論　文　概　要

○ 論 文 題 目　Distinct roles of the alternative sigma factor σ^H in *Listeria monocytogenes*

(*Listeria monocytogenes* における代替シグマ因子 H の特異な役割)

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目　　的:

The purpose of this thesis is to examine the role of the alternative sigma factor H (σ^H) in *L. monocytogenes.* σ^H is widely distributed among the *Firmicutes* group of Gram-positive bacteria and is known for being the main regulator of sporulation and genetic competence. However, *L. monocytogenes* is a non-spore forming species in which competence has not been detected. *L. monocytogenes* lacks most of the genes required to form spores but it has a series of homologous genes that form the DNA-uptake machinery (*comG, comE,* and *comF* operons). Attempts to detect natural transformation in *L. monocytogenes* have failed so far, but the efforts have only focused on the ComK driven competence (main regulator in *B. subtilis* model). σ^H does not have a defined role yet, but some of the competence-related genes have been reported to participate in *L. monocytogenes* phagosomal escape. I hypothesize that *L. monocytogenes* σ^H may act as the regulator of the DNA-uptake machinery and induce the development of competence for transformation.

対象と方法:

In this study, a combination of overexpressing and deletion strains of σ^H and the transcription factor ComK were used to investigate the role of σ^H in *L. monocytogenes.* The contribution of σ^H to genetic competence (common role among non-sporulating *Firmicutes*) was evaluated by gene expression and reporter assays of the DNA-uptake machinery genes and transformation experiments. Finally, the effect of a *sigH* mutant was tested during intracellular survival (an important facet of *L. monocytogenes* life style) by infection of macrophages and epithelial cells.

結　　果:

- Gene expression analysis showed a unique regulation scheme in which σ^H and the transcription factor ComK are involved in the regulation of the DNA-uptake machinery genes. The expression of the *comG* operon can be induced by σ^H and ComK. σ^H is essential for *comEA* expression, but has no effect on *comEC* and the *comF* operon. Both regulators induced the expression at a subpopulation level.
- Synthetic transformation was detected for the first time in *L. monocytogenes.* Transfer of an extracellular plasmid was achieved by the artificial overexpression of ComK. Unexpectedly, transformants were only detected in the absence of σ^H (deletion mutant). σ^H is not required for transformation but rather its presence inhibits the import of extracellular DNA.
- *L. monocytogenes* is an intracellular pathogen and the ComK-mediated activation of some of the DNA-uptake machinery (*comG* and *comEC*) genes was previously shown to contribute to optimal phagosomal escape. σ^H was essential for phagosomal escape in phagocytic and non-phagocytic cells. The suppressive effect caused by the deletion of σ^H could not be compensated by ComK and was independent of the activity of virulence factors. Therefore, σ^H should regulate a novel mechanism of phagosomal escape.

考　　察:

The σ^H factor in *L. monocytogenes* can induce the expression of the DNA-uptake machinery genes, but it is not required for synthetic transformation. Moreover, it seems to negatively regulate the process. Gene expression analysis showed that σ^H is essential for the DNA-receptor (ComEA) expression. It is possible that in *L. monocytogenes*, ComEA binds to the extracellular DNA but instead of bringing it to the channel it prevents its access, which would explain why the deletion of *sigH* is required for transformation. The observed negative effect of σ^H may also be caused by the induction of a nc-RNA that has been showed to bind to mRNA and hide the SD region of some of the DNA-uptake machinery genes.

L. monocytogenes σ^H is essential for intracellular survival. A recent report showed that a σ^H deletion mutant is affected during growth in minimal medium which was attributed to a deficiency in the acquisition or utilization of nutrients. The observed impaired intracellular growth might be attributed to a broad physiological role. Since the deletion of *sigH* had no effect on the activity of virulence factors it is also possible that σ^H regulates a novel mechanism of phagosomal escape.

結　　論:

The results presented in this thesis suggest that the role of σ^H in *L. monocytogenes* has diverged from the rest of *Firmicutes* species. In contrast to its counterparts, σ^H seems to directly or indirectly repress the development of genetic competence and is essential for intracellular survival in phagocytic and non-phagocytic cells. *L. monocytogenes* appears to have adapted the use of σ^H to fit its multifaceted lifestyle.