**RECORD OF GENOMIC ANALYSIS** Research and Development Section

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| --- | --- | --- | --- | --- | --- | --- | --- |
| OLC Reference: | | ROGA-YYYY-MM-DD *Listeria* | |  | Sequencing Analyst: | |  |
| Date received at OLC: | | YYYY-MM-DD |  | | | Bioinformatics Analyst: |  |
| Date sequenced: | | YYYY-MM-DD |  | | | Report author: |  |
|  |  | |  | | | Verified by: |  |
|  |  | |  | | | Location of main file: | Room B5 |

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| --- |
| **Identification Summary:**  2 strains (see Table 1) were submitted for whole-genome sequencing and confirmed to be *Listeria monocytogenes* based on detection of probe sequences (e-probes) indicating the presence of IGS, *hlyA*, and/or *inlJ* genes.  **Quality Control Analysis:**   * Multilocus sequence typing (MLST):   + Isolate BUR-FI-2017-MI-00016-5 was sequence type (ST)-101. The most recent isolates matching this genotype were isolated from RTE Fish-Eel in March 2017 (See RDIMS#9262619).   + Isolate BUR-FI-2017-MI-00032-5 was sequence type (ST)-199. The most recent isolates matching this genotype were isolated from RTE Fish-Eel on 2017-03-17. * To determine degree of relatedness to previous strains isolated at the CFIA with the same sequence type, strains were further analyzed to identify single nucleotide variants (SNVs). This approach compares the entire genome sequence (approximately 3 million bases) to identify the number of nucleotides that are different in at least one strain in the set of strains compared. SNVs were identified using the NML SNVphyl pipeline (v1.0), using 2016-SEQ-1310 as a reference genome. Biorequest # 8732.   + Maximum-likelihood phylogeny based on 141 high quality core genome SNV positions identified amongst 3 genomes over 96.08% of the reference genome is shown in **Figure 1**.   + All of the new isolates have between 27 and 127 SNVs compared to historical isolates with the same genotype (**Figure 1, red boxes**)   Multilocus sequence typing (MLST):   * Isolate BUR-FI-2017-MI-00032-5 was sequence type (ST)-199. Isolates with this genotype are commonly recovered from CFIA’s food testing program (2009 to present).   **Quality Control Analysis:**   * To determine degree of relatedness to previous strains isolated at the CFIA with the same sequence type, strains were further analyzed to identify single nucleotide variants (SNVs). This approach compares the entire genome sequence (approximately 3 million bases) to identify the number of nucleotides that are different in at least one strain in the set of strains compared. SNVs were identified using the NML SNVphyl pipeline (v1.0), using 2016-SEQ-0413 as a reference genome. Biorequest # 8731.   + Maximum-likelihood phylogeny based on 491 high quality core genome SNV positions identified amongst 35 genomes over 92.43% of the reference genome is shown in **Figure 2**.   + All of the new isolates have between 52 and 162 SNVs compared to historical isolates with the same genotype (**Figure 2, red boxes**) * These isolates do not match CFIA *Listeria monocytogenes* control strains (MLST: ST-122 and ST-85) |

**Table 1. GeneSeekr Analysis.**

(Information submitted by OLF PFGE unit is shaded in grey)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PFGE** | | | | | ***Listeria monocytogenes* markers***a* | | | | **MLST***b* | | | | | | | |
| **Strain** | **Genus/ Species** | **Source** | **1'Enzyme** | **2'Enzyme** | **IGS** | | **hylA** | ***inlJ*** | **ST** | | **abcZ** | **bglA** | **Cat** | **dapE** | **dat** | **ldh** | **lhkA** |

● Indicates that marker is present

*aListeria* markers are IGS (intergenic region of the 16S-23S rRNA operon) which is indicative of the genus *Listeria*, and *hlyA* (hemolysin) and *inlJ* (internalin) which are markers of *L. monocytogenes**[**1**]*[*.*](#_ENREF_1)

*b*Multi locus sequence type (MLST) is based on exact sequence matches to alleles within the international 7 gene scheme maintained at http://www.pasteur.fr/mlst[2][. Strains with the same sequence type (ST) have exact matches at all 7 alleles.](#_ENREF_2)

**Table 2:** **Sequence Data Quality:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **SEQ ID** | **Total length*a*** | **Coverage*b*** | **% identity GDCS*c*** | **rSTd** | **Pass/Fail** | **Pipeline version** |

*a*Size of a *Listeria monocytogenes* genome typically ranges between 2.8 and 3.2 Mb. Total length refers to the total number of nucleotides in the assembled sequence data.

*b*Coverage refers to sequencing redundancy. A minimum coverage of 20 indicates that, on average, each nucleotide in the genome is represented in 20 sequence reads.

*c*To ensure complete coverage, sequence data is queried for Genomically Dispersed Conserved Sequences (GDCS) which currently include 53 ribosomal proteins distributed throughout the genome and conserved in all bacterial species (total of ~20000 nt sequence data). The % identity GDCS refers to the cumulative percentage of identical nucleotides to known alleles of these genes.

*d*Ribosomal Multilocus sequence type (rST) is based on the 53 gene scheme available at http://pubmlst.org/rmlst/ [3]. Strains with the same sequence type (ST) have exact matches at all 53 alleles.