

Bacterial diversity associated with freshwater zooplankton

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Summary

Bacterial community compositions (BCC) associated with the cladoceran *Bosmina coregoni* and the cyclopoid copepod *Thermocyclops oithonoides* in oligotrophic Lake Stechlin versus eutrophic Lake Dagow (northeastern Germany) were compared using molecular techniques. We also transplanted the zooplankton from their native lake to the other lake, and studied changes in their associated BCC as a result of the modified ambient environment. Bacterial community composition associated with *B. coregoni* was quite conservative between the oligotrophic and eutrophic lakes, and most of the bacteria belonged to *Alpha*- and *Betaproteobacteria*. In contrast, BCC associated with *T. oithonoides* was highly variable and dependent on the environment, and *Betaproteobacteria* and *Bacteroidetes* were the most represented among the bacteria. *Thermocyclops oithonoides* from the oligotrophic lake was more able to retain its bacteria after being transplanted to the eutrophic lake than vice versa. This suggests that bacteria in oligotrophic water were more firmly attached to the copepod and better in resisting environmental fluctuations than those in eutrophic water.

Introduction

Bacteria may colonize a zooplankton by direct attachment to its body surface (Nagasawa and Nemoto, 1988; Carman and Dobbs, 1997). They may also attach to phytoplankton or other food particles (Simon *et al.*, 2002) and enter the zooplankton's gut via ingestion (Tang, 2005).

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Defecation by the host, on the other hand, releases gut flora to the surrounding environment (Tang, 2005). Thus, there is an active exchange of bacteria between zooplankton and the surrounding water, but the different physicochemical characteristics between these environments may favour different bacteria (Sochard *et al.*, 1979; Delille and Razouls, 1994; Hansen and Bech, 1996). Indeed, the anoxic environment of zooplankton gut and faecal pellets may favour strict anaerobes, which otherwise may not survive in the oxygenated water column (Bianchi *et al.*, 1992; Proctor, 1997; Braun *et al.*, 1999). Hence, zooplankton can be regarded as microbially diverse and active hotspots that are different from the surrounding water.

Because the ambient bacterial community composition (BCC) and food environment can differ substantially between lakes, zooplankton of even the same species may acquire different attached bacteria in different aquatic environments. To better understand this issue we studied the BCC associated with zooplankton in oligotrophic Lake Stechlin (LS) (53°09'03"N, 13°01'40"E) and eutrophic Lake Dagow (LD) (53°09'01"N, 13°03'60"E) in northeastern Germany. To further test the effect of environmental changes, we performed experiments where we transplanted the zooplankton from their native lake to the other lake, and examined changes in their associated BCC.

Results and discussion

In Field Experiment I we compared the BCC associated with female cladoceran from the two lakes (Fig. 1A). Despite the close proximity between LS and LD, their ambient BCC had a similarity of ~85% (Fig. 2). Interestingly, the initial BCC associated with the cladoceran *Bosmina coregoni* with full gut were very similar between lakes and within lake (91–96%), all of which were very different from the ambient bacteria (66%). *Bosmina coregoni* retained > 90% similarity in their BCC relative to the initial BCC after being transplanted from LS to LD. The similarity in BCC associated with *B. coregoni* increased to about 95–98% after gut clearance for both between- and within-lake samples. From Field Experiment I we conclude that the bacterial community associated with

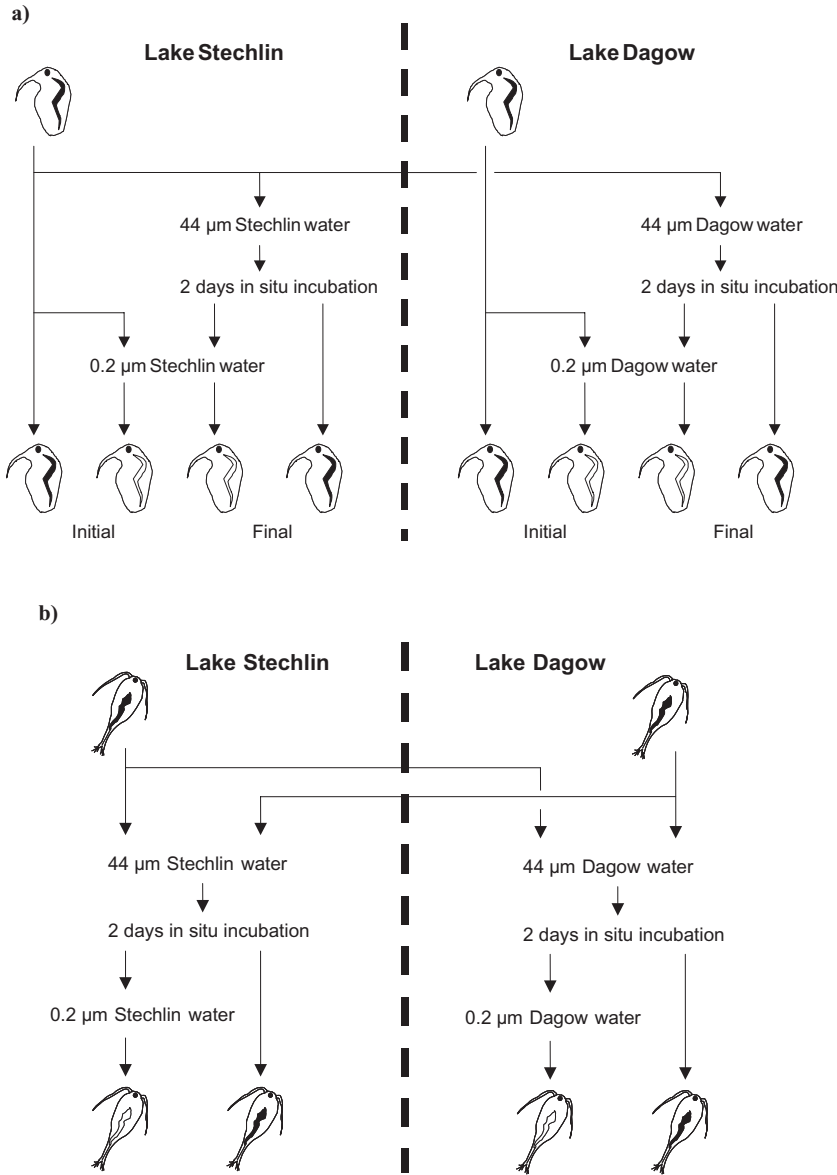


Fig. 1. A. Schematic for Field Experiment I with *Bosmina coregoni*. Live females from Lake Stechlin were incubated in Lake Stechlin (oligotrophic) and Lake Dagow (eutrophic) between 6 and 8 August 2007. Initial and final individuals before (indicated by black gut) and after (white) gut clearance (in 0.2 µm filtered lake water) were collected for DGGE analyses. Live individuals from Lake Dagow were also collected for comparison. B. Schematic for Field Experiment II with *Thermocyclops oithonoides*. Live females from Lake Stechlin and Lake Dagow were incubated in both Lake Stechlin and Lake Dagow between 10 and 12 August 2007. At the end of the incubation, individuals before (indicated by black gut) and after (white) gut clearance (in 0.2 µm filtered lake water) were collected for DGGE analyses.

B. coregoni maintained a rather stable composition ($\geq 83\%$ similarity) irrespective of the lake environment, the ambient BCC or the gut content of the cladoceran.

Dominant bacterial groups on *B. coregoni* in LS and LD belonged to *Alphaproteobacteria* (three *Erythrobacteraceae*, one *Bartonellaceae*, one *Sphingomonadaceae*) and *Betaproteobacteria* (four *Comomonadaceae*), and there was only one band each belonging to *Gammaproteobacteria* (*Aeromonadaceae*) and *Actinobacteria* (*Micrococcaceae*) (Fig. 3). All dominant DGGE bands were present in all treatments and mainly belonged to the genera *Porphyrobacter* (*Alphaproteobacteria*, three dominant bands) and *Hydrogenophaga* (*Betaproteobacteria*, one dominant band). The genus *Porphyrobacter* is typically found in freshwater habitats but has not been reported

occur on zooplankton bodies. However, its chitinolytic capability (Brenner *et al.*, 2005) suggests that it is well adapted to growing on zooplankton bodies or carcasses. The genus *Hydrogenophaga* occurs in different environments degrading a variety of organic compounds (Brenner *et al.*, 2005). Our study further indicates a close association of *Hydrogenophaga* species with zooplankton.

In lakes the zooplankton community is often dominated by cladocerans and copepods, which occupy similar ecological niches. Hence, one may expect that copepods would carry similar BCC as *B. coregoni*. Much to our surprise, however, we obtained very different results with the cyclopoid copepod *Thermocyclops oithonoides* when we did a double-transplant between the eutrophic LD and the oligotrophic LS (Field Experiment II; Fig. 1B). In

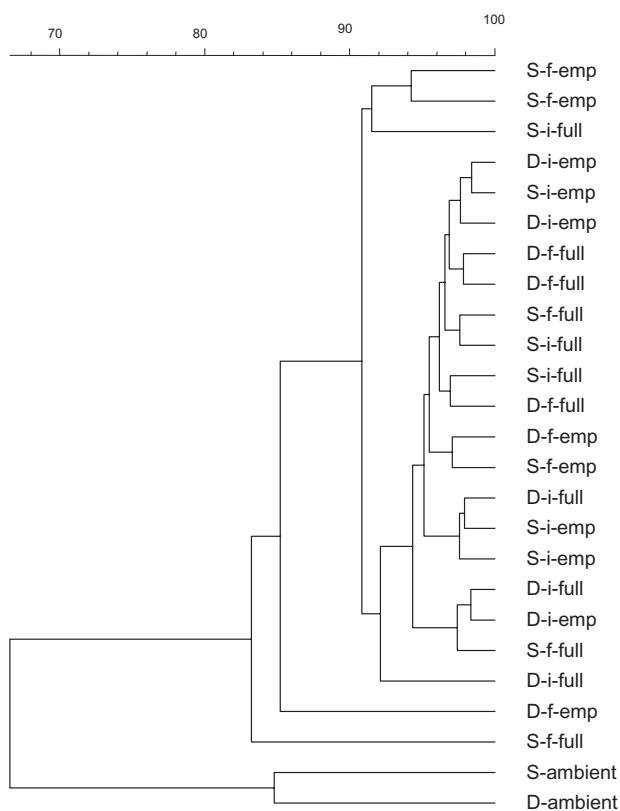


Fig. 2. Cluster diagram of DGGE banding patterns for bacteria associated with *Bosmina coregoni* incubated in different lakes (Field Experiment I). DGGE analysis was conducted with eubacteria-specific primers 341f-gc and 907r. Cluster analysis is based on Pearson's correlation coefficients. D, Lake Dagow; S, Lake Stechlin; i, initial; f, final; full, before gut clearance; emp, after gut clearance; ambient, epilimnion water. Scale bar at the top indicates per cent similarity.

general, both 'native' and transplanted *T. oithonoides* showed much more diverse BCC than *B. coregoni*. *Thermocyclops oithonoides* from LD was incubated in LD and LS for 2 days and its attached BCC retained only 28% and 78% similarity, respectively, between replicates, and there was < 62% similarity between the two groups (Fig. 4). *Thermocyclops oithonoides* from LS retained 16–42% similarity after being incubated in LS, and 28–46% after being transplanted to LD, and there was < 68% similarity between the two groups. This indicates that *T. oithonoides* naturally carried very diverse BCC that reflected the influence of the external environments.

While copepod with full gut maintained diverse BCC in both lakes, the effect of gut clearance on the BCC very much depended on the origin of the copepod. For copepod originating from LD, its associated BCC resemblance drastically increased to 86–92% after gut clearance irrespective of whether the copepod was emptying its gut in native lake water or foreign lake water. In contrast, the BCC associated with copepod from LS

maintained low resemblance (< 30%) after gut clearance regardless of whether the copepod was incubated in LD or LS water. Based on these observations we deduce that many bacteria from the oligotrophic LS were able to firmly attach to the copepod and remain attached even after gut clearance. On the other hand, most bacteria from the eutrophic LD appeared to be only loosely associated with the copepod's gut content and were lost from the copepod after gut clearance.

Bacteria from oligotrophic water tend to benefit from surface colonization more than those in eutrophic water. For example, in oligotrophic Lake Lucerne (Switzerland) growth rate and doubling rate of attached bacteria were 46 times and one order of magnitude higher, respectively, than those of free bacteria; the contrast was much lower in eutrophic Lake Sempach (Friedrich *et al.*, 1999). In eutrophic water, organic substrates are more readily available in dissolved form. In oligotrophic water, however, organic substrates are more concentrated in particulate matters, rendering the ability of bacteria to colonize and remain attached to particle surfaces more essential. This perhaps explains why bacteria originated from the oligotrophic LS were more firmly attached to the copepod and more resistant to change even when the copepod was exposed to a foreign medium.

The main bacteria on *T. oithonoides* belonged to *Betaproteobacteria* (four *Incertae sedis* 5, three *Neisseriaceae*, one *Comamonadaceae*, one *Oxalobacteraceae*) and *Bacteroidetes* (three *Flavobacteriaceae*, two *Flexibacteriaceae*). Three bands affiliated to the *Actinobacteria* (two *Microbacteriaceae*, one *Corynebacteriaceae*) and one band each to the *Alpha-* (unknown) and *Gamma*-*proteobacteria* (*Enterobacteriaceae*) and *Firmicutes* (*Bacillus*) were also found (Fig. 3). *Actinobacteria* are commonly found associated with phytoplankton in LS, LD and other lakes (C. Dziallas, unpubl. research); hence, zooplankton may acquire *Actinobacteria* through ingesting food particles that have been colonized by the bacteria. In contrast, other bacteria such as *I. sedis* 5 and *Bacillaceae* have never been reported being associated with phytoplankton. Their presence on *T. oithonoides* suggests that these bacteria are very rare and perhaps inactive in the ambient water, but they become active and prolific once they are attached to the copepod.

In comparison between the two experiments, the bacterial communities associated with the cladoceran *B. coregoni* appeared to be more independent of the ambient bacterial communities than those associated with the copepod *T. oithonoides*. Both species are crustaceans with similar ecological niches. However, *B. coregoni* is a filter feeder, whereas *T. oithonoides* presumably feeds more selectively; thus, it is expected that the gut flora of *B. coregoni* would be more susceptible to external influences

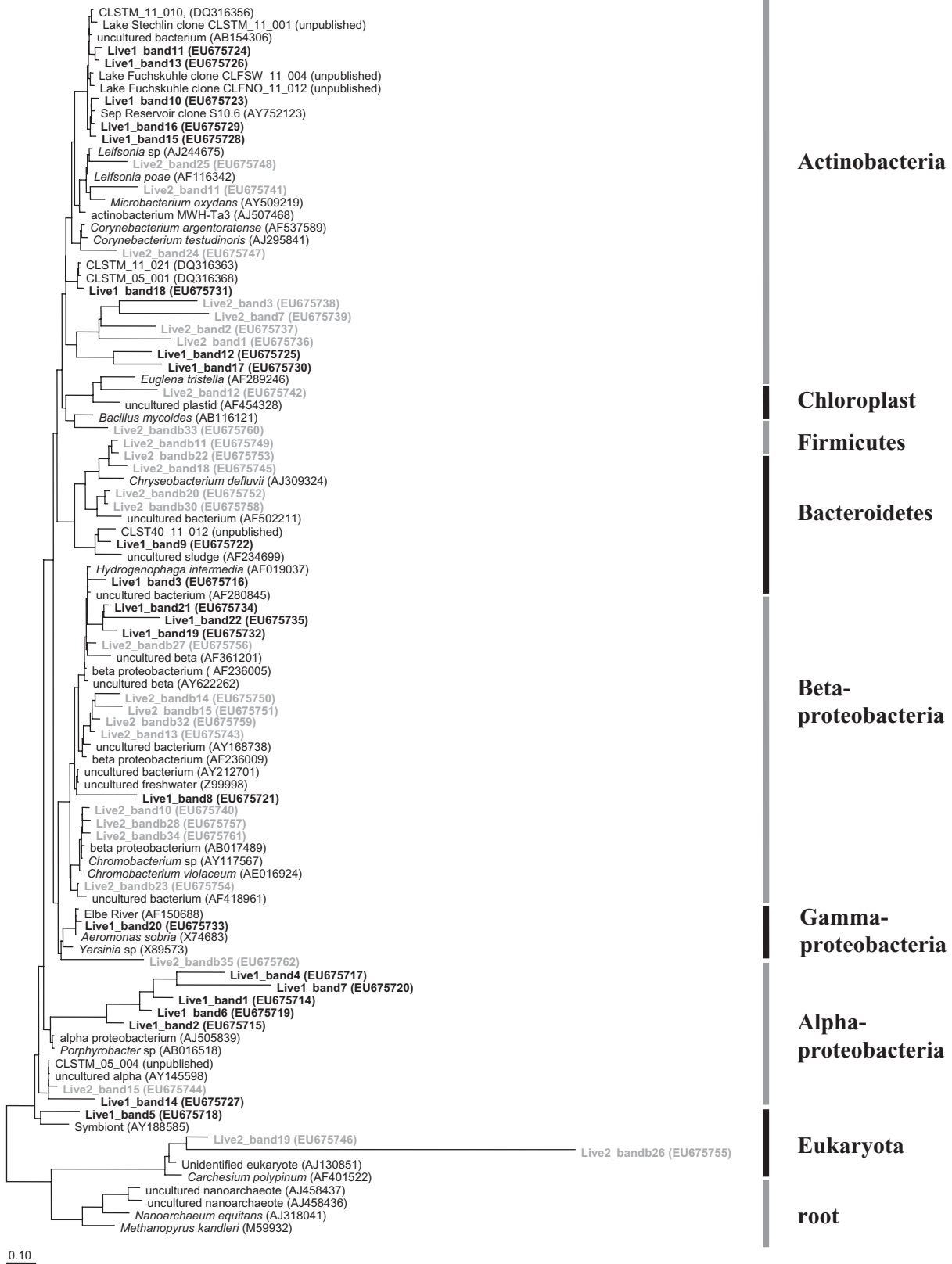


Fig. 3. Phylogenetic tree including 16S rRNA sequences of all DGGE bands from Field Experiment I (bold) and II (grey) (for details see text). Gene bank accession numbers are given in parentheses. The tree is constructed by the ARB software (<http://www.arb-home.de>) using the maximum-likelihood algorithm, which was tested for stability. Scale bar at the bottom represents relative phylogenetic distance.

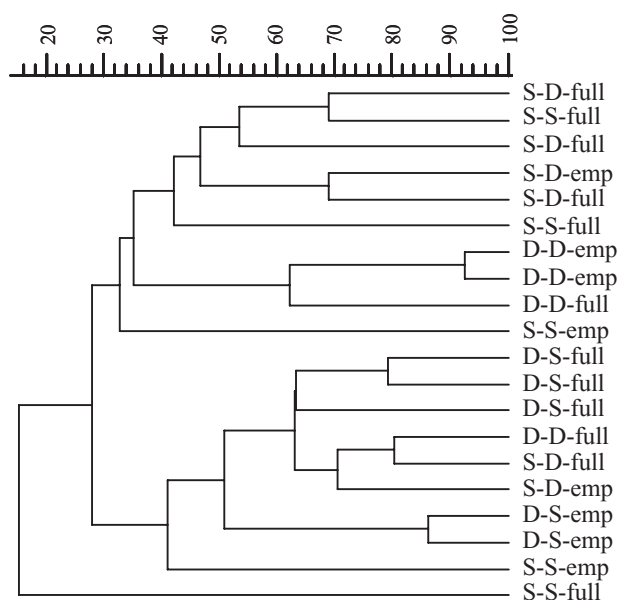


Fig. 4. Cluster diagram of DGGE banding patterns for bacteria associated with *Thermocyclops oithonoides* incubated in different lakes (Field Experiment II). The first letter of the label indicates the origin of the copepods; the second letter indicates the location of the incubation; 'full' indicates before gut clearance; 'emp' indicates after gut clearance. For example, 'S-D-full' represents *T. oithonoides* from Lake Stechlin incubated in Lake Dagow and DGGE was performed before gut clearance. Scale bar at the top indicates per cent similarity.

than that of the copepod. The fact that we observed the opposite suggests that perhaps the internal gut environments are very different between cladoceran and copepod, which select for very different BCC (Harris, 1993). This hypothesis, however, cannot be confirmed without detailed study of the physicochemical characteristics of the gut environments of these zooplankton species.

Previous studies of bacterial colonization of zooplankton have been mainly focused on potentially pathogenic bacteria associated with marine copepods (Maugeri *et al.*, 2004; Belkin and Colwell, 2005; Huq *et al.*, 2005). We also found potentially pathogenic bacteria in our study, such as members of the *Aeromonas* genus associated with *B. coregoni* (Maugeri *et al.*, 2004), and *Chromobacterium* of the family *Neisseriaceae* associated with *T. oithonoides* (Tucker *et al.*, 1979). These observations show that even freshwater zooplankton provide microhabitats allowing for attachment and proliferation of potentially pathogenic bacteria.

In conclusion, the microbial diversity associated with zooplankton is partly governed by species-specific (yet unknown) characteristics of the zooplankton host, the food environment and ambient bacterial communities to which the host is exposed. Permanent or transient associations between diverse bacterial communities and

zooplankton will likely affect many ecological and biogeochemical pathways in the water column, many of which have not been fully investigated.

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