

© AZTI

## SUMMARY

*Listeria monocytogenes* is a well-known pathogen responsible for listeriosis, one of the most serious foodborne diseases, with an increasing trend in the number of cases (including seafood-related cases) in recent years. Bacteriophages show great potential to be used for food safety applications. This Knowledge Output concerns a *Listeria*-specific bacteriophage cocktail, containing five well-characterized complementary bacteriophages, that provides a broad range of activity against *L. monocytogenes* isolates of relevance for the seafood industry. The use of this natural, safe and green bacteriophage cocktail effectively reduces *L. monocytogenes* loads in raw and ready-to-eat (RTE) seafood products and may, therefore, contribute as an additional (bio)control strategy to improve the safety of these products.



## KNOWLEDGE NEED

Control of *L. monocytogenes* occurrence in fresh, minimally processed, and RTE seafood products is an important challenge for the seafood industry and for public health. The application of specific bacteriophages is a promising solution to address this need, but some challenges remain, including the development of cocktails with broader specificity ranges than single monophages, competitively priced production at industrial scale, as well as European Union regulatory status, where there is no clear path yet to register a bacteriophage product.



## RESULTS

One efficient bacteriophage cocktail specifically targeting *L. monocytogenes* has been successfully developed, produced at industrial scale, tested and validated in raw and RTE seafood products. A selection of 16 *Listeria* bacteriophages were characterized regarding the most relevant properties required for food applications. The results on the safety, specificity, efficacy and stability properties of individual phages allowed the formulation of different cocktails that were effective against a broader range of target *Listeria* strains than single monophages. The two most effective cocktails were selected, which lysed over 90% of tested *L. monocytogenes* strains. Their effectiveness for *Listeria* reduction on the surface of contaminated seafood products was then assessed under different conditions. The most efficient cocktail formulation and treatment conditions were then validated in raw and RTE seafood products (raw and cold-smoked salmon) contaminated with naturally occurring low *L. monocytogenes* counts. The cocktail reduced the initial *L. monocytogenes* load and kept it below the legal limit (100 CFU/g) at the end of the shelf-life of both raw and cold-smoked salmon. The production and purification of stable phages was finally optimized and scaled up to industrial commercial levels. The results provide new evidence to support the potential of bacteriophage biocontrol as a natural, green and safe strategy that could act as an additional tool in a multi-hurdle approach to control the prevalence of *L. monocytogenes* in seafood.



## IMPACTS

This knowledge has the potential to be developed into new products for the biocontrol of *L. monocytogenes* in seafood products, which would generate increased revenue for the seafood industry, reduce product loss due to contamination, and sustainably improve the safety of seafood products. Overall, this knowledge could improve consumer trust and seafood consumption habits, contributing to an improved health profile of the European population.

**Contributes to the UN Sustainable Development Goal 12:** Responsible consumption and production.

## END-USERS & APPLICATIONS

➔ **Scientific community:** new knowledge generated by this KO can be used by other researchers working in the field of food safety to support further research on *Listeria* specific bacteriophages.

➔ **Industry - seafood processing companies:** further research at industrial level is needed before being able to apply this new *Listeria* bacteriophage product to produce safer seafood products by the seafood processing industry.

➔ **European Union Food safety authorities / Policy makers:** new knowledge generated by this output can be used to support near-future EU regulation on safety assessment and efficacy of *Listeria* bacteriophages application.

➔ **Scientific community:** academics and scientists working in the area of seafood safety can use the validated datasets and new knowledge to support further research.

## DISSEMINATION AND EXPLOITATION

### Exploitation activities for seafood industry:

- Demonstration of the strategy at **SEAFOOD<sup>TOMORROW</sup>** demonstration workshops, in France, and Spain (February – March 2021) to support uptake and use of the method.
- Video: Bacteriophages for seafood safety (developed by AZTI) [youtu.be/06eY3tkQUuA](https://youtu.be/06eY3tkQUuA)

### Scientific publications:

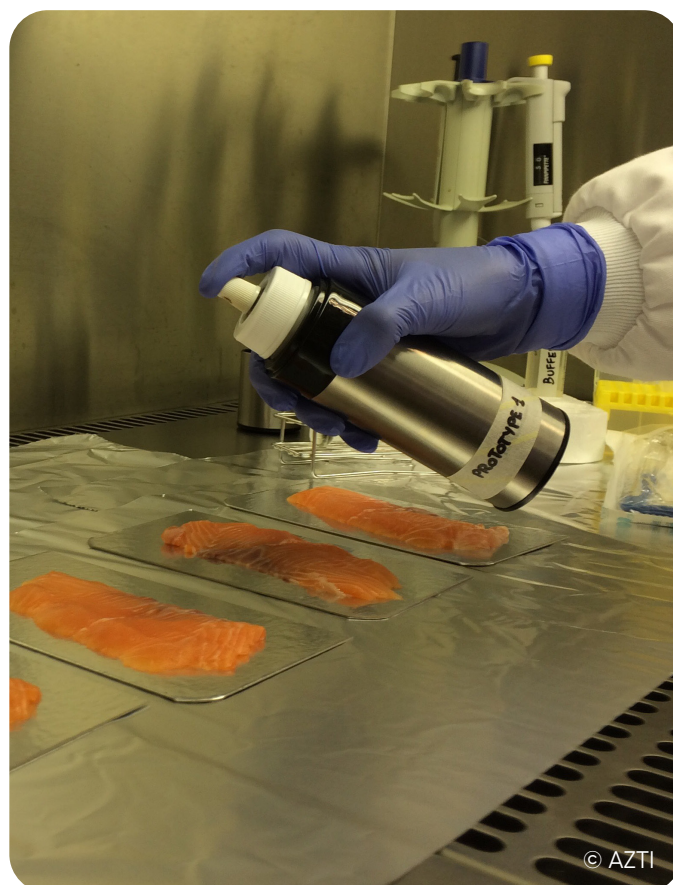
- Scientific publication: Lasagabaster A, Jiménez E, Lehnherr T, Miranda-Cadena K, Lehnherr H. (2020). Bacteriophage biocontrol to fight *Listeria* outbreaks in seafood. *Food and Chemical Toxicology*, 145, 111682. DOI: 10.1016/j.fct.2020.111682.
- Open access version: [zenodo.org/record/4193580#.YCPYLOj7SUI](https://zenodo.org/record/4193580#.YCPYLOj7SUI)
- Open access validated data sets: please see [seafoodtomorrow.eu](https://seafoodtomorrow.eu)

### Exploitation activities for policy makers:

- Monitoring agencies, regulators and policy makers will be reached through the final **SEAFOOD<sup>TOMORROW</sup>** event, info session, and a dedicated EU policy event.

### Dissemination activities for society / all users:

- Project newsletters and news articles
- Promotion on Twitter and LinkedIn



## INNOVATION STATUS

Technology Readiness Level 4 - the technology has been validated in laboratory environment

**Patents and IPR:** patent application planned/foreseen



## FUTURE RESEARCH

More research at semi-industrial and industrial level is needed to assess effectiveness of the defined listeriophage cocktail on contaminated seafood products. The potential development of resistant bacteria during storage must also be assessed to validate this technology in a relevant environment, and to bring it to a higher TRL. For final commercial implementation, the technology must be demonstrated to seafood processing companies that could then trial the method.

## CONTACT AND CONTRIBUTORS

**Contact:** Amaia Lasagabaster, [alasa@azti.es](mailto:alasa@azti.es)

**Contributors:** AZTI, PTC

