Summary

Legionella pneumophila, the causative agent of the Legionnaires' disease, is an environmental strain with a widespread distribution in aquatic habitats. *Legionella* are able to replicate intracellularly in eucaryotic cells, such as macrophages, monocytes and protozoa. The sucsessful colonization of ecological niches, but also the virulence potential of *Legionella* depends on environmental factors. Therefore the investigation of ecological context is expected to provide an understanding of bacterial virulence.

The starting-point of this dissertation is the intensive pigmentation of the culture medium of *L. pneumophila*, witch could be observed in the late stationary phase of bacterial growth. The Legiolysin proteine (Lly) is responsible for this phenotype. This gene product of *L. pneumophila* is known to show an influence on the survival in the environment, which is why Legiolysin has been termed a fitness factor.

In order to investigate the ecological connections of the pigmentation, the *lly*-positive plasmid pEWL 1 was subcloned and sequenced. In this genetic section six further open reading frames (ORF) could be detected besides *lly* by sequence comparison of the derived amino acid sequences. The three directly neighbouring genes of *lly* code for proteins which have functionality with regard to the *lly*-determinant. The three reading frames upstream of *lly* show homologies to proteins, which are involved in transport processes.

The RNA transcript of *lly* could be determined by Northern blot with a lenght of about 1,8 kb. Therefore transcription of the *lly* gene together with the upstream ORF is suggested.

It could be shown by sequence analysis, that the Legiolysine gene responsible for this phenotype, is coding for a *p*-hydroxyphenylpyruvate dioxygenase (HPPD). This enzyme catalyzes the reaction of *p*-hydroxyphenylpyruvate to homogentisate (HGA).

In collaboration with Prof. P. Proksch (Pharmazeutische Biologie, Würzburg) the existence of HGA was demonstrated in culture supernatants of *lly*-positive strains on the basis of high performance liquid chromatography (HPLC). It could be shown, that the legiolysin gene is coding for a protein with a HPPD activity, and, therefore, is involved in the degradation of the aromatic amino acids Phenylalanine and Tyrosine.

Additionally chromosomal integration mutants of the *L. pneumophila* genes *lly* and *mip* ("macrophage infectivity potentiator") were created in *E. coli* K-12 strains. These mutants were subsequently used in ecological long-time studies. The chromosomal integration of *lly* took

place as a locus-specific recombination in the λ att - site of *E. coli* WM 2269. The integration of mip happened in the *fim*-region of the *E. coli* strain AAEC 160.

The second part of the present dissertation is concerned with ecological studies to the persistence of *L. pneumophila* in the environment.
Accordingly, it could be shown, that the expression of *lly* leds to persistence to light-stress. This light protection could be relevant in the environment, but also during the sanitation of water pipes by UV-light.

The association of *L. pneumophila* JR32 and JR32-1 (*lly*-negative) with the cyanobacterium *Fischerella* was observed in microcosms during a course of seven days. *Legionella* are able to persist in association with *Fischerella*, and in the *Fischerella* culture supernatant, respectively. Survival of the bacteria was not possible in fresh *Fischerella* medium. However, the expression of *lly* shows no difference, as could be shown by the coincubation of *L. pneumophila* with *Fischerella*. The growth of bacterial cultures cold neither be detected in supernatants of *Fischerella*, nor in fresh *Fischerella* medium. The adhesive character of the association of *L. pneumophila* with *Fischerella* could be documented by SEM (scanning electron microscopy).

The survival of *Legionella* in suboptimal environment was tested by studying the persistence of *L. pneumophila* and *E. coli* in soil. A rapid decline of CFU (colony forming unit) for *Legionella* could be detected within a short time, as cells could not be cultivated any more after six days. The deficiency of the pathogenetic and enviromental factors Mip, Fla (Flagellin) and Lly had no influence on the persistence of the bacteria in the soil. Additionally the recombinant *E. coli* clones AAEC 160-1 and WM 2269-1 with the genomic *mip* and *lly* integrations, were used in this exreriments. During a course of four weeks, a continuous reduction of CFU could be observed for these strains. Therefore none of these organisms proved successful in colonization of soil samples.

The studies regarding the loss of culturability of *L. pneumophila* and *E. coli* were made in autoclaved potable water and PBS (phosphate buffered saline), respectively, and in two variants of Main river water, one of which was autoclavedwhile the other one was sterilized by filtration. It could be shown, that *L. pneumophila* are able to persist well in potable water and

in the river water. Additionally, not only *L. pneumophila*, but also *E. coli* DH5α entered a viable but nonculturable state (VBNC). During the course of these experiments, the numbers of live cells were determined by fluorescence dyes Moreover, the transition of *L. pneumophila* into the VBNC state was documented by *in situ* hybridization technique with fluorescence marked 16 S rRNA oligonucleotide probes (FISH). This transition into the VBNC state plays an important role to overcome unfavourable phases in natural environments. Finally experiments were made to reactivate the VBNC states. This resuscitation is dependent on species specific triggers and could be achieved by coincubation of *L. pneumophila* with the amoeba *Acanthamoeba castellanii*.