B. Summary

Legionella pneumophila was identified in 1976 as the causative agent of a life-threatening atypical pneumonia. Today the genus comprises about 42 species which are spread worldwide in aquatic biotopes. The bacteria live in association with other microorganisms in biofilms as well as intracellularly in protozoa. They have a dual host system which means that they are able to replicate both in protozoans and in human phagocytes. Several environmental factors are known to play a role in their distribution. The ability to quickly detect and to exactly identify these potential human pathogens is important in order to recognize reservoirs for disease as well as to treat patients in due time.

Fluorescence *in situ* hybridization (FISH) is a technique based on the reaction of a specific oligonucleotide, labeled with a fluorescence marker, with the complementary rRNA target region. In this work a new 16S rRNA-targeted oligonucleotide probe LEGPNE1 for *in situ* hybridization was developed, which is specific for *L. pneumophila*. The specificity and the sensitivity of the probe were evaluated in experiments with different bacterial cultures. LEGPNE1 was demonstrated to be highly species-specific recognizing all tested strains of *L. pneumophila* independently of the serogroup. Non-*pneumophila* reference strains did not hybridize with the probe. Only one mismatch in the sequence was shown to be sufficient for the oligonucleotide to distinguish between complementary and nearly complementary sequences.
The probe was also applied successfully to infected amoebal cells and environmental samples. Different bacteria located intracellularly were recognized specifically by the probe. This allows the *in situ* monitoring of bacterial infection and multiplication rates in amoebae.
As legionellae presumably live most of the time as intracellular parasites, it is also important to be able to detect their hosts. Therefore, the design of new probes was extended to cover two known host amoebal genera, *Hartmannella* and *Naegleria*. Based on comparative sequence analysis the genus-specific 18S rRNA-targeted probes HART498 and NAEG1088 were constructed. Subsequently they were tested in hybridization series with different reference strains and gradually increasing stringency. Amoebal strains which had been identified previously based on their morphological features could be re-confirmed using *in situ* hybridization with these new oligonucleotides.
In situ hybridization experiments of infection assays with *Hartmannella vermiformis* and *Legionella pneumophila* using a combination of 16S and 18S rRNA-targeted probes were done successfully. Interference of the probes with the results of the tests was not observed.

For the analysis of the composition of complex microbial communities a culture-independent and highly specific method is required. This can be achieved by the fluorescence *in situ* hybridization. In order to determine potential preferences of legionellae for water parameters such as pH, temperature, conductivity, or water current, twenty-one different cold water habitats were examined for the presence of *Legionella* using the newly designed 16S rRNA-targeted oligonucleotide probe. The bacteria were shown to be able to tolerate a broad range of the measured parameters. They could be found in nearly all of the habitats investigated independent of the season. The new probe LEGPNE1 was proved to detect *L. pneumophila* in environmental samples highly specifically, even if the cells were in a nonculturable state.
Three *Legionella*-positive sampling sites were examined for the presence of amoebae. Using traditional culture methods followed by morphological determination, eight amoebal genera could be isolated and identified. Most abundant were strains of the apathogenic *Naegleria gruberi*-complex, *Echinamoeba* spp. and *Echinamoeba*-like amoebae. Other species including *Acanthamoeba* spp. (sequence type II), *Hartmannella* spp., *Platyamoeba placida*, *Saccamoeba* spp., *Thecamoeba quadrilineata* and *Vexillifera* spp. were found sporadically. *In situ* hybridization experiments using the new 18S rRNA-targeted oligonucleotide probes HART498 and NAEG1088 confirmed the determinations done by morphological criteria. Concomitant analysis of selected water parameters revealed no preference of the protozoa for certain environmental conditions.

In situ hybridization with rRNA-targeted oligonucleotide probes is a powerful tool to analyze structure and dynamics in biocenosis. However, this technique does not provide much information about the *in situ* function of the detected bacteria. The specific *in situ* detection of mRNA molecules allows to narrow this gap. One problem in the application of this method is the instability and low copy number of mRNA in each cell compared to other molecules like rRNA. Therefore, a signal amplification posterior to the *in situ* hybridization is required in most cases in order to generate a detectable signal. In this work the detection of the mRNA of *mip* in *L. pneumophila* was to be established using a protocol developed for the detection of *iap* in *Listeria monocytogenes*. However, first applications of dot blot and *in situ* hybridizations using DIG-conjugated polyribonucleotide probes and several DIG-labeled oligonucleotides applied simultaneously did not show the neccessary specificity. This technical approach will be essential for further experiments in this field of research.