# Genome analysis of *Listeria ivanovii* strain G770 that caused a deadly aortic prosthesis infection

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## Abstract

We sequenced the genome of *Listeria ivanovii* strain G770, which caused a deadly infection of the thoracic aortic prosthesis of a 78-year-old man. The 2.9 Mb genome exhibited 21 specific genes among *L. ivanovii* strains, including five genes encoding a type I restriction modification system and one glycopeptide resistance gene.



**Keywords:** *Listeria ivanovii*, vascular infection, genome sequence, pathogenesis, type I restriction system
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## Introduction

The genus *Listeria* is composed of 17 species, including two that are recognized pathogens. Of these, *L. monocytogenes* is considered as the only human pathogen causing listeriosis, a highly lethal opportunistic infection caused by ingestion of contaminated food [1]. The second pathogenic species is *L. ivanovii,* which mostly responsible for abortions, stillbirths and neonatal septicaemias in sheep and cattle [1]. However, rare cases of human infection by this species have been published. To date, eight human *L. ivanovii* infections have been reported, including four cases of bacteraemia and one case each of mesenteric adenitis, uterine discharge and stillbirth (Table 1). The eighth case was not detailed [3].

Here we report a rare case of *L. ivanovii* vascular infection in a 78-year-old man. We applied real-time genomics to compare the genetic content of this strain to those of other *L. ivanovii* strains available in public databases.

## Patient and Methods

On 29 November 2014 a 78-year-old man was hospitalized in Timone Hospital, Marseille, France, for suspected infectious endocarditis. The patient had had a mechanical Bentall prosthesis inserted in 2010 as well as a mitral valve regurgitation repaired in 1998. He also had a dissection of the descending thoracic aorta. Over the past 10 days the patient had developed fatigue, fever, chills and concomitant back pain. Transesophageal echocardiography revealed moderate leakages of the mitral and mechanical aortic valves but no vegetation. However, because endocarditis was suspected, empirical intravenous amoxicillin (12 g/d) and gentamicin (3 mg/kg/d) was initiated after blood was collected for culture. On 4 December, as a result of a persistent inflammatory syndrome (C-reactive protein level 264 mg/L), a new aortic paraprosthetic leakage was detected by transesophageal echocardiography. A thoracic computed tomographic scan revealed increased size of the previously known descending aorta dissection. Blood cultures and systematic serology assays (*Aspergillus* sp., *Bartonella* sp., *Brucella* sp., *Legionella pneumophila, Mycoplasma pneumoniae,* Q fever) were negative. Antibiotics were changed to intravenous imipenem (3 g/d) and vancomycin (2 g/d). On 24 December liposomal amphotericin B (3 mg/kg/d) was added to treat a persistent biological inflammatory



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**TABLE 1. Studies reporting human cases of *Listeria ivanovii* infections [2]**

| Clinical condition | Sex | Age (years) | Underlying condition | Year reported | Study |
|---|---|---|---|---|---|
| Unknown | Unknown | 79 | Unknown | 1971 | [3] |
| Uterine discharge | F | Unknown | Pregnancy | 1985 | [4] |
| Mesenteric adenitis | Unknown | Unknown | Unknown | 1985 | [4] |
| Stillbirth | F | Unknown | Pregnancy | 1990 | [5] |
| Bacteraemia | M | 26 | AIDS, lymphoma | 1994 | [6] |
| Bacteraemia | M | 39 | Substance abuse | 1994 | [7] |
| Bacteraemia | M | 64 | Hepatic carcinoma | 2006 | [29] |
| Gastroenteritis, Bacteraemia | M | 55 | Immunosuppression | 2007 | [2] |
| Aortic prosthesis infection | M | 78 | Immunosuppression | 2015 | This study |

syndrome. On 30 December the patient underwent a right posterolateral thoracotomy for resection of a mediastinal abscess. Culture of peroperative specimens were positive for a *Listeria* isolate that was identified as *L. ivanovii* using 16S rRNA sequencing (99.9% identity with GenBank accession no. CP009577). The antibiotic therapy was changed again to intravenous amoxicillin (12 g/d) and gentamicin (3 mg/kg/d). However, on 8 January 2015 the patient developed respiratory distress and was transferred to the intensive care unit, where atrial fibrillation and pleural effusion were diagnosed. On 14 January the patient underwent pleural drainage, right axillofemoral bypass, reimplantation of the subclavian artery on the common carotid artery, removal of the thoracic endoprosthesis and closure of the aortic stump. However, his condition deteriorated, and the patient died on 23 January.

## Genome sequencing

*Listeria ivanovii* strain G770 was deposited in the CSUR collection (WDCM 875) under reference P1995. Genomic DNA of *Listeria ivanovii* was extracted using an EZ-One automate (Qiagen, Hilden, Germany). Genomic DNA was quantified by a Qubit assay with the high sensitivity kit (Life Technologies, Carlsbad, CA, USA) to 10.8 ng/μL. Genomic sequencing was performed using the paired-end strategy. Genomic DNA (1 ng) was used to prepare the paired-end library with the Nextera XT DNA sample prep kit (Illumina, San Diego, CA, USA). The tagmentation step fragmented and tagged the DNA. Then limited-cycle PCR amplification (12 cycles) completed the tag adapters and introduced dual-index barcodes. After purification on AMPure XP beads (Beckman Coulter, Fullerton, CA, USA), the library was sequenced on the MiSeq sequencer (Illumina) in a single 39-hour run in 2 × 250 bp. A total of 455 380 paired-end reads was obtained for this project, with a 871K/mm$^2$ cluster density with a cluster passing quality control filters of 80.5%. The reads were trimmed and filtered according to the read qualities.

## Bioinformatic analysis

The reads obtained after sequencing were assembled using the A5 assembler [9]. Then a finishing step was performed with the Mauve aligner software and CLC bioserver [10]. After assembly and finishing, the size of the genome was 2.9 Mb. Open reading frames (ORFs) were predicted by Prodigal software (http://prodigal.ornl.gov/) with default parameters. Functional annotation was done by comparison of ORF sequences to the GenBank [11] and Clusters of Orthologous Groups (COGs) database using BLASTP. tRNAs and rRNAs were detected using tRNAscan-SE v.1.21 [12] and RNAmmer 1.2 [13], respectively.

The absence of plasmids was verified both by searching the gene annotation for any plasmid-related gene and by mapping all contigs against previously published *Listeria* plasmid sequences.

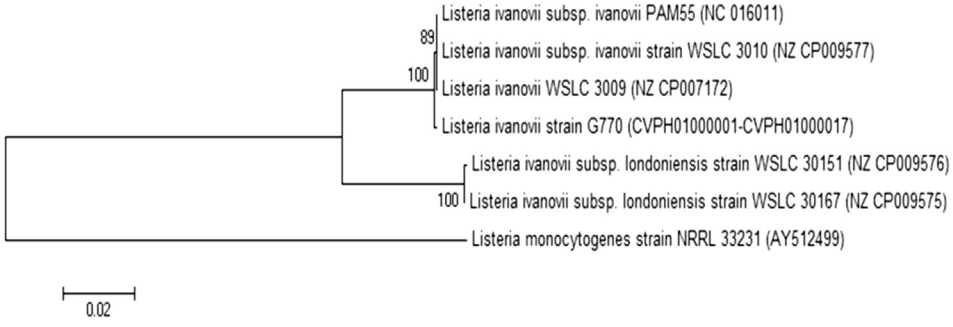


**FIG. 1.** Phylogenetic analysis of *Listeria ivanovii* strains based on comparison of sequences from *hlyA* gene using MEGA 6 software with neighbour-joining method and Kimura two-parameter model. Genome sequence accession numbers are indicated in parentheses. Numbers at nodes indicate results of bootstrap resampling ($n$ = 1000). *Listeria monocytogenes* was used as outgroup. Scale bar = 2% nucleotide differences.

To identify the most closely related *L. ivanovii* genomes, we performed a phylogenetic analysis (Fig. 1) by comparing the sequences of the *hlyA* gene [14] using the neighbour-joining method and MEGA 6 software [15]. Subsequently we compared the genomic content of strain G770 and those of the most closely related *L. ivanovii* strains available in GenBank. The genome sequence similarity between studied strains was evaluated with GGDC software (http://ggdc.dsmz.de).

The analysis of virulence factors was performed by searching the genes already identified as responsible for virulence in *L. ivanovii* (six genes of the LIPI-1 cluster, internalin genes, genes of stress survival islet 1 (SS1)) and by screening every gene that was specific to strain G770 for a putative role in virulence. The CRISPRFinder algorithm was used to identify putative CRISPR (clustered regularly interspaced short palindromic repeat) loci [16].

### Nucleotide sequence accession number

The genome sequence from *L. ivanovii* strain G770 was deposited in GenBank under accession number CVPH01000001–CVPH01000017.

## Results

The draft genome sequence of *L. ivanovii* strain G770 consisted of 17 scaffolds after assembly and finishing. No plasmid was detected. The chromosome size, G+C content and number of genes were 2 965 602 bases, 37.10% and 2946 genes, respectively. Among these genes, 2850 were protein-coding genes and 96 were RNAs (21 rRNAs, 74 tRNAs, one tmRNA). A total of 2433 genes were assigned to COGs (82, 25%). Of these, 2393 genes were assigned a putative function (81.2%), and 553 (18.8%) were annotated as hypothetical proteins.

Phylogenetically, two lineages were identified among *L. ivanovii* strains, including one that was made of *L. ivanovii* subsp. *ivanovii* strains, including strain G770, and a second made of *L. ivanovii* subsp. *londoniensis* strains (Fig. 1). The percentages of nucleotide sequence similarity between strain G770 and *L. ivanovii* strains for the *hly*A gene ranged from 94.35% with the two strains of subspecies *londoniensis* to 99.87% with the other three strains of subspecies *ivanovii*. For 16S rRNA, the percentages of nucleotide similarity were 99.87 and 100% between strain G770 and the strains of the subspecies *londoniensis* and *ivanovii,* respectively. The G770 strain exhibited GGDC values of 99.3% with other *L. ivanovii* subsp. *ivanovii* strains and 51.2 to 51.7% with *L. ivanovii* subsp. *londoniensis* strains (Supplementary Table S1).

As detailed in Table 2, strain G770 exhibited much less differences with strains from *L. ivanovii* subsp. *ivanovii* than with those from subsp. *londoniensis*. Of the G770-specific genes, 21 were absent from all other compared genomes *L. ivanovii* strains (Table 3). In addition, strain G770 had 12, 11 and 13 missing genes with regard to subspecies *londoniensis* (Table 2). Supplementary Table S2 lists the missing in strain G770.

Among the 21 strain G770–specific genes, five encoded proteins similar to type I restriction-modification genes of *L. monocytogenes* and one was mostly similar to *vanZ,* a component of the vancomycin-resistant operon in *Staphylococcus pseudintermedius* (Table 3). Strain G770 also had five specific genes encoding metabolic enzymes such as helicases, transferases and acetyl–coenzyme A synthase. Finally, the strain exhibited six genes encoding hypothetical proteins and one encoding a membrane protein (Table 3).

As other *L. ivanovii* strains, strain G770 had a complete LIPI-1 virulence cluster. However, although the sequences from the five LIPI-1 genes *prfA, LLO, mpl, plcC* and *plcB* of all compared genomes were identical, strain G770 differed from other strains in the *act*A gene sequence (Supplementary Table S3). Nevertheless, the annotated domains of the ActA protein were similar to those of ActA proteins from other strains of the subspecies *ivanovii* (Supplementary Fig. S1).

Strain G770, like all other compared *L. ivanovii* strains, exhibited 16 internalin or internalin-like genes including *inl*A, *inl*B and *inl*C (Supplementary Table S4). In addition, we identified in strain G770 five genes related to the SSI-1 (Supplementary Table S5). These genes were also present in all compared *L. ivanovii* genomes.

The genome of strain G770 harboured one CRISPR region (Supplementary Fig. S2), whereas the other three subspecies *ivanovii* strains exhibited three CRISPR regions each and the subspecies *londoniensis* strains WSLC30151 and WSLC30167 had two and three CRISPRs, respectively [17].

**TABLE 2. Differential gene content among *Listeria ivanovii* strains studied**

| Compared strains | *L. ivanovii* subsp. *ivanovii* PAM 55 | *L. ivanovii* subsp. *ivanovii* strain WSLC 3010 | *L. ivanovii* subsp. *ivanovii* strain WSLC 3009 | *L. ivanovii* subsp. *londoniensis* strain WSLC 30151 | *L. ivanovii* subsp. *londoniensis* strain WSLC 30167 |
|---|---|---|---|---|---|
| No. of additional genes in strain G770 | 42 | 53 | 50 | 438 | 450 |
| No. of missing genes in strain G770 | 12 | 11 | 13 | 513 | 447 |

**TABLE 3. List of genes present in strain G770 but absent or differentially present in other *Listeria ivanovii* subsp. *ivanovii* strains**

| Locus (CVPH01000001–CVPH01000017) | Putative function (COGs category) | Best match with: | Presence in other *L. ivanovii* subsp. *ivanovii* strains | | |
|---|---|---|---|---|---|
| | | | PAM 55 | WSLC 3009 | WSLC 3010 |
| Scaffold1.1_160[a] | Membrane protein (not in COGs) | *Lactobacillus paracasei* | – | – | – |
| Scaffold1.1_161[a] | S-transferase (not in COGs) | *Vibrio tubiashii* | – | – | – |
| Scaffold1.1_162[a,c] | Type I restriction-modification protein subunit M (V) | *Listeria monocytogenes* | – | – | – |
| Scaffold1.1_163[a,c] | Type I restriction-modification protein subunit S (V) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_164[a,c] | Type I deoxyribonuclease HsdR (V) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_165[a,c] | Type I RM HsdR (V) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_166[a,c] | Type I mrr restriction system protein (V) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_167[a] | DNA helicase (L) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_168[a] | DNA helicase related protein (L) | *Listeria marthii* | – | – | – |
| Scaffold1.1_169[a] | Antibiotic resistance protein VanZ (not in COGs) | *Staphylococcus pseudintermedius* | – | – | – |
| Scaffold1.1_170[a] | Hypothetical protein (L) | *Listeria seeligeri* | – | – | – |
| Scaffold1.1_171[b] | Putative secreted, lysin rich protein (not in COGs) | *Listeria ivanovii* | – | – | – |
| Scaffold1.1_172 | Threonine aldoase(E) | *Listeria seeligri* | – | – | – |
| Scaffold1.1_173[a] | Hypothetical protein (S) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_174[a] | PF07510 family protein (S) | *Staphylococcus hominis* | – | – | – |
| Scaffold1.1_175[a] | Hypothetical protein Not in COGs) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_176[a] | DNA-cytosine methyltransferase (L) | *Enterococcus faecalis* | – | – | – |
| Scaffold1.1_177[a] | Hypothetical protein (L) | *E. faecalis* | – | – | – |
| Scaffold1.1_178[a] | DNA mismatch repair protein (Not in COGs) | *Butyrivibrio* sp. *FCS014* | – | – | – |
| Scaffold1.1_179 | PTS cellbiose transporter subunit (S) | *Listeria innocua* | – | – | – |
| Scaffold1.1_180 | Transcriptional regulator (K) | *L. ivanovii* | – | – | – |
| Scaffold1.1_181 | Hydroxyethylthiazote kinase (H) | *L. ivanovii* | – | – | – |
| Scaffold1.1_182 | Phosphome thylpyrimidine kinase (H) | *L. ivanovii* | – | – | – |
| Scaffold1.1_183 | Thiamine-phosphate pyrophosphorylase (H) | *L. ivanovii* | – | – | – |
| Scaffold1.1_184 | 6-Phospho-beta-glucosidase (G) | *L. ivanovii* | – | – | – |
| Scaffold1.1_185 | Membrane protein, putative (not in COGs) | *L. ivanovii* | – | – | – |
| Scaffold1.1_186[a] | F-box/FBD/LRR-repeat protein (S) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_187[a] | F-box/FBD/LRR-repeat protein (S) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_188[a] | Acetyl-coenzyme A synthetase (S) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_189[b] | Transcription antiterminator LicT (K) | *L. seeligri* | – | – | – |
| Scaffold1.1_190[b] | PTS-beta-glucosidase (G) | *L. ivanovii* | – | – | – |
| Scaffold1.1_191[b] | 6-Phospho-beta-glucosidase (G) | *L. seeligri* | – | – | – |
| Scaffold1.1_192[b] | Wall-associated RHS family protein (M) | *L. ivanovii* | – | – | – |
| Scaffold1.1_193[b] | Heat repeat-containing pbs lyase (C) | *L. ivanovii* | – | – | – |
| Scaffold1.1_194[b] | Hypothetical protein (not in COGs) | *L. ivanovii* | – | – | – |
| Scaffold1.1_195[b] | Hypothetical protein (not in COGs) | *L. ivanovii* | – | – | – |
| Scaffold1.1_233[b] | Hypothetical protein (not in COGs) | *L. ivanovii* | – | – | – |
| Scaffold1.1_303[b] | Antibiotic resistance protein VanZ (V) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_347 | Multidrug ABC transporter ATP-binding protein (V) | *L. ivanovii* | + | – | – |
| Scaffold1.1_348 | CRISPR-associated protein Cas6 (L) | *L. ivanovii* | + | – | – |
| Scaffold1.1_349 | CRISPR-associated protein Cst1 (not in COGs) | *L. ivanovii* | + | – | – |
| Scaffold1.1_350 | CRISPR-associated protein (L) | *L. ivanovii* | + | – | – |
| Scaffold1.1_351 | CRISPR-associated protein Cas5 (L) | *L. ivanovii* | + | – | – |
| Scaffold1.1_352 | CRISPR-associated protein Cas3 (R) | *L. ivanovii* | + | – | – |
| Scaffold1.1_354 | CRISPR-associated protein Cas4 (L) | *L. monocytogenes* | + | – | – |
| Scaffold1.1_355 | CRISPR-associated exonuclease Cas1 (L) | *L. ivanovii* | + | – | – |
| Scaffold1.1_356 | CRISPR-associated protein Cas2 (L) | *L. ivanovii* | + | – | – |
| Scaffold1.1_529 | 4-Dihydroxy-2-naphthoate octaprenyltransferase (not in COGs) | *L. ivanovii* | + | – | – |
| Scaffold1.1_918 | Molybdenum cofactor biosynthesis protein D (H) | *L. ivanovii* | – | + | + |
| Scaffold1.1_1236 | Conserved hypothetical protein (not in COGs) | *L. ivanovii* | + | – | – |
| Scaffold1.1_2852[b] | Hypothetical protein (not in COGs) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_2853[a] | Conserved hypothetical protein (not in COGs) | *L. monocytogenes* | – | – | – |
| Scaffold1.1_2855[b] | Hypothetical protein (not in COGs) | *L. monocytogenes* | – | – | – |

COGs, Clusters of Orthologous Groups database.
[a]Genes present only in strain G770.
[b]Genes present in *L. ivanovii* subsp. *londoniensis* strains.
[c]Genes associated to type I restriction-modification system.

## Discussion

To date, only eight cases of human infections caused by *L. ivanovii* have been reported in the literature. Of these, two were diagnosed in patients over 60 years, and most occurred in immunocompromised patients (two pregnant women, and one case each of AIDS, drug abuse, hepatic lymphoma and immunosuppression; Table 1). In the remaining two cases of human *L. ivanovii* infection, no underlying condition was described (Table 1). To our knowledge, we herein present the first case of *L. ivanovii* vascular infection. In an effort to determine whether this unusual presentation was due to an increased virulence of strain G770, we compared the genome of strain G770 to those of other *L. ivanovii* strains.

In *Listeria* species, the LIPI-1 cluster is the major pathogenesis factor. All *Listeria* species lacking this gene cluster are not pathogenic [18]. In *L. ivanovii,* the LIPI-1 cluster is made of six genes, including a pore-forming toxin (listeriolysin O) and two phospholipases (*plcC* and *plcB*) which cooperate to lyse the

membrane of the phagocytic vacuole; an actin-polymerization surface protein (ActA) that is responsible for intracellular bacterial motility and spread; a metalloprotease (mpl) that is involved in the maturation of proPlcB; and a transcriptional activator (PrfA) that controls the expression of LIPI-1 genes [19]. Studies have shown that mutations in the *prfA* gene inhibit bacterial cell-to-cell spread and reduce bacterial virulence in *L. monocytogenes* [20]. Mutations in different parts of ActA, notably the N-terminal region, cause several unusual motility and actin polymerization phenotypes in *L. monocytogenes* [21]. Strain G770 possessed all six genes of the LIPI-1 cluster but varied in *hly* and *actA* when compared to other strains from *L. ivanovii* subsp. *ivanovii* (Supplementary Table S3). The differences in ActA domains are displayed in Supplementary Fig. S1. However, the ActA domains involved in virulence are conserved in all strains [21]. Therefore, the observed differences in cluster LIPI-1 or ActA do not support an increased virulence of strain G770.

Among other known virulence factors of *Listeria* species, internalins such inlA, inlB and inlC are essential for host invasion and cell-to-cell spread [22]. However, strain G770 did not exhibit differences in internalin content (Supplementary Table S4) compared to other *L. ivanovii* strains. In addition, strain G770 also possessed the stress survival islet 1 (Supplementary Table S5), known in *L. monocytogenes* to be linked to tolerance towards acidic, salt, bile and gastric stress [23]. Again, we found no difference in SSI-1 content among studied strains. Finally, we detected CRISPRs in strain G770 (Supplementary Fig. S2). The presence of CRISPR regions confers to *Listeria* species an adaptive immunity that protects them against invading bacteriophages and plasmids [24]. However, no relationship between the number of CRISPRs and virulence has been reported for these bacteria.

In addition, strain G770 exhibited 21 specific genes (Table 3). The best matches for 12 of these genes were found in both *Listeria seeligeri* and *L. monocytogenes.* Only one specific gene (Scaffold1.1_178) was not found in the genus *Listeria,* thus confirming the highly conserved nature of genomes in this genus [22]. Of these additional genes, six may have roles in virulence or resistance of strain G770, including five genes similar to a type I restriction-modification system of *Listeria monocytogenes.* This is the first time that a type I restriction-modification system is found in *L. ivanovii.* Restriction-modification systems effectively allow discrimination of self and nonself in bacteria [25]. These systems protect against phages, plasmids and transposons [25]. In addition, type I restriction-modification systems play roles in various cellular processes including host defence and virulence, and they even control the speed of evolution of the organism [25]. One study showed that bacteria that have acquired a competent restriction-modification system acquire new properties especially during colonization of new habitats [8]. Therefore, we assume that the type I restriction-modification system identified in strain G770 may have conferred to it an increased virulence.

Strain G770 also exhibited a *vanZ* gene. VanZ is an accessory protein that is part of the Tn1546 transposon [26]. The presence of this transposon confers resistance to glycopeptides in *Enterococcus faecium* [26]. In the absence of the other components of the Tn1546 transposon, VanZ confers low-level resistance to teicoplanin (not to vancomycin) [27]. However, no case of resistance to vancomycin has been reported for strains of *Listeria* spp. originating from humans, food or the environment, and strain G770 was not resistant to glycopeptides [28].

Overall, the genomic analysis of *L. ivanovii* subsp. *ivanovii* strain G770, which caused a deadly aortic infection, enabled identification of specific characteristics among other *L. ivanovii* strains, including a type I restriction-modification system that may have conferred to it an increased virulence.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nmni.2016.01.005.

## Conflict of Interest

None declared.

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