The SOS response is a functionally conserved DNA repair system found in a variety of bacteria. The SOS response involves two central regulatory components, RecA and LexA, which coordinate the expression of target genes leading to arrest of cell division, DNA repair and mutagenesis. The SOS response of the food borne pathogen Listeria monocytogenes is presently not well understood. However, we find it likely that the SOS response under these conditions, we hope to elucidate the common factor, which acts as an inducer of SOS response in L. monocytogenes.

**Background and objectives**

The SOS response is an indirect DNA damaging repair system. As such, it is not clear if the SOS response is directly dependent on DNA damage. It is possible that SOS response is indirectly dependent of DNA damage, as has been published elsewhere.

**Screening for SOS response inducing antibiotics**

To identify different antibiotic compounds with the ability to induce SOS response in wild type Listeria monocytogenes, we tested seven different antibiotics, which target L. monocytogenes differently. In the schematics to the left, a wide variety of antibiotics and their targets are presented. From these a number of compounds were chosen for stress experiments and northern blots (labelled in blue). The results shown below confirmed that the DNA damaging agent Mitomycin induced the SOS response. Furthermore the experiment revealed that Cefuroxime as well as Gentamicin also induced the SOS response genes recA and umuD, when cells were stressed for 60 minutes.

**Qualitative studies of cell morphology and SOS induction**

In order to monitor LexA expression in L. monocytogenes, a lexA promoter-gfp reporter system (plexA-gfp) was constructed in a vector plasmid optimized for Listeria monocytogenes. GFP expression as a measure of lexA expression was subsequently monitored in a microscopy during stress with a number of antibiotics. An increase in both GFP fluorescence and cell size was observed. However, cells displayed large variations within the culture. This is a common feature of GFP based reporter systems, which in this case may enhance the feature of the LexA promoter, which must maintain a low but constitutive level of transcription to repress the SOS response under non-stressed conditions.

To quantify the stress response, a FACS (Fluorescence-activated cell sorter) was employed for measuring forward scatter (estimate of cell size) and GFP fluorescence on individual cells. The scatter plots in the left panel shows the measured forward scatter as a function of GFP fluorescence for 10,000 cells.

The FACS scatter plots obtained after 4 hrs. of stress exposure supported the impression gained from the images. Namely that GFP fluorescence increased with cell size and that cell morphology varied with stress type and intensity.

**Quantitative studies employing FACS**

By employing FACS and the strain containing the plexA-gfp construct, it was possible to monitor average changes over time in both GFP emission intensity and cell morphology during antibiotics stress. Due to the mentioned background level of GFP fluorescence expressed by the construct, the quantitative data was normalized by subtracting the average GFP fluorescence of the control at the given time points. This study revealed that 4 of the employed conditions indeed induced lexA and that the induction profiles varied.

**Perspectives**

In order to investigate the role of the SOS response in the survival of and resistance towards antimicrobial agents in Listeria monocytogenes, we have examined the expression of the SOS regulators and target genes. As control we have employed Mitomycin C, which targets the structure and function of DNA directly, and as expected Mitomycin C stress leads to an induction of lexA and cell elongation. As a non-DNA targeting agent, we have been analyzing the cell-membrane targeting beta-lactam Cefuroxime. By following morphology and GFP accumulation we found that Cefuroxime does indeed induce the SOS response as well as an filamentous phenotype. By using a plexA-gfp reporter system and FACS experiments, our preliminary results indicate that antimicrobial agents, which do not target DNA, also leads to induction of the DNA-damaging repair system.

Further studies will be conducted on a wide variety of antibiotics with different cellular targets. By investigating the cellular response towards SOS-inducing antibiotics, we hope to identify a possible common factor responsible for this induction, which is either independent or indirectly dependent of DNA damage.