PEC1.29 **Isolation of stress resistant variants: Lucky shot or directed search?**

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The occurrence of stable stress resistant variants in monoclonal populations of bacteria remains the subject of debate as some research groups reported their isolation while others failed to demonstrate their presence and questioned their existence. Here we present a new strategy that resulted in the isolation of stress resistant variants of *Listeria monocytogenes* under conditions where we previously were not able to find them.

For *L. monocytogenes* strains Scott A and LO28, non-linear inactivation kinetics pointed towards the existence of stress resistant variants. These variants were previously isolated after a high hydrostatic pressure (HHP) treatment, and occurred at a frequency of 10^{-5} – 10^{-6} and appeared to be also more resistant to heat. After a heat treatment these variants could however not be isolated, although significant tailing of the heat inactivation curvature was demonstrated. Furthermore, for strain EGDe previously no stable stress resistant variants could be isolated after a HHP treatment. Either, variants occurred at a very low, undetectable frequency, or the HHP treatment specifically induced the generation of variants, or the detection method was not sensitive enough to select for stable resistant variants. Subsequent detailed kinetic modelling of the inactivation curves of both heat and HHP inactivation showed that the chance to find these variants was different for the various inactivation treatments. Therefore, we designed optimal cultivation and inactivation methods to be able to detect the presence of stable resistant variants. With these methods for both *L. monocytogenes* strains LO28 and EGDe stable resistant survivors were isolated after both heat and HHP treatments. Also under practical conditions, stress resistant variants could be isolated after a heat treatment in milk at 72°C. This research showed a more widespread presence of stable resistant variants in different *L. monocytogenes* populations and demonstrated the strength of kinetic modelling in unravelling the causes of non-linear inactivation and in optimising experimental procedures for hypothesis testing.