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Ecotypes of Planktonic Actinobacteria with Identical 16S rRNA Genes Adapted to Thermal Niches in Temperate, Subtropical, and Tropical Freshwater Habitats

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Seven strains with identical 16S rRNA genes affiliated with the Luna2 cluster (*Actinobacteria*) were isolated from six freshwater habitats located in temperate (Austria and Australia), subtropical (People's Republic of China), and tropical (Uganda) climatic zones. The isolates had sequence differences at zero to five positions in a 2,310-nucleotide fragment of the ribosomal operon, including part of the intergenic spacer upstream of the 16S rRNA gene, the complete 16S rRNA gene, the complete 16S-23S internal transcribed spacer (ITS1), and a short part of the 23S rRNA gene. Most of the few sequence differences found were located in the internal transcribed spacer sequences. Two isolates obtained from habitats in Asia and Europe, as well as two isolates obtained from different habitats in the People's Republic of China, had identical sequences for the entire fragment sequenced. In spite of minimal sequence differences in the part of the ribosomal operon investigated, the strains exhibited significant differences in their temperature response curves (with one exception), as well as pronounced differences in their temperature optima (25.0 to 35.6°C). The observed differences in temperature adaptation were generally in accordance with the thermal conditions in the habitats where the strains were isolated. Strains obtained from temperate zone habitats had the lowest temperature optima, strains from subtropical habitats had intermediate temperature optima, and a strain from a tropical habitat had the highest temperature optimum. Based on the observed temperature responses, we concluded that the strains investigated are well adapted to the thermal conditions in their home habitats. Consequently, these closely related strains represent different ecotypes adapted to different thermal niches.

Many free-living bacterial, archaeal, and protist species have a cosmopolitan distribution and differ in this trait from almost all macroorganisms (9). Closely related strains of several microbial species have been detected in distant habitats (4, 6, 8, 10, 12, 26). For some free-living bacteria and archaea, restricted geographical distributions have been demonstrated for strains inhabiting rare, extreme environments (e.g., hot springs) (17, 24). These distributions might be a result of isolation of populations due to limited dispersal potential resulting from small population sizes combined with the rare occurrence of appropriate habitats separated by wide distances. In the case of free-living microorganisms inhabiting nonextreme environments, the existence of a restricted geographical distribution has rarely been demonstrated (6). For free-living microbes easy dispersal and frequent population of appropriate habitats are assumed (2, 9). Intensive gene flux between habitats should prevent the isolation of populations. On the other hand, local adaptation of populations may prevent colonization of a habitat by less adapted competitors originating from an ecologically different habitat. It is not known, however, if free-living cosmopolitan microbial species consist of many locally adapted populations or if they form a single global population with intensive gene flux.

Intraspecific differences in physiological and ecological traits are well known for free-living microorganisms (3, 14, 18, 21, 22, 23), but so far it has been shown only rarely that this intraspecific variation is linked to local adaptation. For instance, Bronikowski et al. (5) demonstrated that there was a positive correlation between the optimal growth temperatures of *Escherichia coli* and *Salmonella enterica* strains and the body temperatures of their cold-blooded (turtles) or warm-blooded hosts (swine and squirrels). These findings indicate that there is local adaptation of members of the same species to thermal niches, which are different due to differences in the body temperatures of their hosts.

In order to test for local adaptation in free-living bacteria, we isolated seven *Actinobacteria* strains with identical 16S rRNA gene sequences (genotype 1 strains) from freshwater habitats located in different climatic zones. These habitats provide different thermal conditions; therefore, bacteria inhabiting these freshwater systems may be differently adapted. We investigated the thermal adaptation of six of the seven genotype 1 strains and found pronounced differences in the thermal adaptation of the strains investigated. These differences reflect the different thermal conditions of the habitats from which the strains originated. Therefore, we concluded that the strains investigated represent different ecotypes adapted to different thermal niches.

MATERIALS AND METHODS

Sampling sites and isolation of bacterial strains. The seven strains investigated were isolated by the filtration-acclimatization method (10, 11) from surface waters (depth, 0.5 to 1 m) of six freshwater habitats located in Austria, Australia, the People's Republic of China, and Uganda. Details on the sampling habitats are shown in Table 1 and elsewhere (10–12).

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TABLE 1. Characteristics of habitats from which the seven genotype 1 strains were isolated

Habitat	Habitat characteristics	No. of sampling sites	Country	Geographic coordinates	Climatic zone	Monthly avg air temp (°C)[d]		Yearly maximum water temp (°C)
						Minimum	Maximum	
Lake Mondsee[a]	Deep submontane lake in prealpine region	1	Austria	47°50′N, 13°22′E	Temperate	−1.6	18.0	22–28
Lake Wolfgangsee[a]	Deep submontane lake in prealpine region	1	Austria	47°46′N, 13°22′E	Temperate	−1.6	18.0	22–26
Pond in Sydney[b]	Artificial shallow pond	1	Australia	33°52′S, 151°13′E	Temperate	12.0	22.1	26–31
Lake Taihu[c]	Large shallow lake	2	People's Republic of China	31°30′N, 120°20′E	Subtropical	2.2	27.8	29–34
Huqiu (Tiger Hill) pond	Small pond, influenced by a spring	1	People's Republic of China	31°20′N, 120°40′E	Subtropical	2.2	27.8	ND[e]
Pond near Jinja	Shallow natural pond	1	Uganda	0°25′N, 33°12′E	Tropical	20.1	22.4	31[f]

[a] Lake Mondsee and Lake Wolfgangsee are located in the Salzkammergut area. The distance between the two sampling sites is 10 km.
[b] Located in the Sydney Royal Botanic Gardens.
[c] The greatest distance between sampling sites in Lake Taihu and the Huqiu pond is ca. 60 km.
[d] At locations in the same area as the habitats sampled (Salzburg, Sydney, Nanjing, Kampala).
[e] ND, not determined.
[f] Maximum temperature measured in another pond in the area of Jinja (data from Asiyo [1]).

Water temperatures. Data for the water temperatures of Lake Mondsee (1999 to 2003), Lake Wolfgangsee (1999 to 2003), Lake Taihu (1991 to 2002), and the pond in the Sydney Royal Botanic Gardens (2000 to 2004) were analyzed. For Lake Mondsee depth profiles of the water temperatures that were recorded in monthly (cold season) to fortnightly (warm season) intervals were available. Data for Lake Wolfgangsee were obtained at a depth of 0.5 m at 15-min intervals. Data for Lake Taihu were recorded at several sampling points at a depth of ca. 1 m at monthly intervals. Temperature data for the Huqiu pond, as well as the pond near Jinja, Uganda, were not available.

Sequencing of 16S rRNA genes and flanking regions. Parts of the ribosomal operon, including the flanking region (intergenic spacer) upstream of the 16S rRNA gene, the 16S rRNA gene, the 16S-23S internal transcribed spacer (ITS1), and a short fragment of the 23S rRNA gene, of eight Luna2 cluster strains (seven strains with identical 16S rRNA sequences and one reference strain) were directly sequenced. For sequencing of the 16S rRNA gene, standard primers and PCR conditions were used (15). Primer LEAD1 (5′-CCT TGA GAA CTC AAC AGC GTG-3′) was designed for amplification and sequencing of parts of the intergenic spacer (IGS) upstream of the 16S rRNA gene. This primer targets a conserved sequence located ca. 180 to 300 bp upstream of the 16S rRNA genes, which was found in *Streptomyces* spp. (e.g., *Streptomyces griseus*; accession number M76388) *Microbispora bispora* (accession number U83909), *Frankia* spp. (accession number M88466), and *Clavibacter michiganensis* subsp. *sepedonicus* (ongoing genome sequencing project; http://www.sanger.ac.uk/Projects/C_michiganensis/). Amplification with primers LEAD1 and 1492R (15) was performed with Primus or Primus 96^{Plus} thermocyclers (MWG-Biotech) by using 50-μl reaction mixtures containing approximately 100 ng of DNA, each deoxynucleotide at a concentration of 200 μM, 2 mM $MgCl_2$, 1.25 U of *Taq* DNA polymerase (QIAGEN), and each primer at a concentration of 0.2 μM. The cycling conditions were initial denaturation at 94°C for 3 min, which was followed by 30 cycles at 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min. The cycling was finished with an extension step of 7 min at 72°C.

For amplification and sequencing of ITS1, primers 1406Fmod (5′-TGT ACA CAC CGC CCG TCA AG-3′; modified primer 1406F [16]) and L189R (25) were used. In the case of the Luna2 strains investigated the 3′ end of primer 1406F matched its target sequence in the 16S rRNA gene, as well as a sequence in ITS1. This resulted in amplification of two fragments that were different sizes. Fragments obtained from one strain were separated and sequenced. Based on the sequences obtained, primer 1406Fmod specific for the 16S rRNA gene was designed and used for sequencing ITS1 of all the strains investigated. Amplification was performed basically as described above for amplification with primers LEAD1 and 1492R. The only differences were the $MgCl_2$ concentration (3 mM), the primer concentration (0.3 μM), the annealing temperature (41°C), a shorter extension time at 72°C (1 min), and a shorter final extension step at 72°C (5 min). The sequences obtained were aligned and analyzed by using the ARB software package (http://www.arb-home.de [20]). Phylogenetic analysis was performed as described previously (10).

Determination of temperature-dependent growth characteristics of temperature-acclimatized bacterial strains. The growth of six strains was monitored by spectrophotometric measurement of the optical density at 575 nm. Experiments were performed at 15, 20, 25, 27.5, 30, 32.5, 35, 36, 37, and 38°C. Prior to the experiments, bacteria were acclimatized for at least 10 generations to the temperature conditions in the growth experiments. All cultures were grown in liquid NSY medium (11) (3 g liter^{-1}) on a rotary shaker (100 rpm) in the dark. Precultures of acclimatized strains that were used for setting up growth experiments were grown overnight. Cultures used for growth rate measurement were adjusted to an initial optical density of 0.015 to 0.02. Experiments were performed in 100-ml Erlenmeyer flasks in triplicate for each strain. The medium used for the experiments was preincubated for several hours under the same temperature conditions as the temperature conditions in the experiment. Measurement of the optical density was started 30 to 60 min after inoculation and then was performed at 30- to 60-min intervals (depending on the growth rates of the bacteria tested) over a period of 4 to 5 h. Experiments at each temperature were performed simultaneously for all six strains investigated. Experiments at 30 and 32.5°C were repeated three and four times, respectively, and experiments at 25, 36, and 37°C were repeated two times.

For calculation of growth rates the optical density data were ln transformed and analyzed by linear regression. Only optical density values in the range from 0.02 to 0.1 (at least three measurements) were considered for calculation. For previous growth rate measurements (10) the data used for calculation were not restricted to this range of optical density values.

The optimal growth temperature was determined graphically. For all strains investigated there was a linear increase in the temperature-dependent growth curves at the temperature range below 25 to 35°C. With further increases in

TABLE 2. Characteristics of the seven genotype 1 strains and reference strain MWH-Tana7 (16S rRNA genotype 7)

Strain	Habitat	Water temp (°C)[a]	Season of sampling[b]	No. of sequence differences[c]	Optimum temp (°C)	Maximum temp (°C)	Highest growth rate (h^{-1})[e]	Shortest doubling time (h)[e]	Cell size (μm^3)[f]
MWH-Mo1	Lake Mondsee	4.7	w		28.8	35.0	0.344	2.0	0.062
MWH-Wo1	Lake Wolfgangsee	4.5	w	3	27.5	37.0	0.352	2.0	0.059
MWH-Aus1	Pond, Sydney	31.0	s	3	25.0	36.0	0.321	2.2	0.064
MWH-HuqW11	Pond, Huqiu	ND[d]	a	2	31.8	37.0	0.365	1.9	0.057
MWH-Ta1	Lake Taihu (site 1)	7.2	w	3	34.2	37.0	0.442	1.6	0.055
MWH-TaSIIW17	Lake Taihu (site 2)	14.3	a	2	ND	ND	ND	ND	ND
MWH-Uga2	Pond near Jinja	>20[g]	r	5	35.6	37.0	0.388	1.8	0.061
MWH-Tana7[h]	Tana River, Kenya	>20[g]	r	59	ND	ND	ND	ND	0.065

[a] Water temperature at the time of sampling for isolation of bacteria.
[b] w, winter; s, summer; a, autumn; r, rainy season.
[c] Number of different sequence positions in a 2,310-bp fragment of the ribosomal operon compared with the sequence of MWH-Mo1.
[d] ND, not determined.
[e] Growth in liquid NSY medium (3 g $liter^{-1}$) at the temperature allowing the highest growth rate.
[f] Growth in liquid NSY medium (3 g $liter^{-1}$) at ca. 25°C.
[g] The temperature was not measured; the temperature usually does not drop below 20°C.
[h] The genotype I strains and strain MWH-Tana7 have a level of 16S rRNA gene sequence similarity of 99.8%.

temperature the growth curves showed either a plateau or a linear decrease. The optimal growth temperature was defined as the crossing point of the linear regression lines of the increasing part of the growth curve and the plateau line or the decreasing part of the curve.

Determination of minimum and maximum temperatures allowing growth of nonacclimatized bacterial strains. Bacteria were precultured in liquid NSY medium on a rotary shaker (100 rpm) at room temperature. Samples (10 μl) from the precultures were spotted on NSY agar plates, and the plates were sealed with Parafilm and incubated at 5, 10, 15, 20, 30, 35, 36, 37, and 38°C. The plates were inspected for growth weekly for 8 weeks. Formation of macroscopically visible colonies was recorded as growth.

Statistical analysis. Two-way analysis of variance (ANOVA) was used to test for strain-specific differences in temperature response curves, as well as for interactions of the variables temperature and strain with the temperature response. A multiple-comparison procedure was used to test for significant differences in the growth rates of the strains investigated at a given temperature. These pairwise comparisons (Tukey test) were restricted to the temperature-specific data sets, which passed the normality and equal variance tests.

Nucleotide sequence accession numbers. The ribosomal sequences of the eight isolates obtained in this study have been deposited under accession numbers AJ507460, AJ507464, AJ507466, AJ565414, AJ565435, AJ565436, AJ630367, and AJ630368.

RESULTS

The seven strains isolated with identical 16S rRNA gene sequences (Table 2) belong to the Luna2 cluster (*Actinobacteria*) (10), which contains at least 11 different 16S rRNA genotypes (Fig. 1). The minimal sequence similarity within the Luna2 cluster is 95.9% (Fuku N101 and ARK 10165). Members of the cluster were isolated from habitats in Europe, East Asia, East Africa, Central America, and Australia and from Arctic sea ice (10, 11). The seven identical strains represent a 16S rRNA genotype (genotype 1) present in pelagic freshwater habitats located in at least three climatic zones on at least three continents. The strains were isolated from oligotrophic (e.g., Lake Wolfgangsee), oligomesotro-

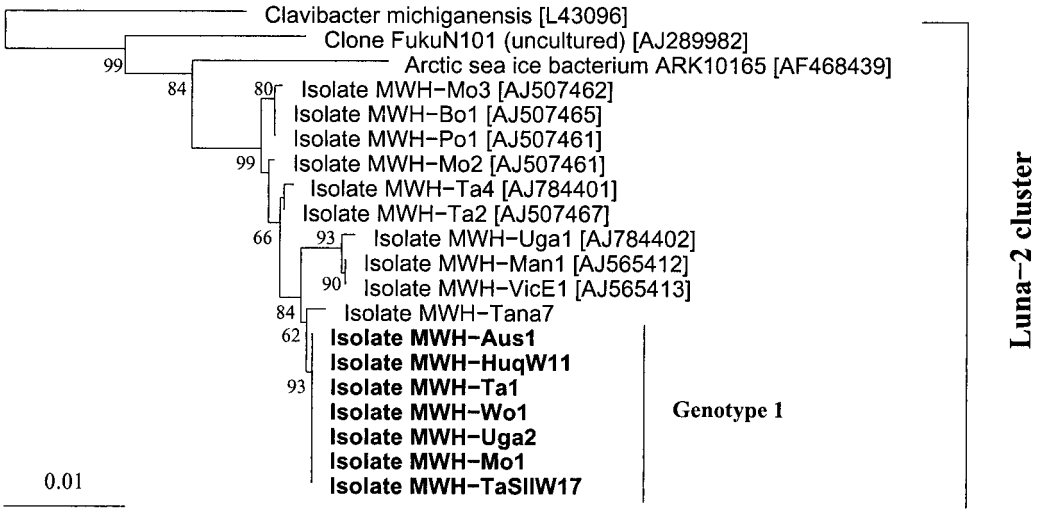

FIG. 1. Neighbor-joining tree showing the phylogenetic relationships of *Actinobacteria* affiliated with the Luna2 cluster. The tree was calculated by using homologous 1,427-nucleotide sequence fragments (*E. coli* positions 51 to 1499) of the 16S rRNA genes. Bootstrap values (1,000 replicates) that are >60% are indicated at the nodes. Scale bar = 1% estimated sequence divergence. The numbers in brackets are accession numbers.

phic (e.g., Lake Mondsee), and hypertrophic (e.g., Lake Taihu, Meiling Bay) habitats. The seven genotype 1 isolates have very similar small cell sizes (Table 2) and identical C-shaped cell morphologies, and they all produce colonies with identical coloration, surface, and border characteristics on agar plates. More detailed descriptions of phenotypic characteristics of three genotype 1 strains can be found elsewhere (10).

Sequence analysis. A 2,310-nucleotide sequence stretch starting 181 or 184 nucleotides upstream of the 16S rRNA gene and ending 198 nucleotides downstream of the 5′ end of the 23S rRNA gene was obtained for the seven genotype 1 strains, as well as the closest reference strain (MWH-Tana7, genotype 7). The 1,525-nucleotide 16S rRNA gene sequences of the genotype 1 strains and MWH-Tana7 differed at three sequence positions (99.8% sequence similarity). More differences were found in the parts of the IGS upstream of the 16S rRNA gene analyzed. The homologous sequences of the seven genotype 1 strains had the same 181-nucleotide sequence, while the homologous sequence of MWH-Tana7 was three nucleotides longer. The partial IGS sequence of MWH-Tana7 differed at 22 to 24 positions (sequence similarity, 87.0 to 88.0%) from the sequences of the other seven strains. The sequences of six of the seven genotype 1 strains were identical, while strain MWH-Mo1 differed at two positions (98.9% sequence similarity). The 16S-23S internal transcribed spacer of the seven genotype 1 strains had the same 406-nucleotide sequence, while the sequence of MWH-Tana7 was three nucleotides shorter. The internal transcribed spacer sequences of the seven strains differed at zero to four sequence positions (sequence similarities, 99.0 to 100%), while the sequence of MWH-Tana7 differed at 29 to 31 positions (sequence similarity, 92.4 to 92.9%). The partial (198-bp) 23S rRNA gene sequences of the seven genotype 1 strains were identical but differed at one position from the sequence of MWH-Tana7. For the entire sequenced fragment of the ribosomal operon the seven genotype 1 strains differed at zero to five positions, while MWH-Tana7 differed at 55 to 59 positions. Strains MWH-Wo1 (Austria) and MWH-Ta1 (People's Republic of China), as well as strains MWH-HuqW11 (People's Republic of China) and MWH-TaSIIW17 (People's Republic of China), had identical sequences. The sequence differences among the genotype 1 strains correlated neither with the geographical origins nor with the climatic conditions in the home habitats.

Thermal adaptation. Due to limited space in the incubator used, the temperature adaptation experiments were performed with only six genotype 1 strains. In the maximum and minimum growth temperature experiments, which were performed without prior temperature acclimatization, no pronounced differences in the temperature ranges of the strains were found. All strains grew at 5°C (the lowest temperature tested), but they performed differently. Very weak growth was observed for strains MWH-Aus1 and MWH-Uga2, which do not experience temperatures below 10 and 20°C, respectively, in their home habitats. The maximum growth temperatures were found to be in a narrow range, from 35.0 to 37.0°C (Table 2). The maximum growth temperatures observed in experiments with and without prior temperature acclimatization were identical. While the temperature ranges of all the strains were very similar (≤5°C to 35 to 37°C), pronounced differences in the optimal growth temperatures and shapes of the temperature response curves were observed (Table 2 and Fig. 2). Some of the strains investigated produced response curves with marked peaks, while other curves had plateau-like shapes. The optimum growth temperatures determined ranged from 25.0 to 35.6°C. With the exception strains MWH-Wo1 and MWH-Uga2, all oft the strains had temperature response curves that were significantly different from each other (as determined by two-way ANOVA). The curves for strains MWH-Wo1 and MWH-Uga2 crossed at 31.5°C and showed that the optimum temperatures were different (27.5 and 35.6°C). At all temperatures less than 31.5°C strain MWH-Wo1 had higher growth rates than strain MWH-Uga2, and at all temperatures more than 31.5°C strain MWH-Wo1 had lower growth rates than strain MWH-Uga2. Furthermore, these two strains had significantly different growth rates at almost all temperatures (as determined by one-way ANOVA).

Besides strain-specific differences, there was also a significant interaction effect of the variables temperature and strain on the temperature responses. Thus, the differences in thermal adaptation of the strains did not simply result from shifts in the temperature response curves to higher or lower temperatures. This interaction effect resulted in strain-specific modifications of the shapes of the temperature response curves.

Growth rates of the strains were also compared separately for five temperatures by one-way ANOVA (Tukey test). For the other temperatures, the data did not pass the test for normality and/or for equal variance; therefore, an ANOVA could not be performed by the Tukey test. An increase in the percentage of significantly different pairs with increasing temperature was observed (Fig. 3). At lower temperatures (i.e., 15 and 20°C) the only tropical strain, strain MWH-Uga2, was involved in the majority of significantly different pairs. At temperatures that were ≤30°C strain MWH-Uga2 had the lowest growth rate of all strains, but at 35°C this strain had the highest growth rate of all strains. At 35°C only 1 of 15 pairs showed no significant difference in the growth rates. The isolates in this pair (MWH-Ta1 and MWH-HuqW11) were from subtropical habitats.

Water temperatures in habitats from which the bacteria were isolated. The surface water temperatures in the dimictic lakes Mondsee (Fig. 4) and Wolfgangsee usually range from 0°C to 22 to 25°. In exceptional years (e.g., 2003) maximum surface water temperatures of 26 to 28°C were measured. The water temperatures in the hypolimnion range from 4 to 6°C. Lake Taihu is a large shallow lake with a maximum water depth of 2.6 m. The shallowness of the lake prevents any long-lasting thermal stratification of the water body. The water temperatures ranged from 1.5 to 34°C (Fig. 4). The temperatures in the pond located in the Sydney Royal Botanic Gardens ranged from 13 to 31°C. Systematic water temperature records were not available for the Huqiu pond or for the pond near Jinja, Uganda. Asiyo (1) reported temperature data for a pond which is located in the same area as the pond near Jinja. She measured a minimum water temperature of 24.6°C and a maximum temperature of 30.6°C. Due to the short period of measurements (May to August 2003), these data may not reflect the real maximum and minimum water temperatures in the pond from which strain MWH-Uga2 was isolated.

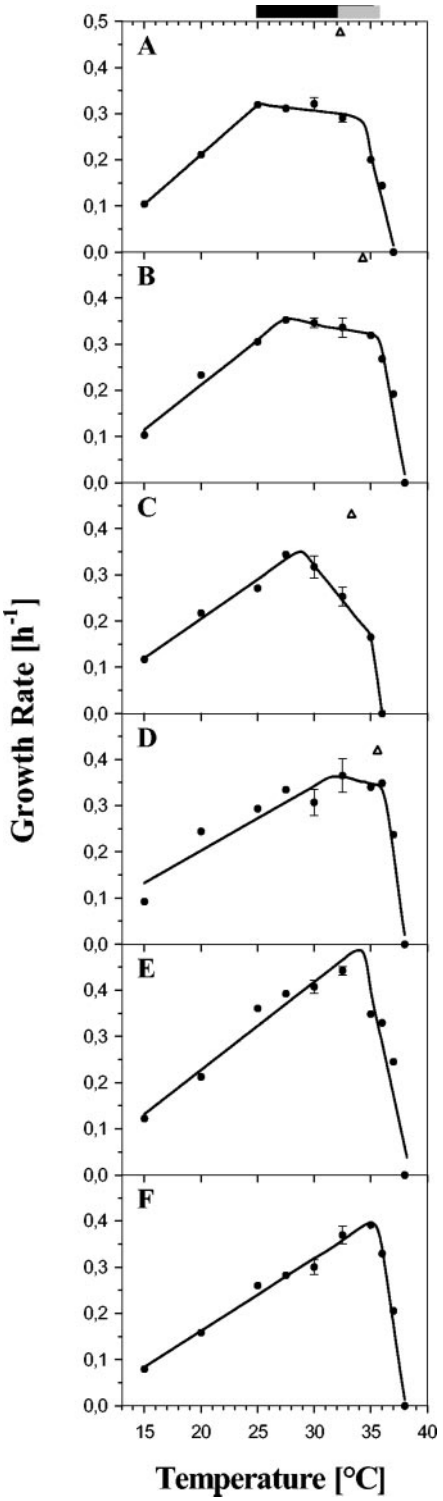

FIG. 2. Temperature response curves for temperature-acclimatized genotype 1 strains. The graphs are sorted (top to bottom) by increasing optimum temperature. (A) Strain MWH-Aus1 (isolated from a habitat in a temperate climatic zone); (B) strain MWH-Wo1 (temperate zone); (C) strain MWH-Mo1 (temperate zone); (D) strain MWH-HuqW11 (subtropical zone); (E) strain MWH-Ta1 (subtropical zone); (F) strain MWH-Uga2 (tropical zone). In panels A to D the crossing points of the linear regressions of the increasing and decreasing parts of the curves are indicated by open triangles. The vertical bar at the top indicates the range of optimum temperatures determined for the six strains. The gray part of the bar represents the temperature range in which the crossing points (A to D) and the optimum temperatures for strains MWH-Ta1 (E) and MWH-Uga2 (F) fall. The error bars indicate the standard deviations of the average growth rates from repeated experiments (only for 30.0 and 32.5°C data).

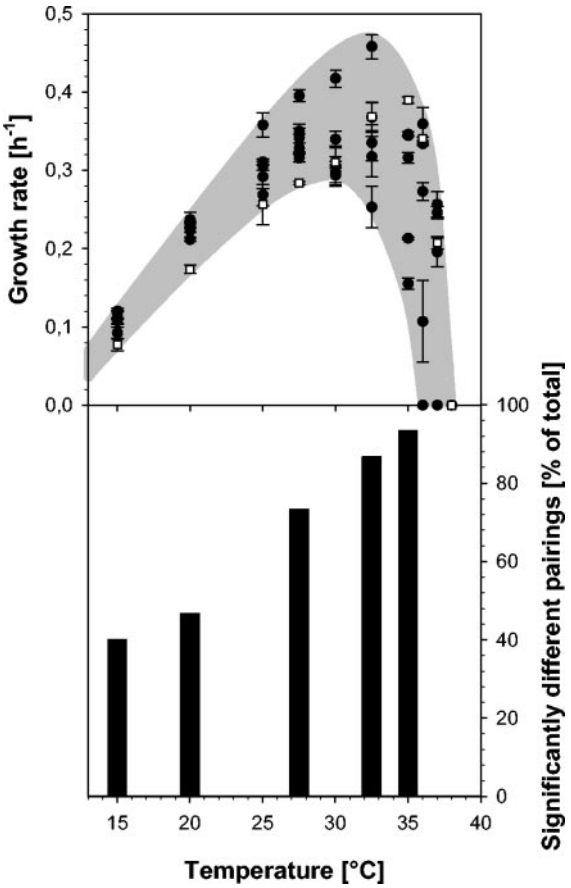

FIG. 3. (Top) Distribution of growth rate data measured for the six genotype 1 strains at different temperatures. Each symbol indicates the mean and standard deviation measured for one strain at one temperature. The data for tropical strain MWH-Uga2 are indicated by open squares. (Bottom) Pairwise comparison of strain-specific growth rates for significant ($P < 0.05$) differences by a one-way ANOVA (Tukey test). The percentage of pairs with significant differences increased with temperature. At lower temperatures the tropical zone isolate MWH-Uga2 is involved in the majority of significantly different pairs.

DISCUSSION

We investigated the thermal adaptation of closely related planktonic freshwater bacteria isolated from habitats located in three climatic zones. The low numbers of differences in the IGS and ITS1 sequences demonstrate the close phylogenetic relationship of the genotype 1 strains. Jaspers and Overmann (13) demonstrated relatively distant relatedness between two groups of strains, all of which had identical 16S rRNA gene sequences. Strains belonging to the two different groups had low levels of genomic DNA similarity, differences in ITS1 detectable by restriction fragment length polymorphism analysis, pronounced differences in cell morphology, and different genomic fingerprints. It is highly likely that these two groups of

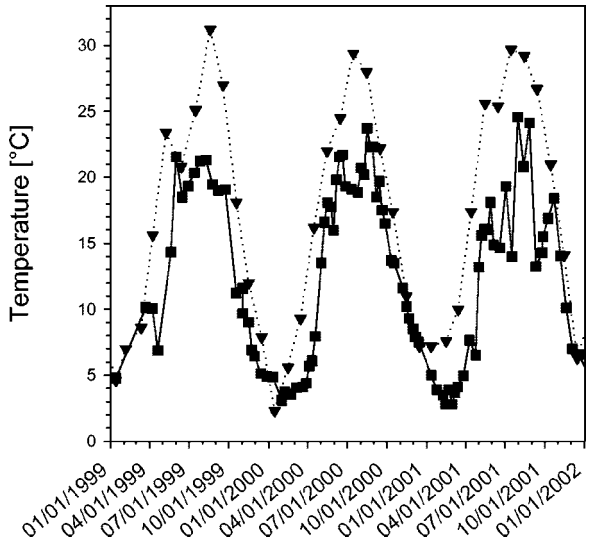

FIG. 4. Water temperatures in the nonstratified Lake Taihu (▼) and surface water (0 m) of Lake Mondsee (■) for 1999 to 2001. The maximum water temperatures reported for these lakes were observed during other years.

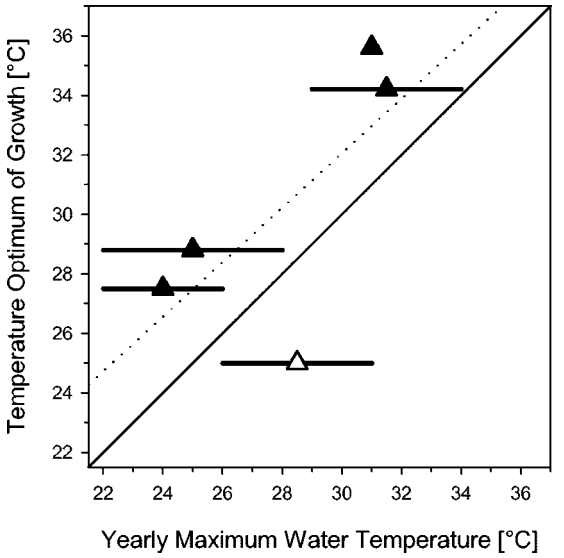

FIG. 5. Yearly maximum water temperatures of habitats plotted versus the optimum temperatures of genotype 1 strains obtained from these habitats. The horizontal bars indicate the ranges of yearly maximum water temperatures observed in different years. The average of the lowest and highest yearly maximum temperatures was plotted versus the optimum temperatures determined (triangles). The dotted line indicates a linear regression of the data plotted. Data for the strain isolated from an artificial pond in Sydney Royal Botanic Gardens are indicated by an open triangle. Water temperature data for several years were not available for ponds in the area of Jinja. Therefore, only the maximum temperature measured by Asiyo (1) in a field study lasting from May to August 2003 is plotted. No water temperature records for the Huqiu pond were available.

strains represent two different bacterial species. We did not perform DNA-DNA hybridization experiments with our strains; thus, we cannot safely conclude that the genotype 1 strains belong to a single species. On the other hand, bacterial strains with DNA-DNA homology values of $<70\%$ and 16S rRNA gene sequence similarity values of $>97\%$ were found in only a few cases (19). In contrast to the strains investigated by Jaspers and Overmann (13), the genotype 1 strains form a homogeneous group in terms of cell morphology, size, pigmentation, colony characteristics, and several other phenotypic traits (data not shown). Furthermore, it is highly unlikely that the few single-nucleotide differences in ITS1 of the genotype 1 strains could be detected by randomly selected restriction enzymes. Thus, the group of strains investigated in our study appears to be a homogeneous group, both in terms of sequence data and in terms of several phenotypic traits. We tentatively concluded that these strains belong to the same species.

Thermal adaptation. Bacterial growth rates increase with temperature up to the optimum temperature, at which the growth rate is maximal. Enzymatic processes are thought to limit further increases in growth rates at temperatures above the optimum temperature. The maximum temperature is reached immediately before an essential enzymatic process is inhibited. Temperature response curves that are shown in textbooks always have a clear peak. In contrast, for three of six strains investigated we observed a plateau-like curve shape (Fig. 2A, B, and D). For these strains the entire range of the plateau or a particular temperature within the range of the plateau could be considered the optimum temperature for growth. For two reasons we argue that the temperature at the onset of the plateau is the ecologically relevant optimum temperature for growth. First, we observed that for strains from temperate zone habitats much larger inoculum volumes were required for successful growth at higher temperatures. The inoculum volumes used for subculturing at lower temperatures ($\leq$30°C) frequently did not result in growth after the same temperature-adapted strains were subcultured at higher temperatures ($\geq$32.5°C). This clearly indicates that temperatures of $\geq$32.5°C are stressful growth conditions for the strains obtained from temperate habitats. In the case of strains MWH-Aus1 and MWH-Wo1 temperatures of $\geq$32.5°C are well within the plateau ranges of their temperature curves. Stressful growth conditions and thermally optimal growth conditions are in clear conflict. Second, a lack of enhanced growth with further increasing temperature indicated that one or several enzymatic processes were suboptimal, thus reducing the fitness of the organism. This is also in conflict with the assumption that the entire range of a temperature curve plateau represents optimum growth temperatures.

The optimum temperature observed generally corresponded to the highest water temperatures observed or estimated for the home habitats of the strains investigated (Fig. 5). For almost all genotype 1 strains the optimum temperature determined was a few degrees higher than the average yearly maximum water temperature. The only exception, strain MWH-Aus1 from a pond in Sydney Royal Botanic Gardens, had an optimum temperature that was several degrees lower than the average and highest observed annual maximum temperature of its home habitat. This strain was isolated from a shallow artificial pond, which may heat up more than similar large but deeper natural ponds having the same area.

Do some features of the temperature curves of genotype 1 strains reflect the temperature adaptation of a common ancestor? The temperature curves for the six genotype 1

strains are significantly different from each other, and strains from different climatic zones clearly have different optimum temperatures. On the other hand, the temperature response curves of all strain also have some common features. Obviously, all genotype 1 strains are able to grow at a similar temperature range, from at least 5°C to 35 to 37°C. Other Luna2 strains from tropical habitats (MWH-Man1 and MWH-VicE1) still grow at 40°C. Another common feature is that the final, linear decreases in the curves to the maximum temperature are very similar for all strains (Fig. 2). Furthermore, for all strains linear increases in the curves toward the optimum temperature were observed. For all strains these increasing lines have similar slopes; thus, the lines have more or less parallel characteristics. The major differences in the temperature curves are in the middle parts of the curves (Fig. 3). Extension of the linearly increasing parts of the curves toward higher temperatures and extension of the final decreasing lines toward lower temperatures result in lines that have crossing points in a narrow temperature range, 32 to 36°C (Fig. 2). These manipulation for strains MWH-Aus1, MWH-Wo1, MWH-Mo1, and MWH-HuqW11 result in curves which are very similar to the peaked curves for strains MWH-Ta1 and MWH-Uga2. Based on these obvious similarities of the curves, one may speculate on the temperature adaptation of the common ancestor of the strains. The ancestor would have been adapted to thermal conditions in subtropical or tropical habitats (i.e., optimum temperature in the range from 32 to 36°C). After dispersal and population of temperate zone habitats, the ability to grow at temperatures more than 30 to 31°C provided no more selective advantage. Therefore, mutations that affected only growth at these higher temperatures may have accumulated in strains inhabiting temperate habitats.

Evolution of temperature adaptation in *E. coli* was found to be slow (7). After 20,000 generations of growth under constant temperature conditions, only small changes in temperature adaptation were observed. These adaptations were much smaller than the differences in temperature adaptations found in the genotype 1 strains investigated. Under environmental conditions that result in generation times of several hours to a few days, the genotype 1 strains may need more than 10 years for 20,000 generations. Assuming a rate of evolution similar to that observed for *E. coli* under constant environmental conditions, the observed thermal adaptations of the genotype 1 strains may be the result of many decades of evolution. Considering these long time spans, the presence of relicts from the ancestral thermal adaptation is not unlikely.

Thermal niches of the strains investigated. The different genotype 1 strains were isolated from water samples with a broad range of temperatures (Table 2); thus, viable genotype 1 strains were present over a wide range of temperatures. This and the temperature adaptations observed may indicate that the genotype 1 strains are well adapted to the entire range of temperatures that occur in their home habitats. On the other hand, the strains differ in adaptation at higher temperatures (Fig. 2, 4, and 6) and potentially also at lower temperatures (e.g., strains MWH-Aus1 and MWH-Uga2). Obviously, the strains obtained from different climatic zones are adapted to different thermal niches. For instance, strain MWH-Mo1 from temperate Lake Mondsee is expected to be the inferior competitor compared to strain MWH-Ta1 from subtropical Lake Taihu when both strains are exposed to temperatures that typically occur in Lake Taihu during the hot season. Due to the different thermal adaptations, the strains obtained from different climatic zones have to be considered different ecotypes. These thermal adaptations may hamper the colonization of habitats by invading strains adapted to habitats with different thermal conditions. Local adaptation in combination with competition may limit the successful invasion of freely dispersed microbes and may lead to restricted geographical distributions of free-living microbes inhabiting nonextreme habitats.

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