selected from the databases of the French Institute for Pig and Pork Industry (Ifip), the French National Reference Laboratory (NRL) and the European Union Reference Laboratory (EURL). Persistent strains shared indistinguishable PFGE profiles and were isolated regularly in the production chain or in the final product during at least one year. When possible, two PP strains were isolated per production site over, at least a one year period. In total 8 PP strains were collected from six companies that process pork meat (delicases producer) and five PP strains were isolated from another production sector (fish processing or cheese production). Each PP strain was associated to a NEP strain that share less than 6 bands of difference on Ascl or Apal profiles. NEP strains were provided by Anses. These strains were isolated from animal at farm, wild animal or raw products.

PFGE typing

The strains were typed using the Anses protocol (Roussel et al., 2014). All the PFGE profiles were analyzed according to the Anses profile interpretation protocol (Roussel et al., 2014). PP and NEP strains Ascl and Apal profiles were selected using BioNumerics software V 7.5 (Applied Maths, Kortrijk, Belgium) according to number of band differences (tolerance and optimisation set at 1%). All PFGE profiles were compared in a UPGMA dendograms using Dice coefficient optimisation and tolerance set at 1%.

Whole genome sequencing

The strain extraction was performed using Promega Wizard® Genomic kit (Promega, Charbonnieres, France) according to manufacturer recommendations with minor modifications. Genomic libraries were prepared for sequencing using the Ion XpressPlus Fragment Library kit (Life Technologies, Marly le Roi, France). The sequencing was performed on an Ion Proton Plateform (Life Technologies).

Automated genome assembly and annotation

Raw read quality was assessed with the software FastQC and trimmed using Trimmomatic 0.33. The de novo assembling was performed using spades 3.1.1 and mira 4.0rcl in parallel. Contig were rearranged on reference genomes using progressivebMauve. Finally the annotation of the genomes was performed using the annotation pipeline Prokka against all reference genomes available for L. monocytogenes on NCBI. Visualisation and analysis of the genetic loci was performed using the sequence alignment module of BioNumerics 7.5.

Bibliographic research of genetic loci potentially involved in the persistence capacity

Major function involved in persistence were selected through reviews dealing with persistence of L. monocytogenes. For each major function involved, significant results from proteomic or mutagenesis studies were prioritize to select genomic loci shown as essential. For each locus, the gene flanking regions were considered.

Results

To date, four strains out of 22 were already sequenced: three PP (1, 6, 20) and one NEP (7). The sequencer raw data were assembled and annotated in order to perform the allele diversity analysis, all assembling data are given in Table 1.

Table 1: technical specifications of assembling and annotation

<table>
<thead>
<tr>
<th>Strains</th>
<th>Number of contigs</th>
<th>Coverage</th>
<th>Contig size</th>
<th>CDS predicted (Prokka annotation pipeline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>197</td>
<td>119,58</td>
<td>3,11</td>
<td>5486</td>
</tr>
<tr>
<td>20</td>
<td>61</td>
<td>85,48</td>
<td>3</td>
<td>3326</td>
</tr>
<tr>
<td>7</td>
<td>213</td>
<td>79,72</td>
<td>3,19</td>
<td>3553</td>
</tr>
<tr>
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<td>2,9</td>
<td>3265</td>
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Genetic loci used for allele diversity analysis were selected through a bibliographic research of significant genes related to three main bacterial functions: cold resistance, biofilm and resistance to disinfectant (Table 2).

Discussion

Whole genome sequencing (WGS) analysis opened the possibility of genome wide approach for foodborne pathogens analysis. The analysis of 14 loci on a large strains panel is made possible by this technology. In the continuation of this study, (i) the WGS variant calling analysis will be performed to detect single nucleotide polymorphisms (SNPs) and insertion deletion using an automated pipeline to detect small insertions and deletions, previously published (Radomski et al., 2015). (ii) The allele diversity analysis of the 14 loci identified will be performed in parallel. Specific mutation found on PP strains will be compared to 180 genomes of the Anses strains reference collection representative of the L. monocytogenes genetic diversity in food products in France over 20 years and 340 genomes publicly available. The final goal is to analyse 30 PP strains at least and a minimum of 10 NEP strains related to each single PP. Finally this work would yield PP specific mutations. Such specific genetic markers would make out the design of a forthcoming quick molecular detection tool for persistence strain detection in food processing environments.
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Epidemiology and control of hazards in pork production chain – SAFEPORK
One health approach under a concept of farm to fork

Table 2: list targeted through bibliographic research for the allele diversity analysis

<table>
<thead>
<tr>
<th>Number</th>
<th>Main function</th>
<th>Genes</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Replication inhibitor</td>
<td>cspD</td>
<td>Replication inhibitor</td>
</tr>
<tr>
<td>2</td>
<td>RNA chaperon protein</td>
<td>cspB</td>
<td>RNA chaperon protein</td>
</tr>
<tr>
<td>3</td>
<td>RNA chaperon protein</td>
<td>cspA</td>
<td>RNA chaperon protein</td>
</tr>
<tr>
<td>4</td>
<td>Aerogel transposon</td>
<td>aacA</td>
<td>Aerogel transposon</td>
</tr>
<tr>
<td>5</td>
<td>Flagellar operon</td>
<td>leuO/T75-0718</td>
<td>Flagellar operon</td>
</tr>
<tr>
<td>6</td>
<td>DNA repair protein</td>
<td>recO</td>
<td>DNA repair protein</td>
</tr>
<tr>
<td>7</td>
<td>Potentially associated protein</td>
<td>hpl</td>
<td>Potentially associated protein</td>
</tr>
<tr>
<td>8</td>
<td>Entomopelidase - melittic agarase</td>
<td>leu5</td>
<td>Entomopelidase - melittic agarase</td>
</tr>
<tr>
<td>9</td>
<td>ABC transporters - export quaternary signal AI2</td>
<td>leu1746</td>
<td>ABC transporters - export quaternary signal AI2</td>
</tr>
<tr>
<td>10</td>
<td>Transposable elements - offsite pump</td>
<td>Tol188</td>
<td>Transposable elements - offsite pump</td>
</tr>
<tr>
<td>11</td>
<td>CpxA-associated protein</td>
<td>CpxA</td>
<td>CpxA-associated protein</td>
</tr>
<tr>
<td>12</td>
<td>RNA chaperon protein</td>
<td>CpxB</td>
<td>RNA chaperon protein</td>
</tr>
<tr>
<td>13</td>
<td>Replication inhibitor</td>
<td>CpxD</td>
<td>Replication inhibitor</td>
</tr>
<tr>
<td>14</td>
<td>UDP-glucose phosphatase</td>
<td>leu1078</td>
<td>UDP-glucose phosphatase</td>
</tr>
</tbody>
</table>

Conclusion

This study is a bioinformatics and statistical challenge, with regard to the amount of data generated by WGS and the complex genetic background that drives persistence.

Acknowledgements

This work was conducted as part of the 2015 activities of the European Union Reference Laboratory for L. monocytogenes and was supported by a grant from the European Commission’s Directorate General for Health and Food Safety (DG SANTE).

References


Although pork is considered an important source of Salmonella infections, the introduction of control programs in pig farms is not obligatory in the EU. To resolve current epidemiological situation, monitoring of pig farms was introduced in Poland in 2014. The paper reports the first year outputs of the survey. Sampling plan targeted breeding and fattening herds (150 each) located in all regions of the country, proportionally to number and size of the farms. Convenience sampling of each herd included: 1) a pair of boots swabs taken during sampling of 2) dust and 3) slurry swabs. Samples were tested according to ISO 6579:2003/A1:2006 followed by serotyping according to White-Kaufman-Le Minor scheme. Salmonella were found in 50 out of 281 herds: 21 out of 147 breeding (14,3%) and 29 of 134 (21,6%) fattening herds. Positive results were more frequently noted in the herds: 1) located in the region with the highest density of pig farms, 2) originating from the biggest farms (≥5000 fatteners or ≥500 sows), 3) fed with commercial feeds, 4) with high health status, and 5) with Al/AO procedure. Irrelevant for Salmonella occurrence were: herd production type, previous record of salmonellosis, and antimicrobial usage. The pathogen was isolated from all three tested samples in seven herds. Two and single sample were positive in, respectively, 10 and 33 herds. Of 14 serovars noted, seven were found in breeding herds and 12 in fatteners. Two farms were contaminated with two serovars. Monophasic Salmonella Typhimurium was the most common serovar found in 32 samples and 21 herds, followed by S. Derby (9 herds) and S. Typhimurium (7 herds). The study revealed higher than expected frequency of Salmonella contamination of pig farms caused by diverse serovars of public health relevance. Some surprising risk factors will be further investigated.

34. Salmonella contamination of pig farm environment, Poland, 2014

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