PSB2.03 **Inactivation-associated formation of reactive oxygen species in heat- and acid-stressed bacteria**

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Recently, the formation of reactive oxygen species (ROS) was suggested to act as a common mechanism of cellular death in aerobically grown bacteria exposed to bactericidal antibiotics. The present study was conducted to assess the role of ROS formation in bacteria exposed to heat and acid, two stresses frequently found in food and food processing conditions.

To assess this role, a protocol for fluorescent imaging of (secondary) ROS formation was developed and optimized for the Gram-negative and Gram-positive model organisms *Escherichia coli* K12 and *Bacillus subtilis* 168. ROS formation upon exposure of mid-exponential phase aerobically grown cells of *E. coli* K12 and *B. subtilis* 168 to heat and acid was assessed by fluorescence microscopy combined with the green-fluorescent probe 3'-(*p*-hydroxyphenil) fluorescein (HPF), that specifically reacts with hydroxyl radicals ($OH\cdot$) and peroxynitrite ($ONOO^-$). HPF was also used in combination with single cell flow cytometry analysis to obtain quantitative information about heterogeneity in secondary oxidative response, and subsequent formation of ROS, in the tested conditions. The results obtained with fluorescent imaging could be correlated with thermal- and acid-induced inactivation of the cells as determined by plate counting at 0, 10, 20, and 30 min after exposure of the cultures to the lethal treatments. Fluorescence microscopy revealed an appearance of green-fluorescent cells throughout the exposure to lethal-heat and acid stress, indicating that hydroxyl radicals and/or peroxynitrite were formed already within the first 10 min. It is conceivable that these lethal ROS originate from a burst of radicals at the electron transfer chain, such as superoxide, upon exposure of the actively respiring cells to the selected stresses.

This study indicates that secondary oxidative stress may be an important factor in heat- and acid-stressed, aerobically grown Gram-negative and Gram-positive bacteria. Furthermore, it provides a new single cell approach to study bacterial behaviour, including (secondary) oxidative stress response and accumulation of ROS under a wide range of stress conditions.