

Differentiation for viable and heat-inactivated *Salmonella Enteritidis* by LightCycler real-time PCR

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Introduction:

Rapid, sensitive and specific results can be achieved by real-time PCR, when detecting pathogens especially in foods. However, there are some problems in respect of a minimal amount of DNA, which must be available for the detection and the impossibility of differentiation between viable and dead cells. These problems can be overcome by comparing PCR's before and after a short preenrichment step. This study is made establishing the minimal enrichment time which is achieved by investigating the growth kinetics of *Salmonella Enteritidis* DSM 9898 and its behaviour in presence of growth stimulating agents. Furthermore, the significance of the difference for the crossing points of viable and heat-inactivated cells is tested.

Material and Methods:

Buffered peptone water was inoculated with 10 cfu/ml of *S. Enteritidis*. To the enrichment medium ferrioxamine E (50 ng/ml) and/or an „enterobacterial autoinducer of growth“ (1 % v/v) were added. Unsupplemented buffered peptone water served as control. The samples were taken every half hour during a period of 4 hours and again after 6 hours.

Buffered peptone water was inoculated with $10^0 - 10^5$ cfu/ml viable and dead (96 °C; 10 min) *S. Enteritidis* for initial concentration. After an incubation time of 4 hours at 37 °C, samples were taken for DNA isolation and following real-time PCR, which was carried out on the *S. invE-A* gene using Hybridization Probes for detection. The assay was repeated four times.

Various mixing ratios (100 % dead, 75 % dead/25 % viable, 50 % dead/50 % viable, 25 % dead/75 % viable, 100 % viable) from viable and heat-inactivated *S. Enteritidis* were tested (100 cfu/ml buffered peptone water) three times.

The differences of the crossing points before and after the enrichment step were compared statistically by student's t-test.

Results:

The growth kinetics of *S. Enteritidis* show that the lag-phase is overcome after an incubation time of two and a half hour. An incubation time of four hours enables the bacteria to reach the exponential-phase and allows to complete the test taking as little time as possible.

No growth stimulating effect can be detected after supplementation with ferrioxamine E and/or an „enterobacterial autoinducer of growth“ compared with unsupplemented samples (FIG. 1). Hence, the further assays are done without supplementation.

The crossing points of the not heated and the heated samples before the preenrichment are between 29 (10^5 cfu/ml) and 46 (10^0 cfu/ml). After the enrichment step the crossing points of the not heated samples are between 18,5 (10^5 cfu/ml) and 37 (10^0 cfu/ml). This corresponds to a rise of about 10 cycles through the enrichment. The crossing points of the inactivated samples stay between 29 (10^5 cfu/ml) and 41 (10^0 cfu/ml) after the incubation.

The crossing points of the mixing ratios lie about 42 before the enrichment, independently of the number of inactivated cells (FIG. 2). After the enrichment the crossing points of the completely inactivated cells stay at 42, as expected. With the increasing number of living cells the crossing points decreases from 36 (75 % dead/25 % viable) to 31 (100 % viable).

The comparison of viable and heat-inactivated cells show a significant difference ($\alpha = 0,05$) between the difference of the crossing points before and after the enrichment step at 37 °C (FIG. 3).

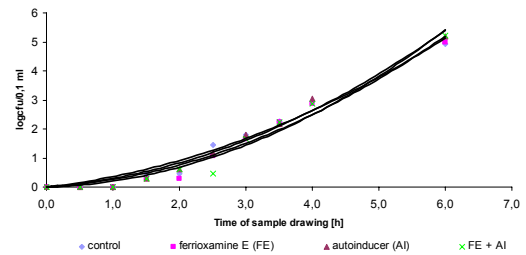


FIG. 1: Kinetics of *S. Enteritidis* DSM 9898 with and without growth stimulation

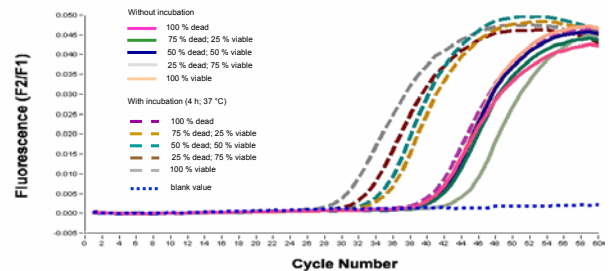


FIG. 2: Amplification curves of various mixing ratios of viable and dead *S. Enteritidis* before and after the preenrichment step (4 h; 37 °C; n = 3)

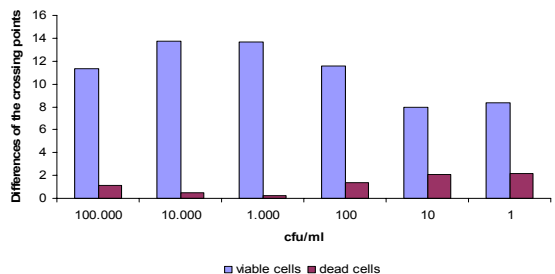


FIG. 3: Differences of the crossing points of viable and dead *S. Enteritidis*, respectively before and after the preenrichment (4 h; 37 °C; n = 5)

Conclusion:

- No growth stimulating effect can be detected after supplementation the culture medium with ferrioxamine E and/or an „enterobacterial autoinducer of growth“.
- A distinction between viable and dead *S. Enteritidis* from pure cultures is even possible at a concentration of 10^0 cfu/ml.
- The detection of 25 % viable *S. Enteritidis* in presence of 75 % dead cells succeeded at a level of 100 cfu/ml.