Ecological Differentiation within a Cosmopolitan Group of Planktonic Freshwater Bacteria (SOL Cluster, *Saprospiraceae*, *Bacteroidetes*)†

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Members of the monophyletic SOL cluster are large filamentous bacteria inhabiting the pelagic zone of many freshwater habitats. The abundances of SOL bacteria and compositions of SOL communities in samples from 115 freshwater ecosystems around the globe were determined by fluorescence in situ hybridization with cluster- and subcluster-specific oligonucleotide probes. The vast majority (73%) of sampled ecosystems harbored SOL bacteria, and all three previously described SOL subclusters (LD2, HAL, and GKS2-217) were detected. The morphometric and chemico-physical parameters and trophic statuses of ecosystems were related to the occurrence and subcluster-specific composition of SOL bacteria by multivariate statistical methods. SOL bacteria did not occur in acidic lakes (pH < 6), and their abundance was negatively related to high trophicity and pH. The subcluster-specific variation in the compositions of SOL communities could be related to the pH, electrical conductivity, altitude, and trophic status of ecosystems. All three known SOL subclusters differed in respect to their tolerated ranges of pH and conductivity. Complete niche separation was observed between the vicarious subclusters GKS2-217 and LD2; the former occurred in soft-water lakes, whereas the latter was found in a broad range of hard-water habitats. The third subgroup (HAL) showed a wide environmental tolerance and was usually found sympatrically with the LD2 or GKS2-217 subcluster. Ecological differentiation of SOL bacteria at the subcluster level was most probably driven by differential adaptation to water chemistry. The distribution of the two vicarious taxa seems to be predominantly controlled by the geological backgrounds of the catchment areas of the habitats.

Which factors control the occurrence and abundance of particular bacterial groups in the environment? Despite fast-growing knowledge in microbial ecology, mainly mediated by methodological innovations bypassing the inherent cultivation problem of microorganisms, we have just begun to unravel the phylogenetic compositions of bacterioplankton communities in freshwater ecosystems (8, 9, 26, 51, 52). However, studies dealing with the compositions of freshwater bacterioplankton communities often use methods with low phylogenetic resolution (1, 8) and deal only with single or rather few habitats (1, 14, 23, 36, 43) or target the whole bacterioplankton community composition by fingerprinting methods (15, 20, 25, 49, 50). While valuable insights into factors shaping the bacterioplankton communities have been achieved (3, 15, 20, 24, 25, 27, 38, 49, 50), these approaches may not always be appropriate enough to meaningfully connect the occurrence and abundance of phylogenetically defined groups of bacterioplankton to the relevant environmental factors in order to gain insights into the ecology of certain bacterioplankton groups. For these reasons, characterizations of ecological niches of certain groups, as well as data on the ecological differentiation between closely related taxa, are rather scarce for free-living bacteria (10, 11), while this kind of knowledge is available for many higher organisms (35, 39, 47).

Lately, we have established the phylogenetic basis of the bacteria affiliated with the monophyletic SOL cluster, which is a dominant group of filamentous bacterioplankton that can be found throughout the year in a broad variety of freshwater ecosystems (34). The lengths of the SOL filaments can vary from 8 to >100 μm (34). It has been shown that the share of the bacterial biovolume of the SOL bacteria can exceed 40% of the total bacterial biovolume (30, 34). Members of the striking SOL morphotype form a monophyletic group (the SOL cluster), which so far divides into at least three phylogenetic subclusters (LD2, HAL, and GKS2-217). These subgroups are characterized by minimal 16S rRNA sequence similarities of 97.6% to 99.7% (intrasubcluster) and 89.9% to 95.5% (intersubcluster). These phylogenetic similarity values justify the assumption that the subclusters represent species-like taxa (33). Environmental sequences clustering within the SOL cluster, but outside of the three well-established subgroups (34), along with the latest fluorescence in situ hybridization (FISH) results, suggest the presence of additional subclusters within the SOL cluster. So far, all bacteria assigned to the SOL cluster via specific FISH probes share the same filamentous morphology. Therefore, members of the different species-like subgroups cannot be distinguished by morphological traits. The set of oligonucleotide probes available for this remarkable component of bacterioplankton (34) enables the resolution of the subcluster-specific composition within the closely related SOL cluster.

The aim of this study was to identify potential ecological differences within a model group of heterotrophic bacterioplankton, which consists of well-defined and phylogenetically closely related subgroups. This goal was reached by applying a

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set of FISH probes with varying phylogenetic resolutions to an extensive set of samples from a broad range of different freshwater ecosystems worldwide.

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MATERIALS AND METHODS

Study sites and sampling. During the years 2002, 2003, and 2004, 140 samples from 115 different freshwater habitats were investigated (see Table S1 in the supplemental material). The probed ecosystems are located in four continents (Eurasia, North America, Africa, and Australia) in three climatic zones (temperate, subtropical, and tropical). The pelagic zones of most of the studied freshwater lakes, rivers, and ponds in Austria, the Czech Republic, Italy, Slovenia, Germany, The Netherlands, Sweden, Finland, Zambia, Uganda, Nepal, Tibet, the People's Republic of China, Hawaii, Mexico, Australia, and New Zealand were sampled with a 10-liter Schindler sampler. Samples from Austrian lakes were kept in 5-liter plastic bottles for less than 2 hours until they were processed in the laboratory. Samples from most of the more distantly located ecosystems were fixed (paraformaldehyde; 2% final concentration) and further processed on site, while a few were transported to Austria untreated. Transcontinental delivery of live samples to the laboratory usually did not take longer than 2 days (maximum, 5 days). Subsamples for total bacterial counts were fixed with formaldehyde (2% final concentration). For FISH, 10 to 30 ml of water was fixed with paraformaldehyde (2% final concentration) for 2 h at room temperature or at 6°C overnight, filtered onto 0.2-μm polycarbonate filters (45 mm; Millipore), dried, and stored frozen at -20°C to await further processing.

Environmental variables. Geographic and morphometric data on habitats (altitude, lake area, and maximum depth) and trophic characterization of Swedish and Austrian freshwater habitats and Tibetan high-mountain lakes were provided by E. Lindström and S. Langenheder, at the Austrian State Department, and Qinglong Wu, respectively. Data on the Bohemian forest lakes were extracted from the work of Vrba et al. (45). Water temperature, pH, and electrical conductivity were measured in the field with a universal meter (Multiline P4; WTW, Weilheim, Germany) whenever possible.

Bacterial abundance. Formaldehyde-fixed samples were stained with DAPI (4',6'-diamidino-2-phenylindole; final concentration, 6 μg ml⁻¹) (31) and enumerated by epifluorescence microscopy (Zeiss Axioplan). Between 300 and more than 1,000 DAPI-stained bacterial cells were counted at ×1,250 magnification. More than 10 microscopic fields (20 to 50 cells per field) were counted for each sample. Filaments of the SOL morphotype were counted separately.

FISH. FISH was performed on polycarbonate filter sections according to the protocol of Alfreider et al. (1). Counting of hybridized filter sections was done by epifluorescence microscopy (Zeiss Axioplan; ×1,250 magnification) as described previously (34). The oligonucleotide probes used for FISH were SAP-309 (specific for *Saprospiraceae*), SOL-852 (specific for the SOL cluster), LD2-1261 (specific for the LD2 subcluster), HAL-844 (specific for the HAL subcluster), GKS-847 (specific for the GKS2-217 subcluster), and HHY-441 (specific for *Haliscomenobacter hydrossis* strain DMS1100). Details of probe sequences, testing, and adequate formamide concentrations have been presented by Schauer and Hahn (34). Probes SAP-309 and SOL-852 were used as positive controls for the assignment of all SOL morphotype filaments to the monophyletic SOL cluster in all of the samples that were hybridized. The SOL subcluster-specific probes LD2-1261, HAL-844, and GKS-847 were used for the enumeration of SOL subcluster bacteria, together with the total SOL morphotype filament counts of DAPI-stained lake water. Probe HHY-441 was used on selected sam-

ples to determine the presence of *Haliscomenobacter hydrossis* (strain DSM1100) in natural freshwater ecosystems.

Statistical analysis. Fifteen of the habitats were sampled repeatedly (two to five times) during the years 2002 to 2004. For these ecosystems, the means of the assessed variables were used. Trophic was coded as an ordered factor in all analyses (oligotrophic < oligomesotrophic < mesotrophic < eutrophic). Hypertrophic Lake Taihu and mesoeutrophic lakes Grabensee, Ibmsee, and Immsee were pooled with eutrophic ecosystems to obtain an even distribution.

We used principal-components (PC) analysis (PCA) for summarizing correlations among the environmental variables and for visualizing differences among the lakes; 76 habitats that had no missing values were considered. Variables were centered and standardized. The significance of PC axes was assessed using the broken-stick model (17). The responses of total bacteria and SOL bacteria to environmental variables were studied by ordinary least squares (OLS) regression and generalized linear models (GLM) with a Tweedie variance function (because of a continuous overpositive y , with positive mass at $y = 0$ distribution) and a canonical link (4, 19), respectively, following (5). Variable selection was based on the Akaike and Bayes information criteria (OLS) and analysis of deviance (GLM) (2). A preliminary detrended correspondence analysis (DCA; detrending by segments) of abundances of filamentous bacteria resulted in an axis length of 2.0 standard deviations, suggesting unimodal response models (41). We therefore used (partial) canonical correspondence analysis (CCA) and 9,999 unrestricted permutations to assess the significance of environmental variables for changes among abundances of SOL bacterial subgroups (22, 40, 41, 42). P values were adjusted (P_{adj}) for multiple testing using a Bonferroni-type test procedure (16). In DCA and CCA, all nonacidic habitats (pH 6.6 to 9.0) with no missing values were considered (53 habitats). Bacterial abundances (number of bacteria ml⁻¹) were $\log_{10}(x + 1)$ transformed to make distributions more symmetric.

All statistical analyses were done in R (32). DCA and CCA were run using the R package Vegan (29).

RESULTS

Statistical analysis of environmental variables. Table 1 summarizes the determined environmental variables. Lakes Taihu and Garda had an unusually high lake area and maximum water depth, respectively. Therefore, they were disregarded in all statistical analyses. PCA sums up correlations among conductivity, pH, altitude, lake area, and maximum water depth (see Fig. S1 in the supplemental material). PCA resulted in two significant axes (broken-stick model), explaining 71.5% of the variance in the five environmental variables. Conductivity and pH were most highly correlated with PC axis 1. The two variables and lake area correlated significantly (Kendall's τ , $P_{adj} < 0.05$). Altitude, lake area, and maximum water depth were correlated with both significant PC axes. Significant correlations were found among altitude, conductivity, and lake area; the last was also correlated with maximum water depth (Kendall's τ , $P_{adj} < 0.05$). PC axis 2 separated the oligotrophic lakes from lakes of higher trophic status (see Fig. S1 in the supplemental material). Trophic changed significantly with con-

TABLE 1. Statistical summary of environmental variables and total bacterial abundances of all 115 sampled ecosystems

Value	Altitude (m a.s.l.)	Lake area (ha)	Maximum depth (m)	pH	Conductivity (μS cm ⁻¹)	Total no. of bacteria (10 ⁶ ml ⁻¹)
Minimum	0	0.1	2	4.5	11	0.19
1st quartile	275	3	6	6.9	31	0.98
Median	553	20	19	8.0	194	1.75
3rd quartile	1,311	214	43	8.4	324	2.69
Maximum	4,987	6,880,000	1,471	9.4	21,700	11.30
Mean	903	105,316	52	7.6	434	2.06
SD	919	738,341	161	1.2	2,207	1.62
Missing values	4	10	27	20	19	4

TABLE 2. Mean abundances of total bacterioplankton in habitats of different trophic status

Trophic status	Total no. of bacteria (10 ⁶ ml ⁻¹)		No. of samples
	Mean	SD	
Oligotrophic	1.17	0.63	63
Oligomesotrophic	1.97	0.77	24
Mesotrophic	2.70	1.12	27
Eutrophic ^a	4.47	3.13	12
ND ^b	2.50	0.75	10

^a Including samples of mesoeutrophic (3) and hypertrophic (1) habitats.^b ND, trophic status remained undetermined.

ductivity, altitude, and maximum water depth (Kruskal-Wallis rank sum test, $P_{\text{adj}} < 0.05$).

Bacterioplankton abundance. Total bacterial abundances ranged from 0.2×10^6 to 11.3×10^6 ml⁻¹ (mean, 2.0×10^6 ml⁻¹) (Table 2). Trophic, altitude, and pH explained 60% of the variance in the total bacterial concentration (OLS, $P < 0.001$); however, pH was significant only after the effects of trophic and altitude had been considered (type II error, $P > 0.05$). Lake Seeleithensee was omitted in this OLS due to an unusually high total bacterial concentration (11.3×10^6 ml⁻¹).

Detection of SOL bacteria and their proportions in abundance and biovolume. In 73% of all 115 sampled habitats, SOL bacteria were discovered and assigned to known subclusters via FISH with cluster- and subcluster-specific oligonucleotide probes. In 52% of the samples, only one subcluster of SOL bacteria was detected. Twenty-four percent of the samples contained two SOL subclusters, while three different subgroups including phylogenetically uncharacterized SOL groups (PUC group) were found in only two habitats (Cenote Laguna, Mexico, and Lake Nkuruba, Uganda). Twenty-two percent of all samples (31 habitats; 27% of habitats) did not contain SOL bacteria. SOL-negative ecosystems could be partly characterized by low pH values (pH < 6; 10 habitats) or short water retention times (only a few days; 4 habitats).

The maximum abundance of SOL bacteria was 21.6×10^4 filaments ml⁻¹, equivalent to 11% of the total bacterial numbers. For all positive samples, on average, 0.81% (1.24×10^4 filaments ml⁻¹) of total bacterial numbers were assigned to the SOL cluster. Using a previously established (34) biovolume conversion factor of 25.7, SOL bacteria contributed an average of 17.3% of the total bacterial biovolume in all positive samples of our data set.

Subcluster-specific composition of SOL bacteria. All three of the SOL subgroups thus far characterized (LD2, HAL, and GKS2-217 subclusters) were detected in our set of freshwater ecosystems. The most abundant and widespread subgroup of SOL bacteria was the LD2 subcluster, which was found in 62% of all samples, accounting for 75% of all SOL bacteria (Table 3). This subgroup was the only subcluster in 56% of the SOL-positive samples, having a mean abundance of 1.4×10^4 filaments ml⁻¹ in the positive samples. The HAL subgroup was found in 22% of the samples, accounting for 7% of the SOL bacteria and having a mean abundance of 0.17×10^4 filaments ml⁻¹ in the positive samples. Members of the GKS2-217 subcluster were found in 12% of all samples, accounting for 11% of the SOL bacteria. This subcluster was the only subcluster in

TABLE 3. Subcluster-specific compositions of investigated SOL assemblages

Parameter	LD2	HAL	GKS2-217	PUC group ^a
Positive samples (%) ^b	62.1	22.1	12.1	8.6
Mean share of SOL bacteria [%] ^c	74.4	7.3	11.0	6.3
Only subgroup (%) ^c	56.0	0.9	6.4	3.7
Mean abundance (10 ⁴ ml ⁻¹) ^d	1.40	0.17	0.25	0.29

^a PUC group, phylogenetically uncharacterized SOL bacteria.^b Percentage of all samples ($n = 140$).^c Percentage of all SOL-positive samples ($n = 109$).^d Calculated from all samples where the respective subgroup was present.

6% of the SOL-positive samples, having a mean abundance of 0.25×10^4 filaments ml⁻¹. In 12 samples, SOL-type filaments could not be assigned to any of the three known SOL subclusters. These thus far phylogenetically uncharacterized (i.e., unknown subcluster affiliation) SOL bacteria accounted for 6% of the SOL cluster, having a mean abundance of 0.29×10^4 filaments ml⁻¹ in the positive samples (Table 3).

Statistical analysis of environmental factors influencing the occurrence and composition of SOL bacteria. SOL bacteria were lacking in all lakes with a pH of <6 and occurred in all investigated lakes higher than 1,700 m above sea level (a.s.l.), except for Lake Weissensee (2,226 m a.s.l.). Focusing on the 64 nonacidic lakes (pH 6.6 to 9.0; no missing values) we found that abundances of SOL bacteria were negatively related to high trophic and pH (GLM, $P < 0.01$; Tweedie error structure, power = 1.6, $\phi = 1.5$).

Members of the SOL cluster occurred in 53 of the 64 nonacidic lakes. Phylogenetically uncharacterized SOL bacteria occurred in only three lakes in this data set. It was the only group of SOL bacteria in the Swedish lakes Mörtsjön and Tvigölingen. Therefore, this group and the two lakes were not considered in the analysis. Conductivity, pH, altitude, and trophic individually explained 34%, 56%, 47%, and 21% of the variation in the abundances of HAL, LD2, and GKS2-217 bacteria, respectively ($P_{\text{adj}} < 0.05$). These four environmental variables together explained 66% of the variation in the abundances of the three SOL subclusters. The only variable that had a statistically unique effect was pH, i.e., after the effects of the other three variables had been removed, it still explained 29% of the variation in the abundances of the three SOL subgroups ($P < 0.001$). Conductivity or altitude had significant conditional effects, i.e., either of them accounted for an additional 7% of the variation in the abundances of the SOL subclusters after taking into account the effects of pH ($P < 0.05$). The two sets of environmental variables (either pH and conductivity or pH and altitude) explained almost as much variation as the full set of four environmental variables. They accounted for 59% of the variation in the abundances of the SOL subclusters LD2, HAL, and GKS2-217.

The environmental variable accounting for most of the variation in the abundances of the SOL subclusters was pH, which is illustrated in Fig. 1. CCA axis 1, which was most highly correlated with pH, separated LD2 from GKS2-217. LD2 occurred at a higher pH and conductivity, whereas GKS2-217

FIG. 1. Canonical-correspondence triplot based on 51 SOL-positive freshwater habitats, the three SOL subclusters, pH, and conductivity. The Swedish lakes Mörtsjön and Tvigölingen exclusively harbored phylogenetically uncharacterized SOL bacteria and hence were excluded. pH and conductivity best explained variation in the three SOL subgroups. Other environmental variables (dashed arrows) were supplementary. Samples were placed according to their pH and conductivity values. The black circles indicate the optima (weighted averages) of the three SOL subclusters in respect to pH and conductivity. Sample and subcluster scores were scaled (factor = 0.4).

was found only in soft-water lakes. HAL was intermediate. SOL subgroups showed a pH tolerance of about 2 (HAL and LD2) and 1 (GKS2-217) orders of magnitude (Table 4). The narrower pH range of the GKS2-217 subcluster did not overlap with the wider one of the LD2 subcluster (Fig. 2). The HAL subgroup was detected in circumneutral (pH 6.6 to 8.6) habitats, overlapping with the respective pH ranges of the other two subgroups (Fig. 2B). The upper range of tolerated pH values of the LD2 subcluster was slightly alkaline. A similar picture can be drawn using electrical-conductivity values (Fig. 3). This variable clearly separated the stenohaline GKS2-217 subcluster from the euryhaline LD2 subcluster. The tolerated conductivity range of the HAL subcluster overlapped with the ranges of the other two subclusters (Fig. 3B).

Sympatric versus allopatric occurrence of SOL subgroups.

The frequently >100- μm -long filaments of the GKS2-217 subcluster were found exclusively in oligotrophic soft-water ecosystems with low conductivity values (<50 $\mu\text{S cm}^{-1}$) in the Austrian Alps and northern Sweden. In seven samples (41.2% of GKS2-217-positive samples), this group was the only SOL subcluster, while in the other habitats it had a sympatric occurrence with the HAL subcluster.

In contrast, the LD2 subcluster was detected in a broad range of morphometrically different habitats with various water chemistries, such as ultraoligotrophic, deep, submontane lakes (e.g., Lake Attersee); hypertrophic bays of large, shallow lowland lakes (e.g., Lake Taihu); the large East African lakes (Lake Victoria and Lake Tanganyika); and oligo- and polyhaline high-mountain lakes in Tibet. In the majority of LD2-

TABLE 4. Minimum and maximum values of environmental variables for SOL subgroups

Subgroup	Parameter	Minimum	Ecosystem ^a	Maximum	Ecosystem ^a
HAL	Altitude (m a.s.l.)	0	Lake Loosdrecht	2,309	Lake ob. Klaffersee
	Lake area (ha)	1	Cenote Laguna	4,620	Lake Attersee
	Maximum depth (m)	2.6	Lake Eckarfjärden	114	Lake Wolfgangsee
	Temp (°C)	4.8	Lake Mondsee	23.3	Lake Nkuruba
	pH	6.6	Lake ob. Klaffersee	8.6	Lake Krottensee
	Conductivity ($\mu\text{S cm}^{-1}$)	14.7	Lake unt. Klaffersee	570	Lake Loosdrecht
LD2	Altitude (m a.s.l.)	0	Lake Loosdrecht	4,987	High mountain lake (Tibet)
	Lake area (ha)	0.1	Pond Hawaii	6,880,000	Lake Victoria
	Maximum depth (m)	1.5	Pond Waldteich	1,471	Lake Tanganjika
	Temp (°C)	2	Lake Mondsee	25.5	Lake Mondsee
	pH	7.7	Lake Egelsee II	9.5	Lake Mondsee
	Conductivity ($\mu\text{S cm}^{-1}$)	64	Lake Falkertsee	21,700	Lake Qinghai
GKS2-217	Altitude (m a.s.l.)	115	Lake Ämthyttsjön	2,309	Lake ob. Klaffersee
	Lake area (ha)	0.2	Lake Bergsee 5	11	Lake Zwerfenbergsee
	Maximum depth (m)	6	Lake Moaralmsee	40	Lake unt. Klaffersee
	Temp (°C)	6	Lake Moaralmsee	10.7	Lake unt. Sonntagkarsee
	pH	6.4	Lake Ämthyttsjön	7.3	Lake Moaralmsee
	Conductivity ($\mu\text{S cm}^{-1}$)	11	Lake Bergsee 5	48	Lake ob. Klaffersee
PUC	Altitude (m a.s.l.)	49	Lake Tvigölingen	1,872	Lake Falkertsee
	Lake area (ha)	0.1	Hawaii pond	6,880,000	Lake Victoria
	Maximum depth (m)	3.4	Lake Mörtsjön	84	Lake Victoria
	Temp (°C)	6.4	Lake Mörtsjön	23.3	Lake Nkuruba
	pH	6.7	Lake S. Snesharen	9.0	Lake Titisee
	Conductivity ($\mu\text{S cm}^{-1}$)	29	Lake Storsjön	535	Cenote Laguna

^a ob., upper; unt., lower.

FIG. 2. (A) Total abundances (log scale) of SOL subclusters versus pH. (B) Range of pH values in which SOL subclusters were detected in the investigated ecosystems. PUC group, phylogenetically uncharacterized SOL bacteria.

positive habitats (70.1%), this subgroup occurred exclusively, while in the rest of the habitats, it had a sympatric occurrence with the HAL subcluster and phylogenetically uncharacterized SOL bacteria (the PUC group).

The third known SOL subcluster (HAL) was almost always found (96.8% of HAL-positive samples) in coexistence with other SOL subclusters (LD2, GKS2-217, and the PUC group).

No sympatric occurrence of members of the LD2 and GKS2-217 subclusters was found in any of the investigated habitats. Consequently, there was no coexistence of all three phylogenetically characterized SOL subgroups in any of the samples analyzed.

Phylogenetically uncharacterized (i.e., unknown subcluster affiliation) SOL filaments (PUC group). All bacteria of the SOL morphotype could be assigned to the SOL cluster by positive hybridization with probes SAP-309 and SOL-852. In 12 habitats, SOL bacteria could not be further assigned to one of the three SOL subclusters with subcluster-specific probes. These habitats included four Swedish soft-water lakes (Lakes Tvigölingen, Mörtsjön, Snesnaren, and Storsjön), two Austrian lakes (Lakes Hinterer Gosausee and Falkertsee), and one German lake (Lake Titisee), with conductivity values between 64 and $147 \mu\text{S cm}^{-1}$, and habitats with higher conductivity values in subtropical (Yangtze river) and tropical (Lake Victoria and Lake Nkuruba in Uganda, an artificial pond in Hawaii, and Cenote Laguna in Mexico) regions.

DISCUSSION

Several studies have investigated the factors controlling the diversity of freshwater bacterioplankton (15, 20, 25, 27, 38, 49, 50). Methé and Zehr (27), for instance, did not find direct effects of acidification and pH in broadly based taxonomic groups but hypothesized that effects might be seen in phylogenetically smaller subgroups. Our study chose a phylogenetically well-defined group of bacterioplankton to test which factors might influence its occurrence and composition.

Niche differentiation inside SOL cluster bacteria. Statistical analysis revealed that environmental factors influence the compositions of the SOL communities in natural habitats. Conductivity, pH, altitude, and trophic were identified as important variables that affect the occurrence and abundance of the known SOL subclusters. Altitude seems to play an important role for the GKS2-217 subcluster, because this subgroup was mainly detected in high mountain lakes located above the tree line. Yannarell and Triplett suggested that spatial differences at the regional and landscape levels explain differences in bacterioplankton community profiles in Wisconsin lakes (50). In the present study, the suggested significance may, however, partly result from covariance among the assessed environmental variables. Submontane and alpine areas, for example, tend to have oligotrophic environments with low conductivity, because of smaller and less intensively used catchments, resulting

FIG. 3. (A) Total abundances of SOL subclusters versus electrical conductivity. Note the log-log scale. Oligo- and polyhaline habitats are marked separately. The conductivity of seawater was set at a value of $50,000 \mu\text{S cm}^{-1}$. (B) Range of conductivity values in which SOL subclusters were detected in the investigated ecosystems. PUC group, phylogenetically uncharacterized SOL bacteria.

in lower ion and nutrient concentrations (37). A GKS2-217-positive ecosystem from northern Sweden, located below the tree line, demonstrates that this subgroup can also be found in oligotrophic soft-water lakes at low altitude. This example suggests that limnochemical characteristics (i.e., pH and conductivity), not landscape level, control the occurrence of two vicarious (6) species-like subgroups and hence, mainly drive the subcluster-specific composition of the SOL communities.

Apart from the dominating effect of the limnochemistry of the habitats, determined mainly by the geological backgrounds of the catchments, biotic factors might be involved in the fine tuning of the composition and abundance of the SOL community. All bacteria affiliated with the SOL cluster possess the same striking filamentous morphology. This similarity suggests that all SOL subgroups undergo comparable grazing pressures, which might originate from crustacean zooplankton (18, 30) rather than from protists, which are the major grazers of many other bacterioplankton groups (13). The large, rigid morphology potentially protects SOL bacteria from grazing by heterotrophic flagellates and most ciliates. Even so, Wu et al. (48) clearly showed that flagellates can be predators of long yet flexible bacterial morphotypes. While grazing pressure presumably is not a differentiating biotic factor between the SOL subgroups, trophic preferences might be. However, the trophic niche of neither subgroup of SOL bacteria has been characterized yet.

In most cases, it is impossible to quantitatively describe the complete ecological niche of an organism, including all chemical, physical, and biotic factors involved. However, only a few variables (dimensions) may be needed to adequately demonstrate ecological differentiation and hence niche differentiation between certain groups of organisms. Gray et al. demonstrated the niche differentiation in *Achromatium* spp. connected to different redox conditions (10, 11). Moore and colleagues (28) demonstrated that the ecological differentiation of cooccurring populations of *Prochlorococcus* characterized by 97% 16S rRNA gene sequence similarity is connected to high- and low-light environments in the North Atlantic, and in a variety of chemolithotrophic bacteria, niche separation is mainly shown by clearly definable trophic niches (21). We demonstrated that adaptation to different limnochemical conditions has played a major role in the ecological differentiation of SOL bacteria.

Ecological amplitude of SOL subclusters. Members of the LD2 subcluster were found over a broad range of habitat types. This wide ecological tolerance, together with numerical dominance, is also reflected in the number of published partial or full-length 16S rRNA sequences of this subgroup retrieved from freshwater ecosystems (34). These facts make the more generalist LD2 subcluster the most common of the known SOL subclusters.

In contrast to the generalist LD2 subcluster, the GKS2-217 subgroup was detected only in a strictly defined type of fresh-

water ecosystem. The preferred habitats of this subcluster were found in the Austrian Alps and in northern Sweden. Other potentially suitable habitats in northern Sweden with appropriate conductivity values either were populated by phylogenetically uncharacterized SOL filaments or possessed pH values that were excessively low. Also, five acidic lakes in the Bohemian forest (45) could be expected to harbor GKS2-217 bacteria, assuming a circumneutral pH. Acidification of these lakes started 50 years ago and may have caused the extinction of GKS2-217 populations.

The HAL subgroup displayed a broader ecological amplitude, comparable to that of the LD2 subgroup. Interestingly, this more generalist SOL subgroup could not be detected in as many samples as the more common LD2 subcluster. Obviously, the appearance of this SOL subgroup is restricted by other, yet unknown abiotic or biotic factors. The only validly described species inside the SOL cluster, *Haliscomenobacter hydrossis* (44), is affiliated with this subgroup. This species is exclusively known from wastewater treatment plants (46) and could not be detected in spot samples of the natural freshwater ecosystems investigated in this work.

A part of the substantially higher ecological amplitude of the LD2 and HAL subgroups compared to the GKS2-217 subcluster could be based on the higher phylogenetic diversity found in the first two groups (34), thus representing phylogenetically and ecologically more diverse clusters of organisms. A higher resolution of the molecular identification tools might therefore reveal more phylogenetically characterized subunits within those well-defined SOL subclusters. Assuming a high enough phylogenetic resolution, such further steps might also reveal potential biogeographic patterns inside the closely related SOL subclusters, as demonstrated for *Cylindrospermopsis* strains (*Cyanobacteria*) by 16S-23S internally transcribed spacer sequences (12) and for anaerobic phototrophic consortia by partial 16S rRNA gene sequences (7).

Phylogenetically uncharacterized SOL genotypes. Phylogenetic analysis revealed partial sequences that clustered within the monophyletic SOL cluster but could not be affiliated with any of the three known SOL subclusters (34). Regarding these partial sequences (AY509351, AY509360, and AY509380; A. Eiler and S. Bertilsson, unpublished), it is more likely that SOL bacteria that did not hybridize with subcluster-specific probes (only 6% of all SOL bacteria) belong to one or a few still uncharacterized SOL subclusters than that the lack of hybridization with subcluster-specific probes is a result of a mutation present in only a portion of SOL subcluster members.

In the present study, environmental variables, such as pH and electrical conductivity, meaningfully explained distribution patterns between closely related bacterioplankton subgroups. Considering these differentiating factors, the diversity of SOL cluster bacteria on the previously defined subcluster level (34) might be still higher, as suggested by the occurrence of phylogenetically uncharacterized SOL filaments in some freshwater ecosystems. A closer look at specific habitats indicates the potential existence of two or more additional SOL subclusters. One seems to inhabit oligo- and oligomesotrophic soft-water lakes in Europe; other potential subclusters might be found in subtropical and tropical hard-water habitats with higher nutrient loads. Besides the SOL subgroups discovered thus far, forming the majority of the monophyletic SOL cluster, there

seem to be other, unknown subgroups that still need to be characterized phylogenetically and ecologically.

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