Summary

Diversity and functions of bacteria associated with springtails

Springtails are tiny invertebrates that inhabit moist environments all over the world. In soil ecosystems, they play important ecological roles by contributing to decomposition processes through the fragmentation of organic matter and the control of soil microbial communities. Some springtail species, such as *Folsomia candida*, are used as model organisms in ecotoxicological research and as bioindicators of soil quality. Decades of research have provided a wealth of information on the anatomy, physiology, ecology, and behavior of these model species, and more recently genomic sequencing studies have offered new insights into their evolution and phylogenetic relationships to other arthropod groups. Like all other animals, springtails constitute a habitat for microorganisms, which may play important roles for the functioning of their hosts. Therefore, associated microbes constitute another aspect of the biology of these animals, which deserves attention.

In this thesis, we studied the microbial community of the springtail *F. candida*. We explored the composition of this community and its potential functions by using a combination of sequence-based and more targeted functional approaches, as well as bioinformatic tools for data analysis. We specifically focused on the identification and description of potential antimicrobial functions, which are ecologically relevant for *F. candida* and may have a variety of applications.

In Chapter 2 we studied the composition of the bacterial communities in two populations of *F. candida*. By using amplicon-based high-throughput sequencing we identified over 800 bacterial groups. Pronounced differences were observed in the relative abundances of bacteria between the two populations, especially in the case of *Wolbachia*, the endosymbiont that causes parthenogenesis in *F. candida*. When applying a method to target bacteria other than *Wolbachia*, we were still able to successfully identify the most dominant bacterial groups, which were shared between the two populations of springtails. While in line with the results of previous studies on the microbial community of *F. candida*, the findings presented in this chapter deepen our understanding of bacterial diversity in this animal.

In Chapter 3 we studied the metagenome of *F. candida*. By using shotgun sequencing and a variety of bioinformatics approaches, we obtained a detailed picture of the pooled genomic content of the gut microbiota of the springtail. The analysis revealed the presence of multiple genes involved in antimicrobial activity and carbohydrate metabolism. These functions may contribute to the digestion of plant material and
to defense against pathogens in the gut of *F. candida*, possibly indicating a supportive role of the microbes for the fitness of their host. In the metagenome, we also identified several homologs to horizontally transferred genes in *F. candida*, suggesting that specific functions may have been transferred from the gut microbiota to the springtail.

In Chapter 4 we investigated the potential for antimicrobial activity of springtails’ bacteria. We isolated and cultivated gut bacteria from four springtail species, and we tested their capacity to inhibit the growth of a variety of microbial pathogens. Of 46 unique isolates identified, 35 showed inhibitory activity, indicating a high potential for antimicrobial production. The isolates were active against insect and plant pathogens, suggesting that they may play a role in host defense or in the control of environmental microbes. The results of this chapter point to springtails’ bacteria as possible sources of biocontrol methods and as interesting targets for drug discovery.

In Chapter 5 we described one *Bacillus* strain isolated from the gut of *F. candida*. We studied its metabolic profile, antibiotic resistance and inhibitory activity against pathogenic microorganisms. The isolate was identified as a *Bacillus toyonensis*, and displayed extremely high resistance to penicillin. Both the living bacteria and their extract inhibited the growth of a variety of pathogenic microorganisms. In line with these observations, genome analysis of the strain revealed an enrichment of resistance genes for β-lactam antibiotics and the presence of secondary metabolite clusters.

The work presented in this thesis expands the knowledge of springtails’ microbiota and constitutes a foundation for future research on this topic. Building on previous findings, which mainly resulted from culturing and 16S amplicon sequencing, we applied a combination of methods to study the bacterial community of *F. candida*. This community appears to be highly diverse and it contains a number of potential functions with possible useful applications as well as high ecological relevance. The finding of many genes associated with carbohydrate metabolism suggests a contribution of gut microbes to digestive processes. The similarity of these genes to foreign genes in the genome of *F. candida* suggests that bacteria may not only break down the plant material in the gut of the springtail, but also transfer genes involved in this process to the host. This would constitute an important adaptation to life in the soil, an environment rich in plant degradation products. A high potential for antimicrobial production was revealed both by metagenome mining and by functional assays. Antimicrobial substances in *F. candida*’s gut may be involved in a variety of processes, namely pathogen defense, microbial interactions, modulation of microbial community composition and communication between microorganisms and their host. These and other processes could be explored by future research aimed at elucidating the function of antimicrobials in springtails and in their associated microbes.