Evaluation of viability of cells of *Listeria innocua* with Raman microspectroscopy after incorporation of heavy water (D_2O)

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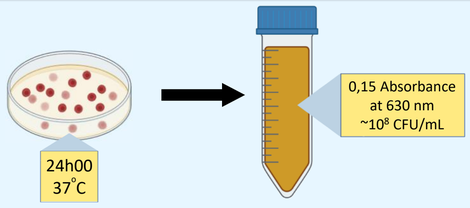
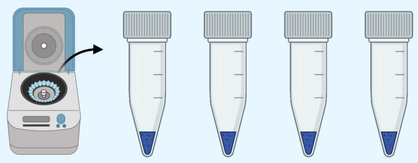
Context

Listeria innocua is a bacteria most frequently encountered in the food industry. This bacteria can pass in a viable but nonculturable (VBNC) state, characterized by a very low metabolic activity and no growth on standard microbiological media commonly used in the food industry but bacteria retain the ability to recover and be potentially pathogenic. VBNC can be detected by comparison of techniques: enumeration on agar vs qPCR vs PMA-qPCR. However, these methods are not very sensitive. A new approach for studying and detecting VBNC bacteria is to use Raman spectroscopy coupled with heavy water (D_2O) labeling. A major concern is that D_2O could also impact the viability state of *L. innocua*. **In this study, we verified the impact of D_2O incorporation on the viability state of *L. innocua*. In a second step, we optimized the labelling for an early detection of the incorporation of this bacteria.**

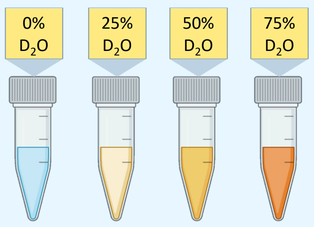
Material & methods



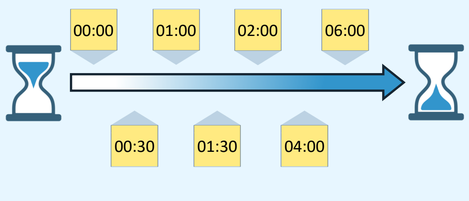
1 *Listeria innocua* resuspended in physiological water



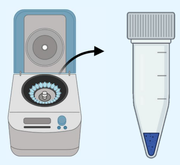
2 *100µL of suspension centrifuged -> remove supernatant*



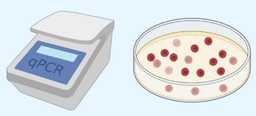
3 *Resuspending cell pellet with 1 mL of trypticase soy broth yeast extract with different percentages of D_2O*



4 *Incubation during 30 minutes to 6 hours*

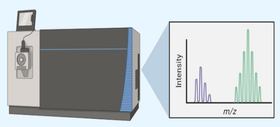


5 *Wash twice bacteria with physiological water*



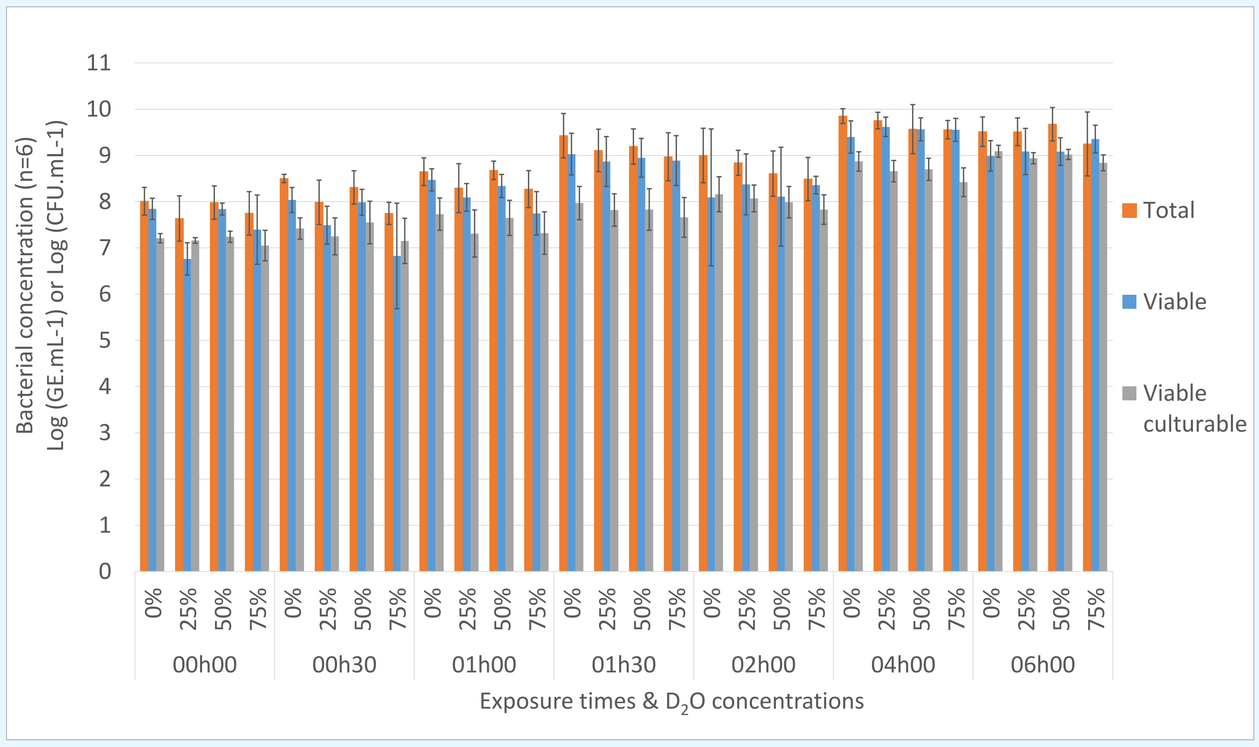
Detection of viability state by quantification of viable culturable (VC) population (by agar), viable population (by PMA-qPCR) and total (by qPCR)

6



Optimisation of heavy water labelling by measurement of the Carbon-Deuterium peak (C-D) between 2040 and 2300 cm^{-1} by Raman spectroscopy

Effect on viability state



Quantification of total, viable, viable culturable *L. innocua* populations. The error bars represent the standard deviation (n=6). The " * " represent a significative difference of viable (blue) and viable culturable (grey) population, $p < 0.05$.

Aim

Verification of the effect of D_2O incorporation on viability state of *L. innocua*

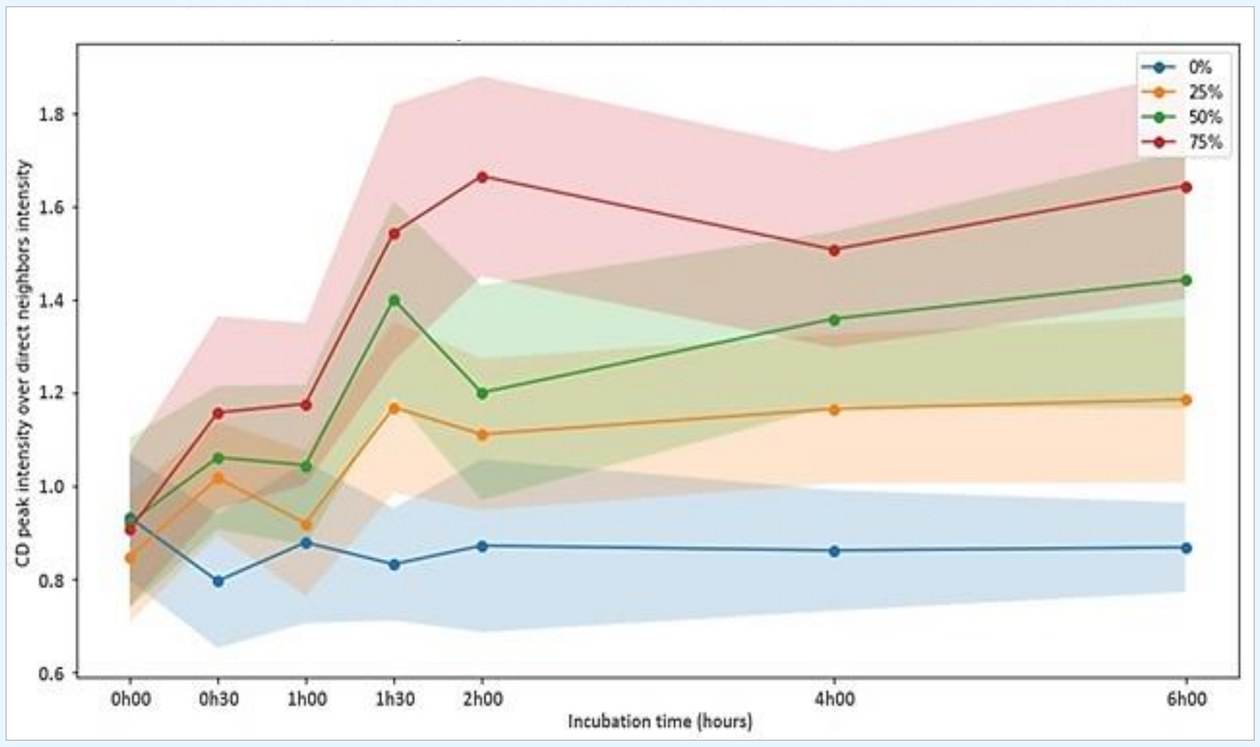
Results

All populations are in VC state until 6h

Conclusion

No effect of D_2O incorporation on viability state of *L. innocua*

Optimization of heavy water labelling



Quantification of the C-D peak in non-deuterated (0% of D_2O (curve blue) and deuterated (25% (curve orange), 50% (curve green), 75% (curve red) of D_2O) bacterial suspension of *L. innocua* over time ($60 > n > 80$).

Aim

Selection of a labelling condition to have a high C-D peak, in the shortest time

Results

High C-D peak is detected from 1h30 for all condition, but is higher the 75% of D_2O condition

Conclusion

Best labelling condition : **1h30 with 75% of D_2O**

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