

Assessing the Applicability of Hygiena™ *Listeria* Molecular Diagnostics Kits Towards the Detection of the Five Novel Non-pathogenic Species of *Listeria* Discovered in Soil and Agricultural Water

Introduction

A new study focusing on the diversity and prevalence of *Listeria* in the US was published in May 2021 in the International Journal of Systematic and Evolutionary Microbiology (1). In this study, researchers discovered five previously unknown *Listeria* species. The researchers were able to characterize 26 isolates originating from soil collected in rural regions in eight different US states and one isolate from an agricultural water source in New York. Whole-genome sequencing and phylogenetic analysis revealed that the 27 isolates could not be assigned to any known *Listeria* species. Instead, the isolates fell into six distinct subgroups that classified into five distinct species:

1. *Listeria cossartiae* subsp. *cossartiae* and *Listeria cossartiae* subsp. *cayugensis*
2. *Listeria farberi*
3. *Listeria immobilis*
4. *Listeria portnoyii*
5. *Listeria rustica*

Three of the novel species; *Listeria cossartiae* subsp. *cossartiae*, *Listeria cossartiae* subsp. *cayugensis*, *Listeria farberi* and *Listeria immobilis* were classified into the *Listeria* clade *sensu stricto* that contains the recognized human pathogen, *L. monocytogenes*. Two of the novel species; *Listeria portnoyii* and *Listeria rustica* were classified into *Listeria sensu lato* clade of non-human and animal pathogens (i.e., believed to be solely environmental bacteria).

Observations

Phenotypic analyses revealed non-pathogenic characteristics for all of the new species. In more detail, these studies assessed: (i) growth rates across the temperature range expected for *Listeria* (0–45°C), (ii) colony phenotypes on selective and differential media, (iii) the *Listeria* identification procedures described in the FDA BAM, Chapter 10 and ISO EN 11290-1 : 2017 including: hemolysis, motility, catalase, oxidase, Gram stain, nitrate reduction, and API *Listeria* (bioMérieux)), (iv) growth under anaerobic conditions, and (v) the biochemical tests included in the API 20E (bioMérieux) and API 50 CH kits (bioMérieux). All five of the novel species are non-hemolytic and negative for phosphatidylinositol-specific phospholipase C (PI-PLC) activity. Additional genomic analyses of the draft genomes revealed the lack of the virulence genes found in *Listeria* pathogenicity island 1 (LIPI-1), and the internalin genes *inIA* and *inIB*. All the above indicate that all the newly characterized isolates are non-pathogenic. To our knowledge none of the novel *Listeria* species has been implicated in foodborne illnesses or outbreaks.

Objective

The applicability of the Hygiena™ *Listeria* molecular diagnostics assays were assessed for the detection of the five novel *Listeria* species by *in silico* analysis. The primers and probes included in the kits were blasted against the whole genome sequences of the novel *Listeria* species on the NCBI platform.

Results

Theoretically, the Real-Time assay will correctly detect the *Listeria cossartiae* sp. subsp. *cossartiae*, the *Listeria cossartiae* sp. nov. *cayugensis*, the *Listeria immobilis* and the *Listeria farberii*, meaning all the new species that fall in the *sensu stricto* *Listeria* clade would be positive. The standard melt curve assay will correctly detect only the *L. farberii* sp. The results are summarized in Table 1.

Clade	<i>Listeria</i> species	Isolate code	Origin	RT Genus <i>Listeria</i> assay	Genus <i>Listeria</i> melt assay
<i>Sensu stricto</i> clade (includes human and animal pathogens)	<i>Listeria cossartiae</i> sp. nov. subsp. <i>cossartiae</i>	FSL L7-1447	soil	YES	NO
	<i>Listeria cossartiae</i> sp. nov. subsp. <i>cayugensis</i>	FSL L7-0993	soil	YES	NO
	<i>Listeria immobilis</i> sp. nov.	FSL L7-1519	soil	YES	NO
	<i>Listeria farberii</i>	FSL L7-0091	soil	YES	YES
<i>Sensu lato</i> clade	<i>Listeria portnoyi</i> sp. nov.	FSL L7-1582	soil	NO	NO
	<i>Listeria rustica</i> sp. nov.	FSL W9-0585	water	NO	NO

Future Studies

The applicability of the assays will be tested with both Hygiena™ BAX® System Assay kits and foodproof® *Listeria* Detection kits once these strains of the novel *Listeria* species are available for laboratory hands-on experiments.

Reference

1. Carlin CR, Liao J, Weller D, Guo X, Orsi R, Wiedmann M. *Listeria cossartiae* sp. nov., *Listeria immobilis* sp. nov., *Listeria portnoyi* sp. nov. and *Listeria rustica* sp. nov., isolated from agricultural water and natural environments. *Int J Syst Evol Microbiol*. 2021 May;71(5). doi: 10.1099/ijsem.0.004795. PMID: 33999788.